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Research Article
Cytotoxic Effect of Biosynthesized Silver Nanoparticles on Ehrlich Ascites Tumor Cells in Mice

[1]Badr E. El Bialy, [2]Ragaa A. Hamouda, [2]Khaled S. Khalifa and [2]Hanafy A. Hamza

[1]Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, University of Sadat City, Sadat City, Egypt
[2]Department of Microbial Biotechnology, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City, Sadat City, Egypt

Abstract

Objective: This study aimed to evaluate the potential anti-cancer effect of silver nanoparticles (AgNPs, of 8-16 nm size range) biosynthesized from *Turbinaria turbinata* marine-alga against Ehrlich Cell Carcinoma (ECC) in mice. **Materials and Methods:** After 1 day of s/c Ehrlich cells inoculation in mice thigh, AgNPs were injected in the same site at doses of 85 and 42.5 µg for each mouse daily for 20 days. Till the end of the experiment, none of clinical signs were observed on mice. **Results:** The AgNPs induced dose-dependent reduction in tumor size. The cytotoxic insult of AgNPs against ECC referred to its potential oxidative damage effect proved by elevation of MDA and H_2O_2 contents in tumor tissue and induction of apoptosis via caspase 3 activation. Also, AgNPs brought the elevated white blood cell count in tumor-bearing mice to near-normal range. The histopathological investigation of tumor tissues revealed presence of severe necrosis of cancer cells especially at higher dose of AgNPs. **Conclusion:** Biosynthesized AgNPs from *Turbinaria turbinata* could control the growth of ECC in mice with limited adverse effects.

Key words: Marine-alga, *Turbinaria turbinata*, silver nanoparticles, cytotoxicity, Ehrlich cell carcinoma, tumor size, oxidative damage, caspase 3, apoptosis, necrosis

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Corresponding Author: Badr E. El Bialy, Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, University of Sadat City, Sadat City, Egypt Tel: +20-1003811701

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cancer is a major health problem threating the life in both developed and developing countries. It is a progressive uncontrolled degenerative disease predisposed by accumulation of toxins through carcinogenic food, smoking, alcohol drinking, toxic medicines environmental pollution and radiation and exposure to infectious organisms that considered being external factors. Also, internal factors as mutations, disturbances in hormonal and immune conditions can induce cancer. Both external and internal factors may act together or in sequence and both attenuated by stressful lifestyle[1,2].

Cancer is still causing mortality despite the discovery of several novel anticancer drugs[3]. But there is increasing demands for anticancer therapy[4]. The discovery and identification of new anti-tumor drug with low side effects on immune system has become an essential goal in many studies of cancer-therapies[5]. Chemotherapy has many drawbacks[6]. To avoid these drawbacks; many attentions have been paid to natural compounds in plants, marine organism and microorganisms.

Nanoparticles (NPs) are clusters of atoms in the size range of 1-100 nm that are different from those of the same molecules in large bulk form[7]. They are of particular shape and size depending on specific requirements and applications[8]. Metallic nanoparticles can be obtained by physical, chemical or biological methods. However, recent efforts have been focused on developing new green chemistry methods of AgNPs synthesis with the advantage of using natural products and avoiding toxic reducing agents, organic solvents and wasteful purifications with high cytotoxic residuals. Biological synthesis is reliable and eco-friendly in which toxic chemicals are not be used in the synthesis protocol[9-11]. Biological synthesis of NPs also has received particular attention as the biologically active molecules involved in the green synthesis of NPs act as functional ligands, making these NPs more suitable for biomedical applications[12].

The biologically synthesized silver nanoparticles have wide range of applications. Their characterization is an emerging field of nanotechnology because of their applications in many fields including biology and medicine[13]. The unique antimicrobial properties of AgNPs[14] have led to their wide application in industrial, household and healthcare-related products[15]. Recently, silver nanoparticles have been extensively used in a various medical fields and nanomedicine due to their antibacterial, antifungal, antiviral, anti-inflammatory and osteoinductive effects as well as their ability to enhance wound healing[16-18].

Seaweeds are the natural and renewable living resources in the marine ecosystem and they are consumed for food, feed and medicine. Seaweeds contain more than 60 elements, macro and micronutrients, proteins, carbohydrates, vitamins and amino acids[19].

Hundreds of biopotential anti-tumor agents have been isolated from marine origin especially from marine algae[20-23]. Bacteria, fungi, plant and algae are biological sources for synthesis of nanoparticles. Micro algae extracts have a great efficiency to synthesize silver nanoparticles with antitumor activity as a green route[24].

In the present study, the biosynthesized AgNPs from *Turbinaria turbinata* as one of marine algae was evaluated for its anti-carcinogenic potency against Ehrlich ascites carcinoma solid tumor in mice.

