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Research Paper

Antitumor Efficacy of Silver Nanoparticles Biosynthesized from Marine Red Seaweed *Halymeniaporphyroides* Boergesen on Dalton's Lymphoma Ascites in Mice

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ABSTRACT

The biosynthesized silver nanoparticles from marine red seaweed *Halymeniaporphyroides*against Dalton's lymphoma ascites (DLA)-inducedtumor inoculation was studied for antitumor activity. The biosynthesized silver nanoparticles from marine red seaweed *Halymeniapor-phyroides* were given orally to Swiss albino mice (50 mg/kg/day) for 14 days showeda significant reduction in body weight, packed cell volume, andviable tumor cell count when compared to the mice of the DLA control group.The haematological parameters of the treatment group with biosynthesized silver nanoparticles also exhibited increased haemoglobin, RBCs, Platelets, and decreased WBCs compared to the DLA control group of mice. Similarly, the biochemical parameters like total cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides (TL), and alkaline phosphatase (ALP) of the treatment control group with biosynthesized silver nanoparticles reversed the parameters to normal levels compared to the DLA control group of mice. The antitumor efficacy of the biosynthesized silver nanoparticles from *Halymeniaporphyroides* was confirmed based on the haematological, biochemical, life span, packed cell volume, cell count, and histopathological analysis.

KEYWORDS: Antitumor, *Halymeniaporphyroides*, Silver nanoparticles, Dalton's lymphoma ascites (DLA)

Introduction

Cancer is a global public health problem characterized by local tissue invasion, uncontrolled cellular growth, and metastasis (Chabner and Collins, 1990) representing a group of various diseases and responsible for 70% of deaths worldwide (Siegel et al., 2020). Cancer treatment options seem to be limited with chemotherapy and other conventional drugs with side effects like somatic cell destructions, alopecia, nausea, and vomiting, etc. (Sak, 2012; Baskar et al., 2014). Currently, researchers are focusing on various studies for the development of drugs for the treatment of cancer (Pucci et al., 2019; Chikara et al., 2018; Singh et al., 2016); one promising approach would be the use of nanotechnology via the green synthesis method with eco-friendly and environmental stable nanoparticles (Heinemann et al., 2021). Marine seaweeds are macroscopic algae that play a crucial role in the green synthesis of nanoparticles and have rich sources of secondary metabolites (Krishnamurthy, 2005b; Vasanthi et al., 2006), bioactive compounds (Holdt and Kraan, 2011; Carpena, 2021), vitamins (Nisizawa, 1988; Dagnelie, 1995), and polyunsaturated fatty acids (PUFA's) (Chandini et al., 2008; Kumari et al., 2010) especially in red and brown seaweeds which acts as capping agents in the biosynthesis of nanoparticles (Vishnu Kiran and Murugesan, 2020). Green synthesis of silver nanoparticles using nanotechnology is widely utilized in medicine for the treatment of various diseases and disorders, therapeutic drug delivery and diagnostic applications in medicine (Arun and Chaudhari, 2013). Various studies have been done in the anticancer activity using silver nanoparticles which enables a platform for the development of new drugs and therapeutic solutions in the treatment of cancer (Al-Sheddi et al., 2018; Vishnu Kiran and Murugesan, 2014; Buttacavoli et al., 2017; Wypij et al., 2021).

In the present study, an attempt has been made for the investigation of *in vivo* antitumor activities of the biosynthesized silver nanoparticles from marine red seaweed *Halymeniaporphyroides*against Dalton's-lymphoma ascites (DLA) induced tumor inoculation concerning haematological parameters, biochemical parameters, cell count and histopathological analysis.

