Anticancer redox action of gallium nanoparticles combined with a low dosage of γ-radiation against hepatocellular carcinoma in male rats

Mohga S. Abdalla[a], El-Sayed M. El-Mahdy[a], Somaya Z. Mansour[b], Sawsan M. Elsonbaty[c], Menna A. Hussien[a]

[a]Department of Chemistry, Faculty of Science, Helwan University, Helwan, Departments of [b]Radiobiology, [c]Radiation Microbiology, National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority, Nasr City, Egypt

Correspondence to Menna A. Hussien, MSc, Department of Chemistry, Faculty of Science, Helwan University, Helwan, 11865, Egypt.
Tel.: 02 25569061;
e-mail: dr.mennaamin@gmail.com

Received: 8 September 2021
Revised: 25 October 2021
Accepted: 27 October 2021
Published: 18 August 2022

Egyptian Pharmaceutical Journal 2022, 21:328–337

Background and objective
Nanotechnology affords a new valuable field for the preparation of intrinsic nano anticancer drugs through green synthesis of plant active extracts supported with gallium nanoparticles (GaNPs) to provide us with a new Ga form of treatment with lower toxicity risk. The current study aimed at evaluation of a new GaNP form with grape seed extract as an anticancer agent against hepatocellular carcinoma (HCC) in rats. Moreover, the effect of the exposure to a low dose of γ-radiation on the treatment and prevention of tumor was studied.

Materials and methods
The cytotoxic effect was measured against the HepG2 tumor cell line. An experimental design was optimized using 80 Wistar male rats (120–150 g) divided into eight groups, with 10 rats each. The animals are administered with diethylnitrosamine to induce HCC and then orally administered with a dose of 38.5 mg/kg from the GaNPs in combination with the exposure of the total body to a low dose of γ-radiation (0.5 Gy).

Result and conclusion
The combination of GaNPs/γ-radiation demonstrated significant cytotoxicity against HepG2 cell line with an IC_{50} of 388.8 μg/ml. Moreover, the results indicated normal structures in the liver architecture, and the conventional biochemical assays showed significant depletion in lipid peroxide, alanine aminotransferase, and aspartate aminotransferase activities and creatinine levels. Additionally, there was a significant increase for the antioxidant state parameter in the form of a pronounced reduction of glutathione level. The ameliorative effect of the treatment was well appreciated by the histopathological alteration results. Therefore, it can be concluded that GaNPs/γ-radiation can serve as a good therapeutic agent for the treatment of HCC that ought to attract more studies.

Keywords:
biochemical parameters, gallium nanoparticles, grape seed extract, hepatocellular carcinoma, HepG2 cell, low-dose γ-radiation

Egypt Pharmaceut J 21:328–337
© 2022 Egyptian Pharmaceutical Journal
1687-4315

Introduction

Hepatocellular carcinoma (HCC) is the world's third most common cause of cancer-related death [1]. The main causes of HCC include chronic hepatitis B and C infections [2]. Other factors that contribute to the formation of HCC include fatty liver disease, iron overload, alcoholism, and exposure to environmental carcinogens [3]. Diethylnitrosamine (DEN) is one of the most prevalent carcinogens and frequently used for HCC induction. Simultaneously, it is widely used in everyday life and surroundings, in tobacco, smoke, processed food, gasoline, and cosmetics [4]. Inorganic nanoparticles (NPs) have recently received more attention as potential cancer-fighting diagnostic and therapeutic systems. Recent studies have shown promising results in both *in vitro* and *in vivo* imaging and tumor therapy, as well. Definite metal nanoparticles and transition metals have been shown to have anticancer properties. Gallium (Ga) is the second most commonly used metal ion for cancer treatment, after platinum. Ga has several radionuclides that have been used in medicine to treat and diagnose illnesses. In addition to protein and DNA synthesis, and DNA inhibition, the activity of enzymes such as serum alkaline

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

phosphatase is inhibited [5,6]. Exposure to Ga concentrations is expected to improve the therapeutic index of cancer diseases. The combination of biomolecules on the surface of the nanocomposite, such as phenols and flavonoids, alleviates cytotoxicity concerns, agglutination, and biological atmospheric instability; prevents cytotoxicity; and the traps reactive oxygen species (ROS). In addition, Ga in the form of nanoparticles overcomes Ga tolerance in cancer therapy [7].

Grape seed extract (GSE) has recently received a lot of attention. Grapes (*Vitisvinifera*) are highly rich in polyphenols, as the seeds contain 60–70% of grape polyphenols, which can be used as nutraceutical agents. Polyphenols, for example, flavonoids and their polymers (proanthocyanidins) are abundant in GSE, making it an excellent source of antioxidants [8,9]. Because of its high polyphenol content and structural variation, GSE has been shown to have cardioprotective, hepatoprotective, anti-diabetic, anti-mutagenic, and anti-inflammatory effects [8,10]. In addition, it has demonstrated promising chemopreventive and anticancer effects in a variety of cancer cells and animal tumor models, including skin, colorectal, prostate, and breast cancers [11,12]. So far, only sporadic efforts have been made to investigate, mainly *in vivo*, the effect of GSE on liver cancer [13,14]. Owing to their intrinsic antitumor properties, metal nanoparticles may help to prevent tumor formation, development, and progression. The application of external stimuli has an extrinsic effect, such as in hyperthermia, where metal NPs are activated by external radiation such as IR or radiograph to form free radicals that destroy cancer cells and also enhance the cytotoxic effect of ionizing radiation [15,16]. Ga is known to be the next most potent anticancer metal after platinum, as gallium nanoparticles (GaNPs) made in environmentally safe ways have shown anticancer efficacy against Ehrlich solid tumors via a redox mechanism [17].

Radiation hormesis is commonly assumed that low-dose radiation in the region of 0.1–0.5 Gy has some physiologic advantage. The low dosage of radiation has been seen to activate the radical detoxification system and improve DNA repair rates. Furthermore, it also raises immunological competence, which promotes the increase of a wide range of cytotoxic cells (lymphocytes), resulting in a decrease in the occurrence of metastatic cancer [18]. GaNPs combined with a low dose of γ-radiation (RAD) have previously been shown to prevent the production of cytotoxic effects on cancer cells [17,19]. Taking into account the aforementioned evidence on the potential of GaNPs as an anticancer agent, the present study was suggested to explore its potential therapeutic effect separately or combined with a low dose of γ-radiation against chemically induced hepatocellular carcinogenesis.