MATERIALS AND METHODS

Animal housing: Thirty mice, 5-6 weeks old female Swiss Albino mice, weighing 18-23 g were purchased from AL-Zyade Experimental Animals Production Center, Giza, Egypt. Upon arrival, the mice were randomly transferred to polycarbonate cages containing sawdust bedding and allowed to acclimatize for 10 days before the start of the experiment. They were housed under the standard conditions of room temperature (18-20°C) and light (12 h light/12 h dark, cycles) and received food and tape water *ad libitum*. Animal rearing and handling and all experimental design were approved by the Research Ethical Committee, Faculty of veterinary medicine, University of Sadat City, Egypt. All the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals avoiding any stress.

Animal grouping: Ehrlich Ascitis Carcinoma (EAC) bearing donor mouse was obtained from National Cancer Institute. One milliliter of freshly ascitic fluid was drawn from a donor mice (7-14 days-old EAC) and diluted with 9 mL normal saline in sterile test tube. The EAC cells were thereafter propagated weekly for 3 weeks by intraperitoneal injection of 0.2 mL (1×10^6 EAC cells) of EAC suspension into three mice to ensure that the ascitic fluid will still propagated.

Thirty female albino mice were randomly divided into five groups, six animals each. The G1, control negative mice injected daily with normal saline; G2, Ehrlich tumor control mice (injected once with 0.2 mL of EAC cells suspension containing about 1×10^6 viable cells mouse^{-1}, then injected with normal saline); G3, injected daily with AgNPs in a dose of

85 µg mouse^{-1}; G4, Ehrlich tumor-induced mice injected daily with AgNPs in a dose of 85 µg mouse^{-1} and G5, Ehrlich tumor-induced mice injected daily with AgNPs in a dose of 42.5 µg mouse^{-1}. All treatments were by s/c injection in the right thigh of the hind leg daily for 20 days. Treatment with AgNPs began at 24 h after tumor induction. Weights of mice of each group were recorded separately at the beginning and at the end of the experiment.

Samples collection: After completion of the experiment, all mice were fasted overnight, weighed (for calculation of body weights difference) and blood samples were collected in plain glass centrifuge tubes without anticoagulant by heparinized capillary tubes from retro-orbital veinus plexus under light ether anaesthesia. Blood samples were left to clot at room temperature and centrifuged at 3000 rpm for 15 min. Sera were then, separated and stored at -20°C as aliquots for further biochemical analysis (ALT and AST activities). Whole blood samples were immediately collected in ethylene diamine tetra acetic acid (EDTA) coated vials for haematologic analysis of potential haematologic toxicity.

Blood parameters included determination of the number of white blood cells and red blood cells and haemoglobin concentration according to the routine haematological procedures adopted by Feldman *et al.*[25].

The animals were euthanized by cervical dislocation for collection of tissue samples of the liver and induced tumor of each mouse were collected and preserved in 10% formol saline for histopathological examination and caspase 3 immunohistochemistry.

Specimens of tumor tissue also were stored at -80°C and homogenized in cold phosphate buffered saline 7.4 pH using teflon tissue homogenizer (Dounce Tissue Grinder 40 mL, 32×140 mm, Omni International) for malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) measurement as indication for lipid peroxidation and generation of free radicals.

Serum and tissue biochemical estimations: Diagnostic kits for assaying serum ALT, AST, MDA and H_2O_2 in tumor tissue homogenates were purchased from the Biodiagnostic Company, Dokki, Giza, Egypt.

Serum ALT and AST were estimated according to Reitman and Frankel[26]. The MDA was determined according to the procedure described by Ohkawa *et al.*[27] and Satoh[28]. The H_2O_2 was estimated according to Aebi[29].

Caspase-3 immunohistochemical analysis: For immunohistochemistry, cancer tissue samples were fixed in 10% formalin for 24 h and automatically processed and routinely embedded in paraffin blocks. The paraffin blocks were cut into 4-5 µm thickness. Deparaffinization and antigen recovery were carried out.

Paraffin sections were placed on mounted on electrically positively charged slides, paraffin removed by xylene and ethanol washes for tissue rehydration. Sections were subjected to microwaves for 15 min at a high energy setting in 0.1 M sodium citrate buffer, pH 6.0 to enhance immunoreactivity. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 min followed by a casein-based protein block (Dako cytomation) to minimize nonspecific staining. Then sections were incubated with rabbit anti-human cleaved caspase-3 (Promega) or rabbit monoclonal Ki-67 (clone SP6; Lab Vision; 1:100 dilution in Large Volume Ultr Ab Diluent) for 2 h at room temperature.

The Dako Envision+HRP/DAB+System (Dako cytomation) was used to produce localized, visible staining. The slides were lightly counterstained with Mayer's hematoxylin, dehydrated and cover slipped, examined using olympus vanox microscope (Tokyo, Japan). Positive nuclei for caspase-3 accumulation were stained brown. Expression of caspase-3 was scored according to staining intensity and number of stained cells as follows: (score 0 or -ve) for no staining or very weak staining, (score 1 or +) for weak to moderate staining detected in 10-20% of carcinoma cells, (score 2 or ++) moderate to strong staining in 21–50% of cells and (score 3 or +++) for strong staining in >50% of cells[30,31].