Materials & Methods

Collection and Preparation of Seaweed Extract

The marine red alga *Halymeniaporphyroides* Boergesen were collected from the intertidal regions of

Mandapam (Latitude 9°17" N, longitude 79°11"E); Southeast Coast of Tamilnadu. Collected seaweed was washed with seawater for eliminating impurities such as sand, rocks, epiphytes, and epifauna. The washed samples were preserved with 5 -10 % formaldehyde in seawater and transported to the laboratory in a box containing slush ice. The fumes of the formaldehyde would help to fix and preserve the seaweed material. In the laboratory, the samples were washed thoroughly in running tap water to remove salt and washed three times using distilled water which may remove metallic compounds and it was shade dried at room temperature (37^0C) for 10 days. The dried seaweed materials were crushed by using mortar and pestle to get the powder form and it was stored in an air-tight container. About 1 g of crushed seaweed powder was added with 100 ml of distilled water in a 250 ml conical flask and boiled for 5-10 minutes at 60-80°C. The crude extract was collected and stored at 4°C for experimental use (Rajesh et al., 2017).

Biosynthesis of Silver Nanoparticles

The crude extract of the experimental marine red seaweed *Halymeniaporphyroides*was used for the synthesis of silver nanoparticles. Silver nitrate ($AgNO_{3.}$) (SD fine) was used for the synthesis of silver nanoparticles and double-distilled, deionized water was used for all the experiments. The silver nanoparticle formation was carried out by taking 500 mg of dry, shade dried powder samples of *Halymeniaporphyroides*in a 250 mL Erlenmeyer flask with 10^{-3} M aqueous $AgNO_{3-}$ solution and was incubated at room temperature. The pH was checked during the reaction, and it was found to be 5.09. Nearly 95% of bioreduction of $AgNO_3$- ions occurred within 24 hrs at stirring conditions.The biosynthesis of silver nanoparticles was characterized by UV Vis spectroscopy; size and morphology by employing SEM and TEM, structure from X-ray diffraction (XRD) technique, stability of silver nanoparticles from Thermogravimetric analysis (TGA), and biomolecules involved in the capping agent of silver nanoparticles from Fourier transform infrared (FT-IR) spectroscopy (Vishnu Kiran and Murugesan, 2020).

Experimental Laboratory Animal's Acclimatization and Delection

The complete study was done by purchasing 20 to 25 g of male Swiss albino mice from KMCP College of Pharmacy animal experimental laboratory. Micro nylon boxes were used for housing the laboratory animals under a controlled temperature of 25°C and 65-70%humidity was maintained in 12 hours of dark/light cycles. The laboratory mice feeding was done according to the standard diet protocol with water *ad libitum*. The animal laboratory experiments protocols were approved by the Institutional Animal Ethics Committee with reference (IAEC/KMCP/155/2014). The Swiss albino mice were quarantined for 15 days with a healthy diet and in a hygienic environment as per the standard practice, before the experimental commencement (Unnikrishnan and Kuttan, 1990).

Tumor Induction Technique using DLA Cell Line

In the present study, cell lines induced cancer in mice was used to evaluate the anticancer activity of the biosynthesized silver nanoparticles from marine red seaweed *Halymeniaporphyroides*. The induction of tumors in animals was done using various techniques such as chemical induction using DMBA/Croton oil, etc. (Agarwal et al., 2009), cell line induction using sarcoma-180, ULCA fibro sarcoma and Jensen sarcoma, mouse lung fibroblast cells L929, Dalton's Lymphoma Ascites (DLA), Ehrlich Ascites Carcinoma (EAC) (Appleman et al., 1950; Chitra et al., 2009) methods.The Dalton's Lymphomaascites (DLA) cell was supplied by Amala CancerResearch Center, Trissur, Kerala, India. The intraperitoneal transplantation method was adopted for maintaining the cells *in vivo* in the Swiss albino mice. The tumor cells were transformed to the grouped animal by aspirating the DLA cells from the peritoneal cavity of the mice using saline.Finally, the dilutions were made, and the cells were counted where the total cell volume of 1 x 10^6 was given intra-peritoneally to the mice and allowed for seven days for the tumor formation before the commencement of the experiment.