Materials and methods

Materials

Grape seed extract was obtained from a pharmacological source (gravital capsules). DEN and Ga-nitrite were purchased from Sigma-Aldrich Chemical Corporation, St. Louis, Missouri, USA, dissolved in deionized water, and used in the preparation of GaNPs.

γ-irradiation

Whole-body γ-irradiation of rats was performed using the Canadian gamma cell-40 (137Cs) at the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt. Rats were exposed to a single dose of γ-radiation (0.5 Gy) at a dose of 0.912 rad/s.

Experimental animals

Wistar male rats weighing 120–150 g were obtained from Nile Pharmaceutical and Industries Co. (Amiria, Cairo), and kept at the animal house of the NCRRT. Rats were housed in plastic cages, with five rats in each cage. They were fed a commercial diet (21% protein) and excess of drinking water ad libitum. The temperature was maintained at 22±3°C at the animal house. They were allowed to acclimatize to the environmental conditions such as temperature, pressure, humidity, good ventilation, and illumination for one week before the experiment. All rats were cared in accordance with the Ethical guidelines of NCRRT in accordance to the 'guide for the care and use of laboratory animals' published by the US National Institute of Health [20].

Green synthesis of gallium nanoparticles

First, 8.0 g of grape seed powder was mixed with 100 ml of distilled water, and the resulting mixture was placed in a water bath for 30 min at a temperature of 60°C. Next, the solution was filtered with Whatman filter paper No. 1 and then the filtrate (extract) was stored at 4°C. GaNP synthesis was performed according to the method described by Mohseni *et al.* [21]. At the initial stage, a freshly prepared Ga-nitrite solution (1 mM, alkaline pH) was added to the seed extract solution at a ratio of 1 : 4.

In vitro cytotoxicity of gallium nanoparticles on HepG2 cell line

It was obtained by calculating the IC50 for GaNP samples versus the HepG2 cell line by the sulforhodamine-B cytotoxicity test [22].

Cell line and cell culture

The Egyptian National Cancer Institute, Cairo University, provided the Human HCC HepG2 cell line. It was grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin antibiotic, and 1% L-glutamine from Life Technologies, Gibco (Grand Island, New York, USA). To impact cell release from the culture flask, cells were grown in 5% CO2 at 37°C and then treated with 0.25% trypsin EDTA.

Cell viability assay

In shortly, cells were grown in a 96-well plate for 1 day before being inoculated with a new medium containing various concentrations of GaNPs (0–50 g/ml) and incubated for another 1 day. HepG2 cells were fixed with ice-cold 10% trichloroacetic acid at 4°C, stained for 30 minutes at room temperature with 0.4 percent sulforhodamine-B test, a fluorescent dye for the measurement of cellular proteins in cultured cells, and dissolved with 10 mMTris base solution. At 540 nm, the absorbance of a soluble dye was measured spectrophotometrically.

***In vivo* study**

Determination of LD_{50}

Determination of LD_{50} was performed on male Wistar rats. The LD_{50} of the newly synthesized GaNPs was determined as a preliminary step for *in vivo* study according to Akhila *et al.* [23].

Experimental design

In the present study, 80 male Wistar rats were divided into eight groups, with 10 rats each, as follows: group 1 (control, C), animals received 1 ml of physiological saline orally by gavage; group 2 (RAD), rat's whole body was exposed once to low dose of γ-radiation (0.5 Gy); group 3 (GaNPs), rats received GaNPs (10% of LD_{50} dose 38.5 mg/kg) orally by gavage; group 4 (GaNPs+RAD), rats received GaNPs, five times a week for 6 weeks, and exposed to 0.5 Gy γ-radiation dose; group 5 (DEN), each animal received DEN (dissolved in 0.9% normal saline), orally by gavage (20 mg/kg, five times/week for 6 weeks); group 6 (DEN+GaNPs), rats received DEN as group 5 and then were treated with GaNPs for 6 weeks as in group 3; group 7 (DEN+RAD), rats received DEN as in group 5 and then were exposed to 0.5 Gy γ-radiation; and group 8 (DEN+GaNPs+RAD), rats received DEN as in group 5 and then were treated with GaNPs for 6 weeks as in group 3 and finally exposed to 0.5 Gy γ radiation.

Sample processing

At 24 h after the last treatment, all animals were anesthetized with urethane (1.2 g/kg body weight, Sigma-Aldrich, St Louis) [24]. The rats' blood was collected, and liver was immediately isolated, washed with ice-cold physiological saline, dried, and preserved for subsequent analysis at −80°C. Tissue samples of the livers were fixed in a 10% neutral buffered formalin solution for histopathological investigation. In ascending ethanol concentrations, tissue specimens were dehydrated, cleared in xylene, implanted in paraffin wax, and sectioned at a thickness of 5 microns and stained by hematoxylin and eosin [25].

Biochemical assays

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured calorimetrically using an assay kit by Spectrum, Diagnostic, Egypt. The level of creatinine in serum was determined using colorimetric assay of Biodiagnostic Co., Egypt. Reduced glutathione (GSH) was quantified in the liver tissue according to the method of Beutler *et al.* [25]. Lipid peroxide (LPx) level was evaluated by determination of malondialdehyde (MDA) concentration in the liver tissue homogenate [26].

Histopathological analysis

Liver samples were fixed in 10% formalin for 24 h, dehydrated by ethanol, and then samples were embedded in paraffin beeswax. Sections of 4-μm thickness by slide microtome were deparaffinized and stained with hematoxylin and eosin stain for examination through a light microscope [27].