Histopathological examination: Autopsy samples were taken from the liver and Ehrlich tumor cells in subcutaneous tissue of mice in different groups and fixed in 10% formol saline for 24 h. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56° in hot air oven for 24 h. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stain for routine examination through the light electric microscope[32].

Statistical analysis: The present data were presented as Mean±SEM. Statistical significant of treatment effect of AgNPs on the investigated parameters exposed mice were determined by one way ANOVA followed by Duncan's test for *post hoc* analysis at $p \leq 0.05$ using SPSS version 16.

RESULTS

Difference in body weight gains of different groups: Along the experiment, there were no observable clinical signs on mice of all groups or deviation in their behaviors or feeding habits.

By calculation of body weight gains (Fig. 1), it was noticed a significant increase in body weight gain of group 2, Ehrlich tumor control mice (7.8±0.73 g) in comparing with control negative group (3.2±0.25 g) and AgNPs treated G3 (3.7±0.37 g) while significant reductions in body weight gains of Ehrlich tumor groups treated with AgNPs (5.0±0.72 and 5.3±0.43 g in G4 and G5, respectively) in comparing with Ehrlich tumor control group were recorded. Differences in body weight gains between experimental groups referred to absence of tumor in groups 1 and 3 or variation in tumor size in groups 2, 4 and 5 as revealed from camera photographed pictures in Fig. 2.

Haematological parameters: In considering of haematological parameters (Table 1), haemoglobin concentration and RBCs count showed insignificant changes between different groups.

The number of WBCs in G2 recorded highly significant elevation ($11.3\pm1.2\times10^3$) in relation to that of G1 ($4.07\pm0.8\times10^3$). While significant reduction of WBCs count in Ehrlich tumor groups treated with AgNPs (6.64±0.5 and $6.87\pm0.7\times10^3$ in G4 and G5, respectively) in comparing with Ehrlich tumor control G2. The WBCs count of G3 treated with AgNPs in a dose of 85 µg was around normal range in comparing with control negative group (Table 1).

Results of serum and tissue biochemical estimations: In this study, ALT and AST showed insignificant elevation in Ehrlich tumor control group in comparing with control negative group. All groups treated with AgNPs recorded normal ranges of ALT but elevation in AST in groups 4, 5 that was significantly in G4 Ehrlich tumor treated with AgNPs in a dose of 85 µg (137±1.6) in comparing with control negative group (112±3.5) (Table 1).

Malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents of solid Ehrlich tumor homogenate showed significant elevations in Ehrlich tumor groups treated with 85 and 42.5 µg AgNPs (G4 and G5) in comparing with untreated Ehrlich tumor group G2 (Fig. 3a, b). The recorded MDA and H_2O_2 levels were 99.14±.0.98 and 5.18±0.06 for G4 and 96.99±0.73 and 5.05±0.05 for G5, respectively. However, levels of MDA and H_2O_2 recorded for G5 were 90.68±0.86 and 4.43±0.23, respectively.

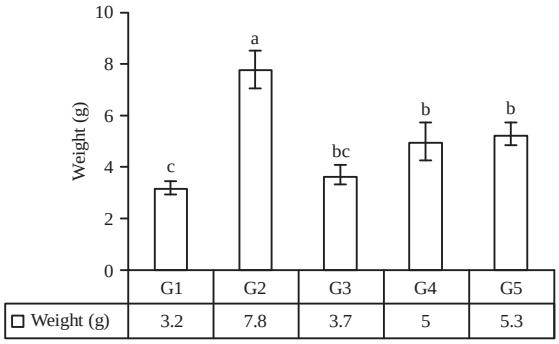

Fig. 1: Mean±SEM of body weight gains (g) in control negative group (G1), Ehrlich tumor mice (G2), Mice treated with AgNPs in 85 µg (G3), Ehrlich tumor mice treated with 85 µg AgNPs (G4) and Ehrlich tumor mice treated with 42.5 µg AgNPs (G5). Different letters mean statistical significance at $p \leq 0.05$ according to one-way ANOVA followed by Duncan's test for *post hoc* analysis

Fig. 2: Solid tumor in the right thigh of a life Ehrlich tumor mouse (G2)

Immunohistochemical reaction for caspase-3: Immunohistochemical analysis is shown in Fig. 4 where Ehrlich tumor mice received 85 µg AgNPs was highly expressed for caspase-3 (Score 3, +++) (Fig. 4c) compared to moderate expression (Score 2, ++) of Ehrlich tumor mice received 42.5 µg AgNPs (Fig. 4b) and slight staining for caspase-3 (Score 1, +) in untreated Ehrlich tumor mice (Fig. 4a). Liver of all treated groups showed negative reaction for caspase-3 (Score 0, -ve) (Fig. 4d).