Treatment Protocol

The treatment protocol consists of four groups and the Swiss albino mice of six no's in each group were divided equally. The three groups of the experimental laboratory animals were injected with DLA with PBS (1×10^6 cells per mouse) intraperitoneally, and the remaining one group was left as a normal control group. The groups are elaborated as described in Table 1. In the present investigation, drug treatment (silver nanoparticles synthesized by *Halymeniaporphyroides*) was given after 24 hrs of inoculation into the peritoneal cavity, and it was continued once in a day for 14 days. On day 14, i.e. after the last dose, all mice from each group were sacrificed; the blood was withdrawn from each mouse by cardiac puncture method, and the biochemical investigations described below were carried out using COBASMIRA PLUS-S, Auto analyzer, Roche, Switzerland.

TABLE 1

Treatment protocol.

Groups	Description	Treatment protocol
I	Normal control	Received normal diet and water ad libitum
II	Tumor control	Received normal diet and water ad libitum
III	Positive control	Treated with 5-fluorouracil at 20 mg/kg body
IV	Treatment control	Administered with biosynthesized AgNps from *Halymeniaporphyroides*[50 mg/kg] orally [LD50 OECD Guidelines].

Cancer Cell Count

The fluid (0.1 mL) from the peritoneal cavity of each mouse was withdrawn after the experimental period by a sterile syringe, diluted with 0.8 mL of ice-cold normal saline or sterile phosphate buffer solution and 0.1 mL of trypan blue (0.1 mg/mL) (Mary et al., 1994) and the total number of the living cells were counted using a Haemocytometer as follows:

$$\text{Cell counts} = \frac{\text{No. of cells dilution}}{\text{Area} \times \text{Thickness of liquid film}}$$

Bodyweight

All the mice were weighed, from the beginning of the 15th day of the study, and average body weight was determined.

Life Span

The percentage of increase in lifespan (Santhosh Kumar et al., 2007) was calculated by the following formulae

$$\%\text{ILS} = \frac{\text{Life span of treated group}}{\text{Life span of control group}} - 1 \times 100$$

Hematological Parameters

The collected blood sample was analyzed for hematological parameters such as WBC, RBC, platelet count and hemoglobin count and the serum was analyzed for cholesterol, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alanine phosphatase (ALP).

Determination of Cholesterol

The total serum cholesterol in blood was estimated as per the method of Zlatkis*et al.*,(1953) (Zlatkis et al., 1953). The serum sample 0.1 mL was taken in a test tube and added with glacial acetic acid of 6.0 ml with coloring reagent of 0.4 mL which was mixed well. Similarly, 0.1 mL of water as blank, and 0.1 mL of standard cholesterol solution (2 mg /ml) was runsimultaneously. Then tubes were allowed to cool at room temperature and were recorded for optical density at 540 nm usinga Shimadzu-UV-visible spectrophotometer.

Estimation of Triglycerides

The blood (1 mL) from the normal control, cancer control, positive control group, and the treatment control group was withdrawn intravenously and centrifuged at 2000 rpm for 10 minutes at 4°C, post which the serum was collected and used for triglycerides determination. The assay of serum triglycerides was carried out as described above for the preparation of its standard curve, except the triolein was replaced by 50 µL of serum. The amount of serum triglycerides was extrapolated from the standard curve between triolein concentrations in the range of 0.5 to 14.0 mM /A_{510}.

Determination of AST (Aspartate Aminotransferase)

AST (Aspartate aminotransferase) from the liver in the blood serum was assayed according to the method of Reitman and Frankel (1957) (Reitman and Frankel, 1957).

Principle

SGOT (AST) catalyzes the following reaction

α-Ketoglutarate + L-aspartate ⟶ L-glutamate+oxaloacetate

Oxaloacetate so formed is coupled with 2, 4 Dinitrophenyl hydrazine (2, 4 DNPH) to give the corresponding hydrazine, which gives a brown color in alkaline medium and this is measured calorimetrically.

Determination of ALT (Alanine aminotransferase)

ALT (Alanine aminotransferase) from the liver in the blood serum was assayed according to the method of Reitman and Frankel (1957) (Reitman and Frankel, 1957).

Principle

ALT catalyzes the following reaction

α-Ketoglutarate + L-alanine ⟶ L-glutamate + pyruvate.