Statistical analysis

Statistical analysis of the results was performed using the statistical package for Windows, version 15.0 (SPSS Software, Chicago, Illinois, USA). The results for continuous variables were expressed as a mean±SE. Values were compared by one-way analysis of variance. Post-hoc testing was performed for intergroup comparisons using the least significant difference test, and *P* value less than or equal to 0.05 was considered statistically significant.

Results

In-vitro cytotoxicity of gallium nanoparticles on HepG2 cell line

GaNPs cytotoxicity was tested on the HepG2 cell line. The produced GaNPs sample was examined with

different doses and shown to have a cytotoxic impact on HepG2, with an IC50 value of 388.8 μg/ml (Fig. 1).

In vivo studies

Effect on liver

The activities of liver enzymes ALT and AST were determined in the serum of different groups using colorimetric methods. Serum of DEN-intoxicated rats showed a significant increase in ALT (62.7±1.5) and AST (113.3±1.7) activities compared with normal control animals. Rats administrated GaNPs or exposed to radiation showed amelioration ($P<0.001$) in enzyme activity in intoxicated rats compared with DEN-intoxicated rats. A significance difference was noted for ALT (23.0±1.0) and AST (54.7±1.7) activity levels in DEN+GaNPs+RAD group when compared with DEN-intoxicated rats (Tables 1 and 2; Fig. 2).

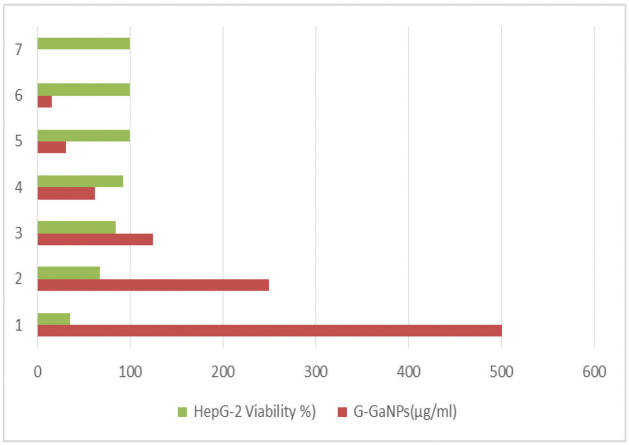
Impact on kidney

The level of creatinine was assessed calorimetrically in the serum of different groups. Creatinine level or rats administrated with DEN recorded a significant ($P<0.001$) elevation (1.4±0.42) compared with the control normal rats (0.9±0.05). On the contrary, GaNP-treated (1.0±0.12), radiation exposure (1.2 ±0.22), or DEN+GaNPs+RAD (0.9±0.35) groups showed significantly reduced creatinine level compared with DEN group (Table 3, Fig. 3).

Oxidative stress

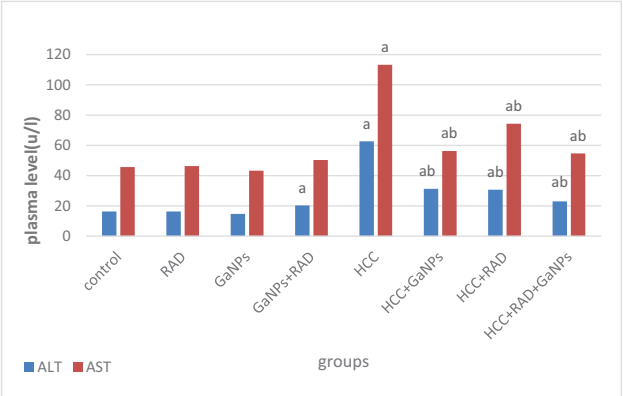
Oxidative stress state of the liver was assayed through determining GSH and MDA levels in liver tissue homogenate using colorimetric methods. The data showed significant reduction in GSH level of rats

Figure 1



Evaluation of cytotoxicity gallium nanoparticles G-GaNPs) against HepG2 cell line.

Figure 2



Plasma ALT and AST activity (U/l). Each bar represents mean±SD. [a]*P* value less than 0.05 compared with control; [b]*P* value less than 0.05 compared with DEN group. ALT, alanine aminotransferase; AST, aspartate aminotransferase; DEN, diethylnitrosamine.

Table 1 Statistical analysis for the plasma alanine aminotransferase (U/l) activity

Parameters	Groups							
	Control	RAD	GaNPs	GaNPs+RAD	HCC	HCC+GaNPs	HCC+RAD	HCC+GaNPs+RAD
Plasma ALT level (U/l)	16.3±0.7	16.3±0.7	14.7±0.7	20.3±0.9[a]	62.7±1.5[c]	31.3±1.8[cf]	30.7±1.5[cf]	23.0±1.0[bf]
% change from control	–	0	−9.8	24.5	284.7	92.0	88.3	41.1
% change from HCC	–	–	–	–	–	−50.1	−51.0	−63.3

ALT, alanine aminotransferase; GaNP, gallium nanoparticle; HCC, hepatocellular carcinoma. [a]significant difference at $P<0.05$ in comparison with control group. [b]significant difference at $P<0.01$ in comparison with control group. [c]significant difference at $P<0.001$ in comparison with control group. [f]significant difference at $P<0.001$ in comparison with HCC group.

Table 2 Statistical analysis for the plasma aspartate aminotransferase (U/l) activity

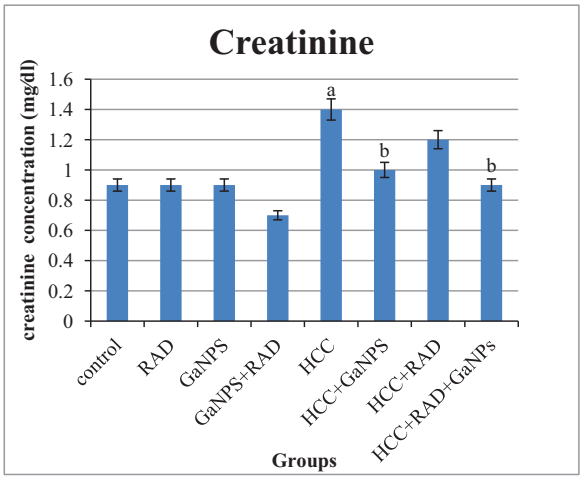
Parameters	Groups							
	Control	RAD	GaNPs	GaNPs+RAD	HCC	HCC+GaNPs	HCC+RAD	HCC+GaNPs+RAD
Plasma AST level (U/l)	45.7±1.3	46.3±0.7	43.3±0.3	50.3±0.3	113.3±1.7[c]	56.3±0.7[cf]	74.3±4.1[cf]	54.7±1.7[cf]
% change from control	–	1.3	−5.3	10.1	147.9	23.2	62.6	19.7
% change from HCC	–	–	–	–	–	−50.3	−34.4	−51.7