Liver histopathological alterations: Normal histological structure of the central vein and surrounding hepatocytes were recorded in liver of control negative mice (Fig. 5a).

Table 1: Mean levels of serum biochemical and haematological parameters in control negative group (G1), Ehrlich tumor mice (G2), mice treated with AgNPs in 85 µg (G3), Ehrlich tumor mice treated with 85 µg AgNPs (G4) and Ehrlich tumor mice treated with 42.5 µg AgNPs (G5)

	Groups				
Parameters	G1 control negative group	G2 Ehrlich tumor mice	G3 mice treated with 85 µg AgNPs	G4 Ehrlich tumor mice treated with 85 µg AgNPs	G5 Ehrlich tumor mice treated with 42.5 µg AgNPs
WBCs×10^3	4.07±0.8^b	11.30±1.2^a	5.28±0.9^b	6.64±0.5^b	6.87±0.7^b
RBCs×10^6	3.77±0.29^a	3.55±0.25^a	3.31±0.21^a	3.54±0.26^a	3.90±0.14^a
Hb (g dL^{-1})	11.29±0.89^a	10.63±0.77^a	9.94±0.66^a	10.60±0.79^a	11.69±0.43^a
ALT (U mL^{-1})	38.66±0.67^a	44.00±3.06^a	40.00±2.31^a	38.00±1.33^a	38.00±0.67^a
AST (U mL^{-1})	112.00±3.53^a	125.00±12.33^{ab}	110.00±3.53^a	137.00±1.67^b	131.00±3.79^{ab}

Different letters mean statistical significance at p≤0.05 according to one-way ANOVA followed by Duncan's test for *post hoc* analysis

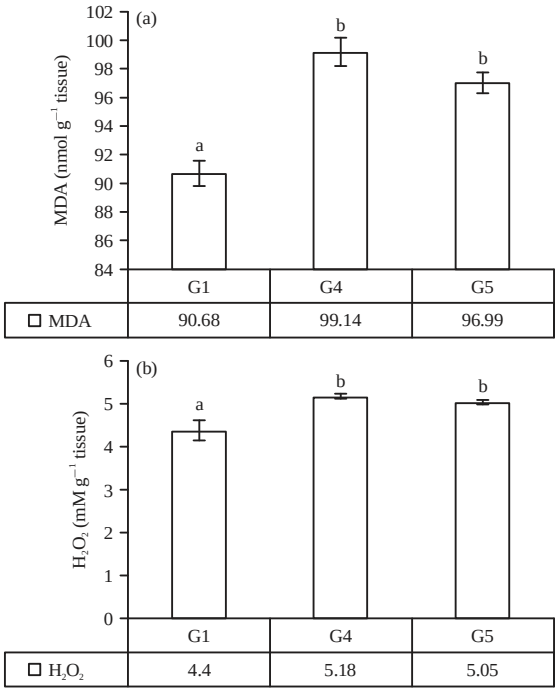

Fig. 3(a-b): Mean±SEM of MDA (nmol g^{-1} tissue), H_2O_2 (mM g^{-1} tissue) concentrations in (a-b) Ehrlich tumor mice (G2), Ehrlich tumor mice treated with 85 µg AgNPs (G4) and Ehrlich tumor mice treated with 42.5 µg AgNPs (G5). Different letters mean statistical significance at p≤0.05 according to one-way ANOVA followed by Duncan's test for *post hoc* analysis

Diffuse kupffer cells proliferation was noticed in between the hepatocytes in liver of tumor control mice and tumor mice treated with AgNPs in (Fig. 5b, d, e). Few inflammatory cells infiltration with kupffer cells proliferation were detected in the portal area in liver of mice treated with 85 µg kg^{-1} b.wt., AgNPs (Fig. 5c).

Ehrlich tumor cells in subcutaneous tissue histopathological alterations: Tumor cells were observed in the subcutaneous tissue with few areas of necrosis and all of them surrounded by inflammatory cells infiltration of group 2 mice (Fig. 6a, b).

Wide area of necrosis was detected in the tumor cells of mice treated with 42.5 µg AgNPs in group 5 (Fig. 6c). Necrosis was noticed in most of the tumor cells treated with 85 µg AgNPs in group 4 mice (Fig. 6d, e).

DISCUSSION

Owing to high death rate associated with cancer and serious side effects of chemotherapy and radiation therapy, many cancer patients seek alternative and/or complementary methods of treatment. Marine algae are one of the natural resources in the marine ecosystem. They contain various biologically active compounds which have been used as source of food, feed and medicine. Until now, more than 2400 marine natural products have been isolated from seaweeds of subtropical and tropical populations[33]. Algae extract has great efficiency to synthesize the silver nanoparticles as a green route[24].

Silver nanoparticles showed different degrees of *in vitro* cytotoxicity at using different human cell lines[34]. The proposed mechanism by which metallic nanoparticles lead to cytotoxicity was considered to be through the induction of Reactive Oxygen Species (ROS)[35]. The cytotoxic effects of AgNPs promote them to be used as anti-cancer therapy.

Silver nanoparticles were prepared by reduction of silver ions (Ag^+) present in $AgNO_3$ using *Turbinaria turbinata* algal extract. The biosynthesized AgNPs were then characterized by UV-visible spectroscopy, x-ray diffraction (XRD), transmission electron microscope (TEM), scanning electron microscopy, energy dispersive x-ray spectroscopy (EDX) and Fourier-transform infrared (FTIR). The preparation and reduction of $AgNO_3$ was performed in a previous study[36].

The green synthesis of AgNPs by using *Turbinaria turbinata* algal extract was indicated by a colour change from bale yellow to brown. After 48 h from addition of $AgNO_3$

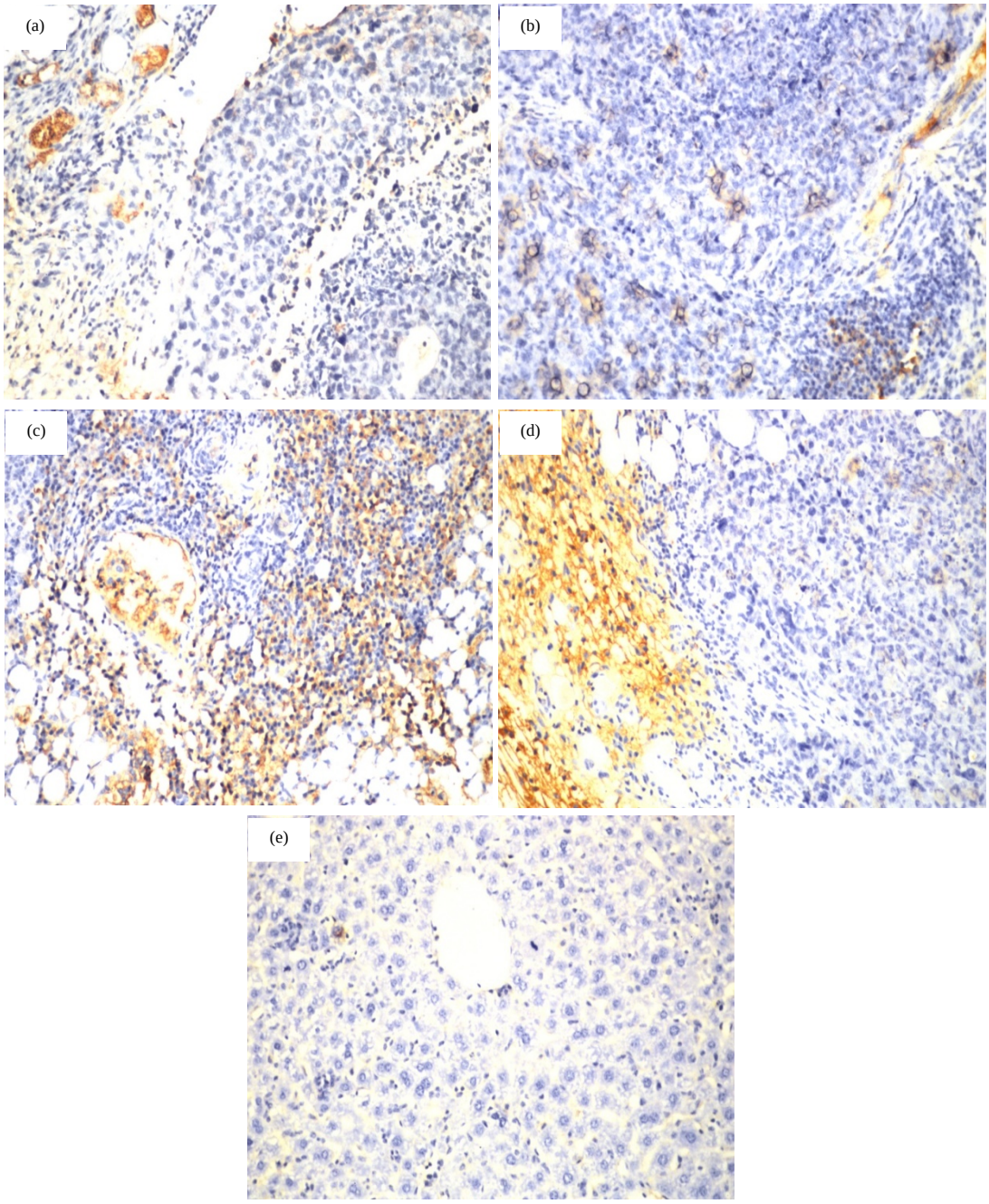

Fig. 4(a-e): Immunohistochemistry for caspase-3 (X40). (a) Slight staining for caspase-3 (Score 1, +) in untreated Ehrlich tumor mice (G2), (b) Moderate caspase-3 expression (Score 2, ++) of Ehrlich tumor mice received 42.5 µg AgNPs (G5), (c) High expression for caspase-3 (Score 3, +++) of Ehrlich tumor mice received 85 µg AgNPs (G4), (d-e) Liver of all treated groups showed negative reaction for caspase-3 (Score 0, -ve)

to algal aqueous extract, a broad emission peak of AgNPs at 428 nm was monitored by UV spectroscopy. Scanning and transmission electron microscopes images showed that AgNPs were spherical in shape, well distributed and with a range of 8-16 nm in size.