AST, aspartate aminotransferase; GaNP, gallium nanoparticle; HCC, hepatocellular carcinoma. [c]significant difference at $P<0.001$ in comparison with control group. [f]significant difference at $P<0.001$ in comparison with HCC group.

intoxicated with DEN (24.3±2.3) compared with control healthy rats (77.1±3.3). Treating DEN-intoxicated rats with GaNPs (47.8±2.9) or RAD (49.6±2.5) significantly elevated GSH level when compared with DEN-treated rats (24.3±2.3) (Table 4 and Fig. 4a).

LPx as a result of uncontrolled oxidative stress was determined calorimetrically by assessing MDA level. The level of MDA showed a significant elevation in rats intoxicated with DEN (52.0±2.6). Comparable results were obtained upon treatment with GaNPs or RAD, which revealed amelioration in MDA level compared with untreated DEN rats. Moreover, combined treatment with DEN+GaNPs+RAD (19.6 ±0.9) significantly reduced MDA level compared with DEN-intoxicated group (52.0±2.6) (Table 5, Fig. 4b).

Figure 3



Plasma creatinine activity (mg/dl). Each bar represents mean±SD. [a]*P* value less than 0.05 compared with control; [b]*P* value less than 0.05 compared with DEN group. DEN, diethylnitrosamine.

Histopathological findings

Histopathological examination of liver sections of control rats showed normal hepatic architecture (Fig. 5a). However, liver sections of RAD-treated (Fig. 5b) and GaNPs-treated rats demonstrated congestion in central vein (cv) and sinusoids (h) (Fig. 5c). Moreover, GaNPs+RAD-treated rats revealed normal histological structure (Fig. 5d). On the contrary, animals treated with DEN showed a focal area of anaplastic hepatocytes, whereas the other cells forming acini were observed associated with fibroblastic cell proliferation dividing the degenerated and necrosed hepatic parenchyma into nodules (Fig. 5e). It is noted also that the liver sections from rats treated with DEN and exposed to a dose of 0.5 Gy γ-radiation exhibited focal nodular area in the hepatic parenchyma and showed dysplastic hyperplastic hepatocytes. There was congestion in the portal vein and inflammatory cells with dilated bile ducts in the portal area (Fig. 5f). In addition, the animals treated with DEN and administrated with GaNPs showed diffuse ballooning degeneration and focal necrosis in the hepatocytes associated with inflammatory cell infiltration and fibrosis in the portal area, besides congestion in both central and portal veins (Fig. 5g). Furthermore, the rats that

Table 3 Statistical analysis for the plasma creatinine (mg/dl) activity

Parameters	Groups							
	Control	RAD	GaNPs	GaNPs +RAD	HCC	HCC +GaNPs	HCC +RAD	HCC+GaNPs +RAD
Plasma creatinine level (mg/dl)	0.9 ±0.05	0.9 ±0.15	0.9 ±0.06	0.7±0.15	1.4 ±0.42[a]	1.0±0.12[d]	1.2±0.22	0.9±0.35[e]
% change from control	–	0.0	0.0	−22.2	+55.6	+11.1	+33.3	0.0
% change from HCC	–	–	–	–	–	−28.5	−14.2	−35.7

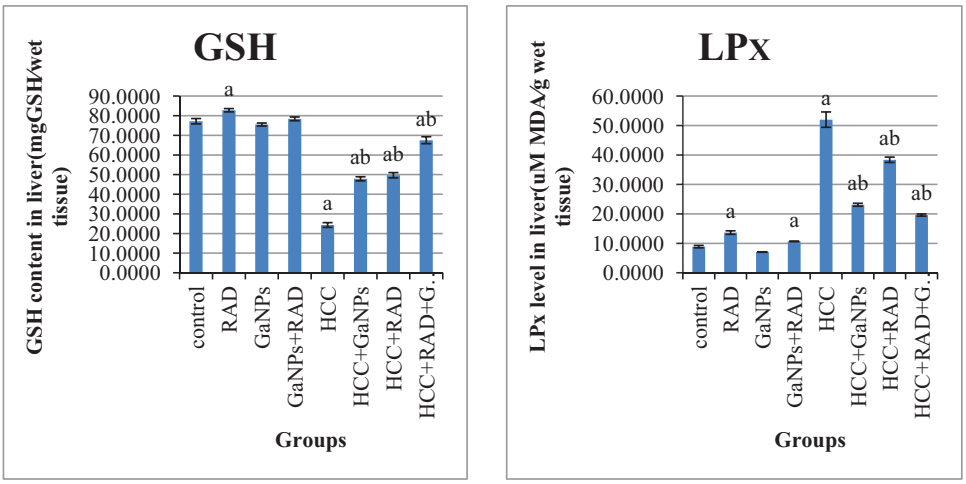
GaNP, gallium nanoparticle; HCC, hepatocellular carcinoma. [a]significant difference at P<0.05 in comparison with control group. [d]significant difference at P<0.05 in comparison with HCC group. [e]significant difference at P<0.01 in comparison with HCC group.

Table 4 Statistical analysis for the liver glutathione (mg glutathione/g wet tissue) level

Parameters	Groups							
	Control	RAD	GaNPs	GaNPs +RAD	HCC	HCC +GaNPs	HCC +RAD	HCC+GaNPs +RAD
Liver GSH level (mg GSH/g wet tissue)	77.1 ±3.3	82.7 ±2[b]	75.5 ±1.8	78.3±3.3	24.3 ±2.3[c]	47.8±2.9[cf]	49.6 ±2.5[cf]	67.5±4.3[cf]
% change from control	–	7.2	−2.0	1.5	−68.4	−38.0	−35.6	−12.4
% change from HCC	–	–	–	–	–	96.7	104.1	177.7

GaNP, gallium nanoparticle; GSH, glutathione; HCC, hepatocellular carcinoma. [b]significant difference at P<0.01 in comparison with control group. [c]significant difference at P<0.001 in comparison with control group. [f]significant difference at P<0.001 in comparison with HCC group.