Also, in a previous study, the anti-tumor activity of AgNPs biosynthesized by algal extract was determined *in vitro* against Ehrlich Ascites Carcinoma (EAC) cell line by Khalifa *et al.*[36]. The Different concentrations (42, 56, 69, 85 and 98 µg mL^{-1}) of green synthesized AgNPs inhibited the

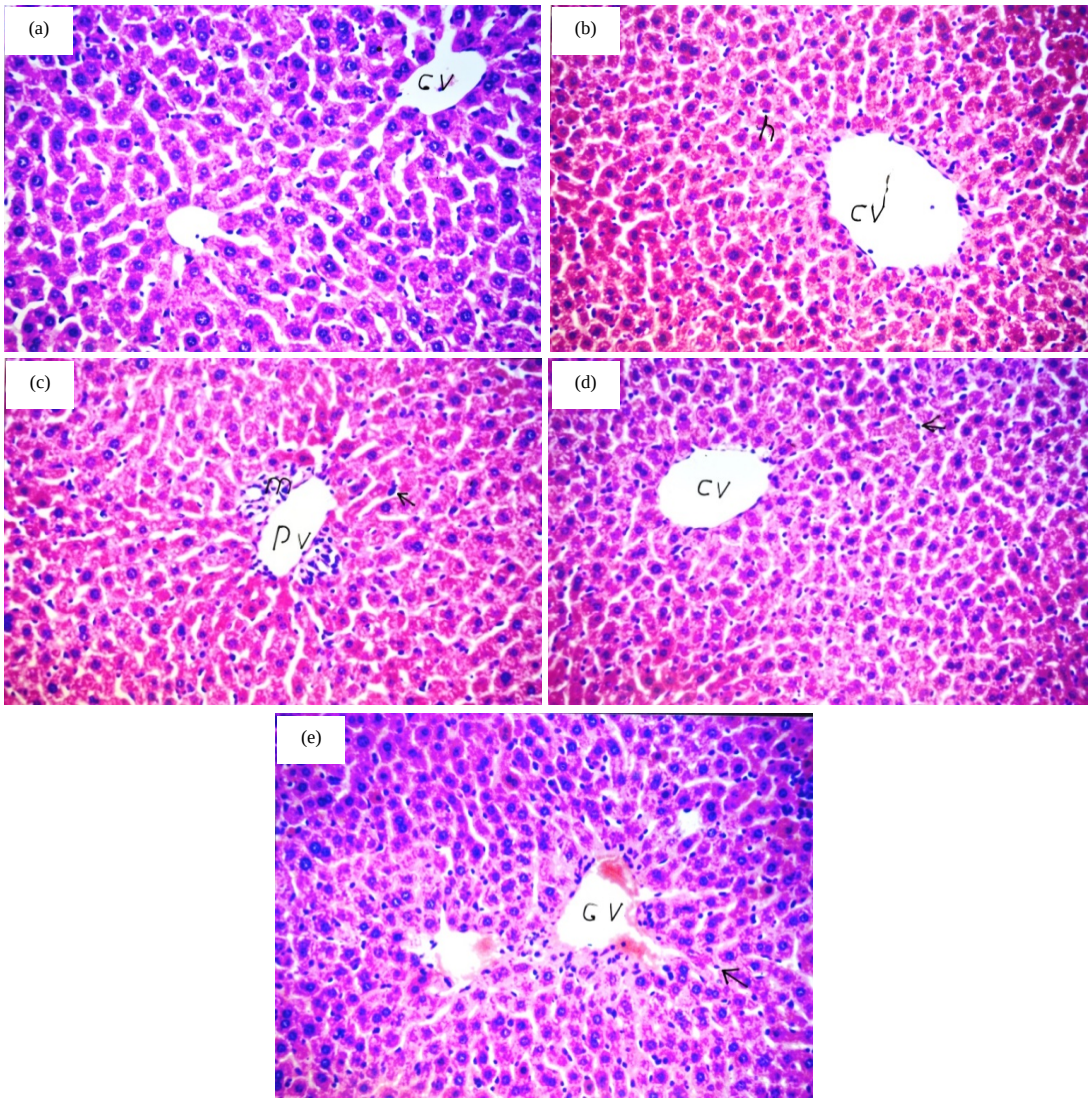

Fig. 5(a-e): (a) Normal histological structure of the central vein and surrounding hepatocytes of control negative mice, (b, d, e) Diffuse kupffer cells proliferation in between the hepatocytes in liver of tumor control mice and tumor mice treated with 85 and 42.5 µg AgNPs in (c) Few inflammatory cells infiltration in the portal area with kupffer cells proliferation in liver of normal mice treated with 85 AgNPs (H and E X40)

proliferation of EAC cells in percentages of (67, 77, 85, 92 and 99%), respectively. The present study was designed for *in vivo* evaluating the potential use of the biosynthesized AgNPs to induce cancer cells cytotoxicity in the lowest dose and submaximal lethal dose of *in vitro* study. This was done by their SC injection early after one day of SC cancer cells inoculation in the thigh of mice.