Figure 4



(a) (b)

(a) GSH content in liver (mg GSH/g wet tissue), and (b) LPx level in liver (μM MDA/g wet tissue). Each bar represents mean±SD. [a]*P* value less than 0.05 compared with control; [b]*P* value less than 0.05 compared with DEN group. DEN, diethylnitrosamine; GSH, glutathione; LPx, lipid peroxidation; MDA, malondialdehyde.

Table 5 Statistical analysis for the liver lipid peroxidation (μM malondialdehyde/g wet tissue) level

Parameters	Groups							
	Control	RAD	GaNPs	GaNPs+RAD	HCC	HCC+GaNPs	HCC+RAD	HCC+GaNPs+RAD
(μM MDA/g wet tissue)	8.6±0.7	13.3±1.0[c]	7.0±0.18	10.6±0.2[a]	52.0±2.6[c]	23.1±1.2[cf]	38.3±2.2[cf]	19.6±0.9[cf]
% change from control	–	54.6	−18.6	23.2	504.6	168.6	345.3	127.9
% change from HCC	–	–	–	–	–	−55.5	−26.3	−62.3

GaNP, gallium nanoparticle; HCC, hepatocellular carcinoma; MDA, malondialdehyde. [a]significant difference at P<0.05 in comparison with control group. [c]significant difference at P<0.001 in comparison with control group. [f]significant difference at P<0.001 in comparison with HCC group.

received DEN and then administrated with GaNPs and exposed to a single dose of γ-radiation showed infiltration with focal inflammatory cells in the hepatic parenchyma associated with diffuse Kupffer cell proliferation in between the hepatocytes (Fig. 5h).

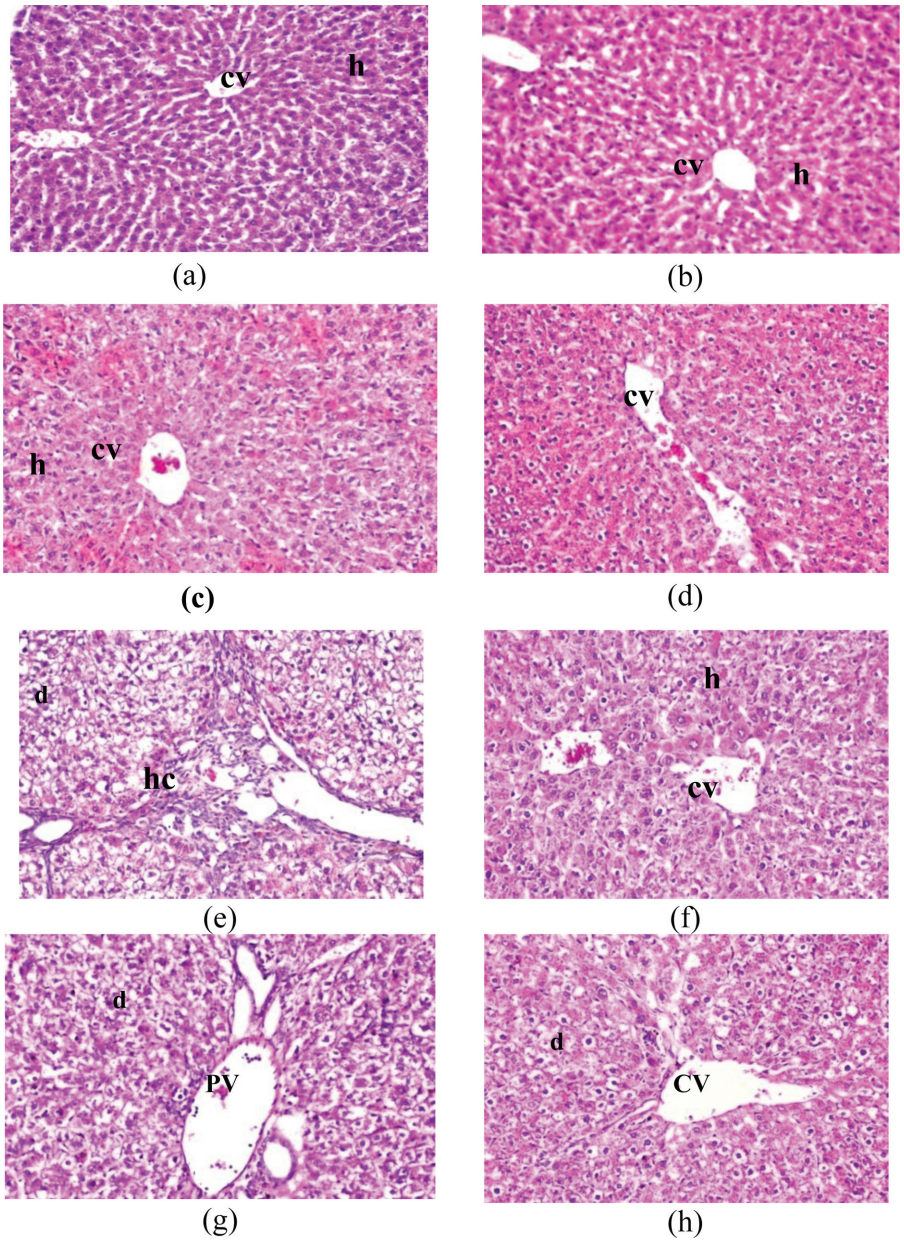
Discussion

Based on the fact that a combination of therapies is a successful and more effective strategy for cancer treatment, chemotherapy was combined with radiotherapy, increasing the effects of cancer treatment and making cancer therapy more effective. Chemo-radiotherapy can improve both chemotherapy and radiotherapy effectiveness. In addition, guided treatments with NPs and radiotherapy could be an effective strategy in cancer therapy to overcome limitations in conventional chemotherapy. Because of their tiny size, NPs are able to permeabilize cells effectively, which facilitates activity and *in vivo* distribution. Because they are not captured by the reticuloendothelial system, smaller NPs accumulate more in the tumor regions and also have a prolonged *in vivo* half-life [28,29]. Targeted NPs for HCC therapy are more effective than for other types of cancers as most of which might end up in the liver and spleen [30].