At the end of the experiment, significant increase was found in weight gains of Ehrlich tumor group 2 mice in relation to control negative and AgNPs treated groups. We referred this increase to absence of tumor tissue or variations in tumor size as there was no significant change between control negative G1 and non-tumor G3 mice treated with AgNPs or in another mean AgNPs in a dose of 85 µg had no effect on the change of body weight gains.

The morphology of AgNPs formed by *Turbinaria turbinata* showed that the size range is 8-16 nm. This small size of obtained silver particles have capability of distribution and accumulation in tumor cells after its injection at the site of tumor. Devi *et al.*[37] reported that the cytotoxic effect is inversely proportional to the size of AgNPs.

In this study, to investigate the mechanism by which AgNPs emitted its cytotoxic effect on EAC inoculated in thigh of mice, we measured the levels of the MDA and H_2O_2 as

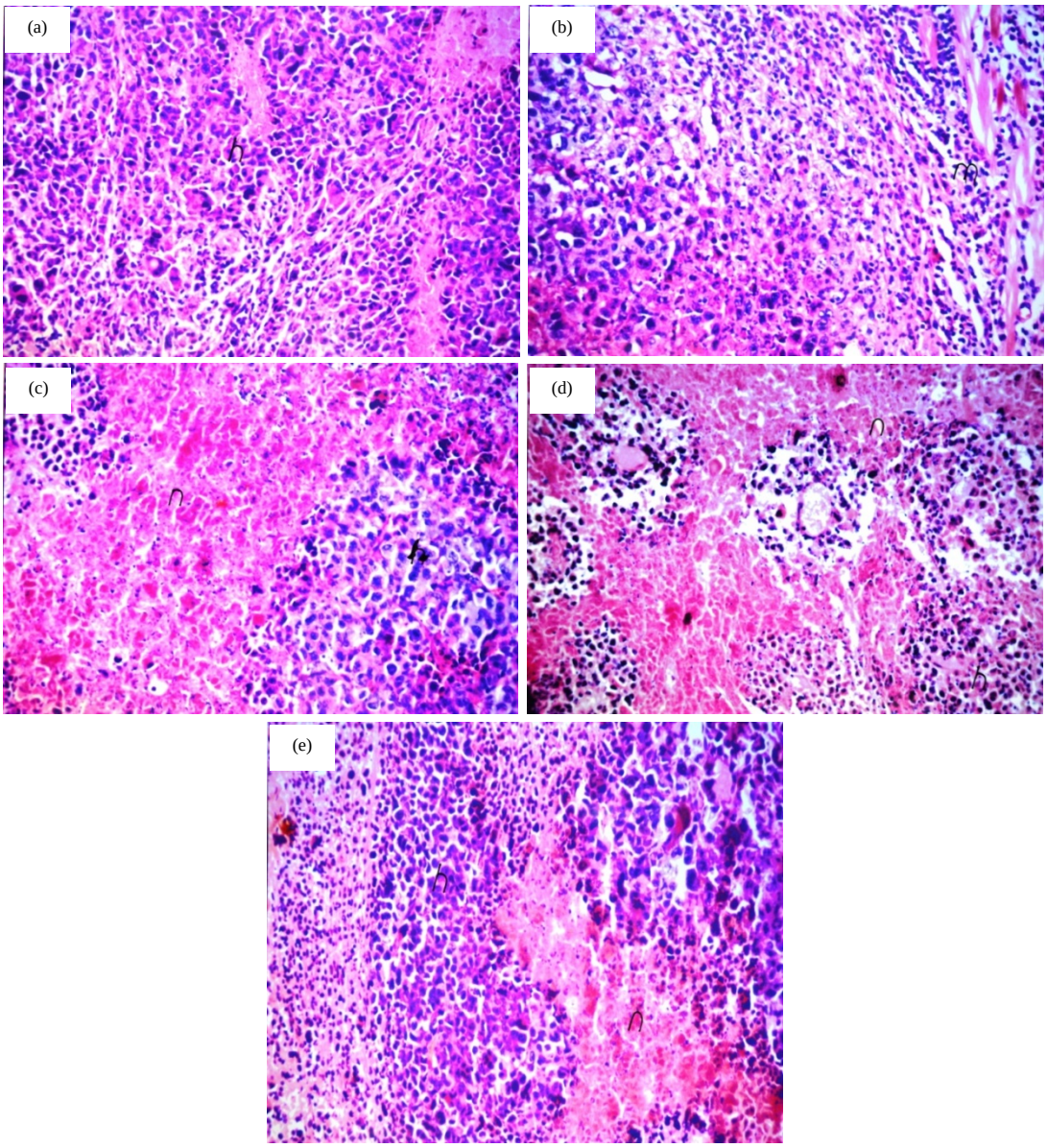

Fig. 6(a-e): Photomicrograph of the induced s/c Ehrlich tumor mass showing (a-b) Anaplastic cells with few areas of necrosis surrounded by inflammatory cells infiltration in group 2 mice, (c-d) Necrosis in most of the tumor cells of mice treated with 85 µg mouse^{-1} AgNPs in group 4 and (e) Wide area of necrosis in the tumor cells of mice treated with 42.5 µg mouse^{-1} AgNPs in group 5 (H and E X40)

indicative for lipid peroxidation and free radicals production in tumor tissue. It has been found that AgNPs significantly induced elevations in MDA and H_2O_2 contents in the solid tumor tissue of tumor bearing treated mice than those of tumor control untreated group.

A significant increase in hydrogen peroxide and superoxide productions in normal human lung fibroblast cells (IMR-90) and human glioblastoma cells (U251) after treatment by AgNPs was recorded by AshaRani *et al.*[34]. Also, it has been observed that AgNPs lead to an increase in ROS associated with DNA damage, apoptosis and necrosis[35,38-40].

The caspase-3 protein is a member of the cysteine-aspartic acid protease caspase family[41]. Sequential activation of caspases play a central role in cell apoptosis. Caspase-3 is activated in the apoptotic cell both by extrinsic (death ligand) and intrinsic (mitochondrial) pathways[42,43].

Most cytotoxic anticancer agents cause tumor regression, at least in part, by inducing apoptosis. This study revealed

another mechanism by which AgNPs could elicit anti-cancer effect. It was apoptotic effect on tumor tissue by activation of Caspase-3 protein. Immunohistochemistry of tumor tissue illustrated dose dependent activation of caspase-3 by AgNPs. Cell apoptosis enhanced by caspase-3 as it can cleave a wide range of cellular substrates including structural proteins and DNA repair enzymes. It also can activate an endonuclease caspase-activated DNAse, which causes DNA fragmentation that is characteristic of apoptosis[44].