Previous research has shown that GaNPs produced by *Bacillus helveticus* bacteria have antiproliferative capabilities in MCF-7 [31] and HepG2 cell lines [32]. *In vivo* studies demonstrated antiproliferative and proapoptotic effects against Ehrlich solid tumors in mice and HCC in rats [31,32]. This prompted us to do more research on GaNP antitumor mechanisms as a viable alternative drug for cancer therapy and a radio-sensitizing agent.

In vitro antiproliferative activity of GaNPs synthesized by GSE showed less cytotoxicity (IC_{50}=388 g/ml) than that synthesized by Lactobacillus *helveticus* (IC_{50}=8.0 g/ml) against HepG2. This can be explained owing to the reduced capability of the material used in the synthesis as a reducing and capping agent for Ga [33].

Figure 5



Histopathological examination of liver (a–h), (a) normal liver histology of a rat fed the standard diet. (b) Normal liver histology of a rat exposure to 0.5 Gy. (c) Rats administrated GaNPs showing congestion in central vein (cv) and sinusoids (h). (d) Rats administrated GaNPs and then exposed to 0.5 Gy. (e) Rats administrated DEN. (f) Rats orally gavaged with DEN and then exposed to 0.5 Gy, showing area focal in hepatic parenchyma of dysplastic hepatocytes forming a nodule. (g) Rats orally gavaged with DEN and GaNPs showed degeneration in the hepatocytes (d) with focal necrosis in hepatic parenchyma and inflammatory cells in the portal area and congestion in central and portal veins (pv). (h) Rats received DEN and GaNPs, then exposed to 0.5 Gy, showing few inflammatory cells infiltration in hepatic parenchyma with diffuse Kupffer cell proliferation. DEN, diethylnitrosamine; GaNP, gallium nanoparticle.

Along with reduced GSH, imbalance between ROS formation as a result of DEN administration and antioxidant scavengers seriously damaged biological systems and promoted carcinogenesis by injuring tissues, causing chromosomal instability, altering biochemical compounds, eroding cell membranes, and causing mutations, which are involved in all stages of carcinogenesis, that is, initiation, promotion, and progression [34].

Ga has the ability to prevent tumor development by competing with ferric and magnesium ions. It inhibits DNA synthesis, alters cellular iron acquisition via binding to transferrin, and interacts with the iron-dependent enzyme ribonucleotide reductase [35]. Moreover, Ga causes DNA fragmentation that leads to apoptosis [31]. Mineral iron is necessary for cellular respiration. Iron-produced ROS trigger a variety of signaling pathways [36]. By competing with Mg for

DNA binding, Ga may interact with DNA, recording a 100-fold higher affinity than Mg [37]. Ga binds to the phosphate group and nucleic acids in DNA, causing DNA structural changes. According to previous research [38], Ga activates caspases and causes apoptosis via the mitochondrial route, as well.

Results in this study showed a marked decrease in GSH level accompanied by an increase in DEN rats treated with GaNPs alone or combined with exposure to radiation compared with the DEN-untreated group. The drop in GSH level might be attributed to Ga high oxidative damage or its employment in the detoxification of free radicals produced by GaNPs, which increases LPx [39,40]. The induction of LPx is closely associated with the reduction in GSH concentration. The anticancer action of the GaNPs is thought to be induced by toxicity, oxidative stress, and inflammation that produces ROS, which are implicated in a number of cellular processes ranging from apoptosis and necrosis to cell proliferation and carcinogenesis [41]. The interaction of reduced GSH and Ga to create oxidized Ga or Ga–GSH complex may cause a decrease in reduced GSH concentration.

These findings suggest that when animals are exposed to radiation throughout their bodies, nonenzymatic antioxidant levels increase to varying degrees in different organs. Increased LPx in the liver is seen following low-dose irradiation of the whole body, followed by a rise in the redox agent GSH. Increased expression of mRNA for γ-glutamylcysteine synthase, a rate-limiting enzyme in GSH production, is believed to be the cause of the increase in GSH levels. The immediate rise in GSH concentration in the liver following low-dose whole-body radiation indicates that GSH works as a first line of defense to protect cells from increasing oxidative stress induced by ionizing radiation [42,43].

In the current investigation, the HCC group showed marked elevation in liver marker enzymes ALT and AST in plasma compared with normal control group. Our data were in harmony with other previous studies [44,45]. Jahan *et al.* [46] reported that DEN is a hepatotoxin and a carcinogen. In DEN-treated rats, an increase in the ALT and AST activity is linked to hepatotoxicity and carcinogenesis, along with the development of preneoplastic alterations, and the severity and advanced stage of liver cancer.

The combined treatment with GaNPs, synthesized by grape seed, and the low dose of γ-radiation on the HCC induced animals has led to a significant reduction in the ALT and AST activity compared with the HCC group [47]. Furthermore, a significant increase in ALT activity and creatinine level of tumor-bearing rats in the serum compared with normal healthy control indicates hepatic and renal damage as a result of HCC tumor formation. These increases have been linked to cancer cell invasion in important organs, which results in liver overload and renal impairment as a result of degenerative alterations in the epithelial lining of renal tubules [48]. Administration of GaNPs and/or R to tumor-bearing rats displayed a significant decrease in ALT activity and creatinine levels. The effect of GaNPs on tumors that reduce the production of toxins from tumors might explain the decrease in ALT activity and creatinine levels [49]. Previously, it was discovered that a low dose of γ-radiation (0.5 Gy) modulates ALT activity [42] and suppresses a high level of creatinine [50]. They hypothesized that the enhanced effect of low-dose γ-radiation on the antioxidant system may reduce the liver and kidney damage caused by tumor formation.