Silver nanoparticles induce apoptosis and inhibit the pathway mediating Dalton's lymphoma ascites tumor cells proliferation and viability[45]. It was hypothesized that cell apoptosis was the mechanism induced by the biosynthesized AgNPs against breast cancer cells[46].

From the results of histopathological investigation, there were sever necrotizing effects on tumor cells induced by AgNPs especially at the higher dose.

Previous studies proposed many cytotoxic mechanisms of AgNPs as disruption of the mitochondrial respiratory chain leading to production of ROS and interruption of ATP synthesis, which in turn cause DNA damage[34], impairment of sulfur and phosphorus containing essential macromolecules such as proteins and DNA[47] and interaction with thiol rich enzymes[48].

It was found that mice bearing Ehrlich solid tumor treated with AgNPs and exposed to gamma radiation significantly ameliorated superoxide dismutase and catalase activities and reduced glutathione with an increase in malondialdehyde and nitric oxide levels compared to tumor group, with induction of apoptotic via a mechanism involved caspase-3 activation. Moreover, AgNPs showed antiangiogenesis effect[49].

Silver nanoparticles in the present study did not induce any alterations in RBCs count or hemoglobin concentration between the controls, tumor controls and tumor-treated mice but reduction in the elevated white blood cell count in tumor-bearing mice compared with tumor- bearing untreated mice. These data indicate absence of adverse effect of AgNPs except effective control of white blood cells that possess the immunologic constituents[45]. Furthermore, AgNPs did not induce significant changes in ALT but elevation in AST that may give prediction of hepatic or muscular changes. But until now, histopathological alterations of hepatic tissue were only kupffer cells proliferation and few inflammatory cells infiltration.

Apoptosis of the liver had not been recorded as indicated from negative reaction of hepatic cells to AgNPs caspase-3 protein.

Therefore biosynthesized AgNPs that injected in thigh, the site of tumor, had no or less systemic side effects but local cytotoxic effects on tumor tissue. De Lima *et al.*[50] also concluded that biogenic silver nanoparticles are generally less cyto/genotoxic *in vivo* compared with chemically synthesized nanoparticles so its systemic side effects are limited.

CONCLUSION

In conclusion biosynthesized silver nanoparticles from *Turbinaria turbinata* marine-alga could control the growth of Ehrlich Cell Carcinoma (ECC) in mice via their potent cytotoxic and apoptotic activities against tumor cells with limited side effects on liver and blood of mice.

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