The histopathology findings corroborated the changes in the biochemical markers. In the present study, histopathological pictures of HCC liver sections revealed a focal region of anaplastic cellular hepatocytes with other cells forming acini, as well as fibroblastic cell proliferation splitting the degraded and necrosed hepatic parenchyma into nodules, which is in agreement with Youssef *et al.* [51]. DEN-induced necrosis varies from vacuolar degeneration, spotty necrosis, confluent necrosis, nodules of ghost necrotic malignant hepatocytes, and nodules of solid HCC containing giant hepatocytes with large nuclei.

HCC groups treated with GaNPs or radiation were in agreement with the biochemical parameters, providing evidence of the hepatic improvement effect on the liver architecture with mild degeneration, and exhibited a pronounced antitumor effect. Moawed *et al.* [32] found that the rats treated with GaNPs alone or in combination with γ-radiation had hepatocyte degeneration, focal necrosis, and inflammatory cell infiltration, as well. In addition, the hepatic parenchyma exhibited a widespread proliferation of Kupffer cells in the spaces between the hepatocytes.

Based on the aforementioned findings, it can be concluded that the combination of GaNPs and low-dose γ-radiation may have significant anticancer properties against hepatocarcinogenesis.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1 Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Globalcancer statistics, 2012. CA Cancer J Clin 2015; 65:87–108.

2 Martel C, Ferlay J, Franceschi S, Vignat J, Bray F, Forman D, Plummer M. Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. Lancet Oncol 2012; 13:607–615.

3 Mittal S, El-Serag HB. Epidemiology of HCC: consider the population. J Clin Gastroenterol 2013; 47:S2–S6.

4 Park D-H, Shin JW, Park S-K, Seo J-N, Li L, Jang J-J, Lee M-J. Diethylnitrosamine (DEN) induces irreversible hepatocellular carcinogenesis through overexpression of G 1/S-phase regulatory proteins in rat. Toxicol Lett 2009; 191:321–326.

5 Kulkarni S, Pandey A, Mutalik S. Liquid metal based theranosticnanoplatforms: application in cancer therapy, imaging and biosensing. Nanomedicine 2020; 26:102175.

6 Mikuš P, Melník M, Forgácsová A, Krajčiová D, Havránek E. Gallium compounds in nuclear medicine and oncology. Main Gr Met Chem 2014; 37:53–65.

7 Chakraborty A, Boer JC, Selomulya C, Plebanski M. Amino acid functionalized inorganic nanoparticles as cutting-edge therapeutic and diagnostic agents. Bioconjug Chem 2017; 29:657–671.

8 Rababah TM, Hettiarachchy NS, Horax R. Total phenolics and antioxidant activities of fenugreek, green tea, grape seed, ginger, rosmary, gotu kola, and ginkgo extracts, vitamin E and tert-butylhydroquinone. J Agri Food Chem 2016; 52:5183–5186.

9 Nassiri-Asl M, Hosseinzadeh H. Review of the pharmacological effects of Vitisvinifera (grape) and its bioactive compounds. Phytother Res 2009; 23:1197–1204.

10 Liu W, Xu C, Sun X, Kuang H, Kuang X, Zou W, *et al.* Grape seed proanthocyanidin extract protects against perfluorooctanoic acid-induced hepatotoxicity by attenuating inflammatory response, oxidative stress and apoptosis in mice. Toxicol Res 2016; 5:224–234.

11 Raina K, Singh RP, Agarwal R, Agarwal C. Oral grape seed extract inhibits prostate tumor growth and progression in TRAMP mice. Cancer Res. 2007; 67:5976–5982.

12 Meeran SM, Vaid M, Punathil T, Katiyar SK. Dietary grape seed proanthocyanidins inhibit 12-O-tetradecanoyl phorbol-13-acetate-caused skin tumor promotion in 7,12-dimethylbenz(a) anthracene-initiated mouse skin, which is associated with the inhibition of inflammatory responses. Carcinogenesis 2009; 30:520–528.

13 Hamza AH, Abdulfattah HM, Mahmoud RH, Khalil WK, Ahmed HH. Current concepts in pathophysiology and management of hepatocellular carcinoma. Acta Biochim Pol 2015; 62:573–580.

14 Sherif AA, Abdelhalim SZ, Salim EI. Immunohistochemical and biochemical alterations following administration of proanthocyanidinextract in rat's hepatocellular carcinoma. Biomed Pharmacother 2017; 39:1310–1319.

15 Porcel E, Liehn S, Remita H, Usami N, Kobayashi K, Furusawa Y, *et al.* Platinum nanoparticles: a promising material for future cancer therapy. Nanotechnology 2010; 21:85103.

16 Danhier F, Feron O, Preat V. To exploit the tumor microenvironment: passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. J Control Release 2010; 148:135–146.

17 Kandil EI, El-Sonbaty SM, Moawed FS, Khedr OM. Anticancer redox activity of gallium nanoparticles accompanied with low dose of gamma radiation in female mice. Tumor Biol 2018; 40:1–18.

18 Feinendegen LF. Evidence for beneficial low level radiation effects and radiation hormesis. Br J Radiol 2005; 78:3–7.

19 Feinendegen L, Hahnfeldt P, Schadt EE, Stumpf M, Voit EO. Systems biology and its potential role in radiobiology. Radiat Environ Biophys 2008; 47:5–23.

20 National Research Council (US) Institute for Laboratory Animal Research. Guide for the care and use of laboratory animals. Washington, DC: National Academies Press (US); 1996.

21 Mohseni MS, Khalilzadeh MA, Mohseni M, Hargalani FZ, Getso MI, Raissi V, Raiesi O. Green synthesis of Ag nanoparticles from pomegranate seeds extract and synthesis of Ag-Starch nanocomposite and characterization of mechanical properties of the films. Biocatal Agri Biotechnol 2020; 25:101569.

22 Vichai V, Kirtikara K. Sulforhodamine B colorimetric assay for cytotoxicity screening. Nat Protoc 2006; 3:1112–1116.

23 Akhila JS, Deepa S, Alwar MC. Acute toxicity studies and determination of median lethal dose. Curr Sci 2007; 93:917–920.

24 Banchroft JD, Stevens A, Turner DR. Theory and practice of histological techniques. 4th ed. London: Churchil Livingstone 1996. 125.

25 Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963; 61:882–888.

26 Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituricacid reaction, Anal Biochem 1979; 95:351–358.

27 Banchroft JD, Stevens A, Turner DR. Theory and practice of histological techniques. New York London San Francisco, CA: Churchill Livingstone; 1996.

28 Silpa S. Nanotechnology-present revolutionary biotechnology. Int J Pharma Res Health Sci 2016; 4:1261–1267.

29 Bhattacharya R, Mukherjee P. Biological properties of 'naked' metal nanoparticles. Adv Drug Deliv Rev 2008; 60:1289–1306.

30 Nguyen KT. Targeted nanoparticles for cancer therapy: promises and challenges. Nguyen J Nanomed Nanotechnol 2011; 2:5.

31 KandilE I, El-Sonbaty SM, Moawed FSM, Khedr OMS. Anticancer redox activity of gallium nanoparticles accompanied with lowdose of gamma radiation in femalemice. Tumor Biol 2018; 40:1–14.

32 Moawed FSM, El-Sonbaty SM, Mansour SZ. Gallium nanoparticles along with low-dose gamma radiation modulateTGF-b/MMP-9 expression inhepatocellular carcinogenesis in rats. Tumor Biol 2019; 41:1–9.

33 Chandramohan S, Sundar K, Muthukumaran A. Reducing agents influence the shapes of selenium nanoparticles (SeNPs) and subsequently their antibacterial and antioxidant activity. Mater Res Express 2019; 6:0850i2.

34 Shaarawy SM, Tohamy AA, Elgendy SM, Elmageed ZY, Bahnasy A, Mohamed MS, *et al.* Protective effects of garlic and silymarin on NDEA-induced rats hepatotoxicity. Int J Biol Sci 2009; 5:549–557.

35 Sanvisens N, Bano MC, Huang M, Puig S. Regulation of ribonucleotide reductase in response to iron deficiency. Mol Cell 2011; 44:759–769.

36 Nakamura T, Naguro I, Ichijo H. Iron homeostasis and iron-regulated ROS in cell death, senescence and human diseases. Biochim Biophys Acta Gen Subj 2019; 1863:1398–1409.

37 Manfait M, Collery P. In vitro study by Raman spectroscopy of conformation of DNA under the influence of magnesium and gallium ions. Magnesium-B 1984; 4:153–155.

38 Chitambar CR, Purpi DP, Woodliff J, Yang M, Wereley JP. Development of gallium compounds for treatment of lymphoma: gallium maltolate, a novel hydroxypyrone gallium compound induces apoptosis and circumvents lymphoma cell resistance to gallium nitrate. J Pharmacol Exp Ther 2007; 322:1228–1236.

39 Yang M, Chitambar CR. Role of oxidative stress in the induction of metallothionein-2A and heme oxygenase-1 gene expression by the antineoplastic agent gallium nitrate in human lymphoma cells. Free Radic Biol Med 2008; 45:763–772.

40 Joseph TP, Janine PW, Chitambar CR. Gallium nitrate as a novel agent for the treatment of mantle cell lymphoma: target and mechanisms of action. Proc Am Assoc Cancer Res 2005; 46:1383–1384.

41 Chitambar CR. Gallium-containing anticancer compounds. Future Med Chem 2012; 4:1257–1272.

42 Avti PK, Pathak CM, Kumar S, Kaushik G, Kaushik T, Farooque A, *et al.* Low dose gamma-irradiation differentially modulates antioxidant defense in liver and lungs of Balb/c mice. Int J Radiat Biol 2005; 81:901–910.

43 Kawakita Y, Ikekita M, Kurozumi R, Kojima S. Increase of intracellular glutathione by low-dose γ-ray irradiation is mediated by transcription factor AP-1 in RAW 264.7 cells. Biol Pharm Bull 2003; 26:19–23.

44 Hassan SK, Mousa AM, Eshak MG, Farrag AH, Badawi AM. Therapeutic and chemopreventive effects of nano curcumin against diethylnitrosamine induced hepatocellular carcinoma in rats. Int J Pharm Pharm Sci 2014; 6:54–62.

45 Mohammed FZ, Sultan AS, Abas AM. Chemopreventive and therapeutic effect of capsaicin against diethylnitrosamine induced liver injury and hepatocellular carcinoma in rats. Int J Biol Pharma Res 2014; 5:630–642.

46 Jahan MS, Vani G, Shymaladevi CS. Anti-carcinogenic effect of *Solanum trilobatum* in Diethylnitrosamine induced and Phenobarbital promoted hepatiocarcinogenesis in rats. Asian J Biochem 2011; 6:74–81.

47 UmaMaheswari M, Rao PG. Antihepatotoxic effect of grape seed oil in rat. Indian J Pharmacol 2005; 37:179–182.

48 Hashem MA, Mohamed HM, Magda SH. Clinicopathological, pathological and biophysical studies on the effect of electromagnetic field on the Ehrlich tumor cells implanted in mice. Egypt J Comp Clin Pathol 2004; 17:117–147.

49 Salem A, Noaman E, Kandil E, Badawi A, Mostafa N. Crystal structure and chemotherapeutic efficacy of the novel compound, gallium tetrachloride betaine, against breast cancer usingnanotechnology. Tumour Biol 2016; 37:11025–11038.

50 Fahmy HA, El-Azime Ash ABD, Gharib OA. Possible ameliorative role of low dose of radiation against cisplatin induced oxidative stress and tissue damage in male rats. Eur J Biol Med Sci Res 2013; 1:10–18.

51 Youssef MI, Maghraby H, Youssef EA, El–Sayed MM. Expression of Ki 67 in hepatocellular carcinoma induced by diethylnitrosamine in mice and its correlation with histopathological alterations. J Appl Pharm Sci 2002; 2:52–59.