Pyruvate so formed coupled with 2, 4 Dinitrophenylhydrazine (2, 4 DNPH) to give the corresponding hydrazine, which gives a brown colour in alkaline medium and this can be measured calorimetrically.

Determination of ALP (Alanine phosphatase)

Estimation of Serum ALP Activity is used for *in vitro* quantitative determination of alkaline phosphatase (ALP) in serum and plasma.

Principle

ALP

p-nitrophenylphosphate + H_2O ALP Phosphate ⟶ Phosphate + p-nitrophenol

ALP catalyzes the hydrolysis of p-Nitrophenyl phosphate (pNPP) to p-nitrophenol. pNPP is colorless, but p-Nitrophenol has a strong absorbance at405 nm.

Histopathological Examination of Liver

The liver was cleaned and fixed at 10% neutral buffered formalin solution. After dehydration in graded ethanol solutions and in toluene, they were embedded in paraffin. Tissue sections of 3–5 µm thickness were stained with Haematoxylin and eosin (H.E.) for histopathological examination. Other sections were stained with Periodic acid staining (PAS) for histopathological examination.

Statistical analysis: The experimental results wereexpressed as the mean ± S.E.M. The haematological andbiochemical parameters were subjected to statisticalanalysis by one-way Analysis of Variance to determinethe significant difference between the groups. ANOVA was done with Graph pad Prism software. All PairwiseMultiple Comparison Procedures by Student-Newman-Keuls Method. Data were accepted as the statisticallysignificant difference was obtained at $p<0.05$.

Results

In vivo Tumor Cell Suppression Activity

The *in vivo* tumor cell suppression activity of biosynthesized silver nanoparticles from marine red seaweed *Halymeniaporphyroides*was analyzed using Dalton's Lymphoma Ascites cell line (DLA) inoculated in male Swiss albino mice at 50 mg/kg body weight. While transforming the tumor cells into the grouped animals, the DLA cells were aspirated from the peritoneal cavity of the mice using saline. Cell counts were done and further dilutions were made so that the total cell count should be 1 x 10^6 mL, this dilution was given intraperitoneal and the tumor was grown in the mice for a minimum of seven days before starting treatments.

Parameters such as life span, body weight, cancer cell count were measured during the treatment period. The biochemical, haematological, and histopathological studies of the tested animals indicated that the tumor cell suppression activity possessed by the biosynthesized silver nanoparticles from *Halymeniaporphyroides*.

Effect of Silver Nanoparticles on Tumor Cell Growth

In the DLA tumor control group, the average life span of the animals was found to be 48.00%, whereas the silver nanoparticles biosynthesized using *Halymeniaporphyroides* at a dose of 50 mg/kg body weight increased the life span up to 82.00%. However, the average life span of 5-Fluorouracil treatment at 20 mg/kg was found to be 90.00%, indicating its potent tumor cell suppression activity. The antitumor nature of silver nanoparticles biosynthesized from *Halymeniaporphyroides* at a dose of50 mg/kg body weight was evident by the significant increase in body weight of animals when compared to the DLA bearing mice. It was also supported by the significant reduction in packed cell volume and viable tumor cell count in the treatment when compared to the DLA tumor control (Table.2 and 3).

Effect of Silver Nanoparticles on Haematological Parameters

Haematological parameters of the experimental animals were analyzed from all the experimental laboratory animal groups. The red blood cells (RBC's), haemoglobin, platelets were decreased while the white blood cell (WBC's) count was significantly increased in the DLA control group compared to the normal control group. The biosynthesized silver nanoparticles from *Halymeniaporphyroides* at a dose of 50 mg/kg body weight significantly increased the Hb content (9.68 ± 0.86 gm/dL), RBC (3.22 ± 0.52 Mill/cumm), platelets (2.08 ± 0.32 Lakhs/cumm) and significantly decreased the WBC count (12.05 ± 1.82 cells/ mL × 10^3) to about normal level (Table.3).

The results suggest that the biosynthesized silver nanoparticles from the experimental seaweed exhibited tumor cell suppression activity at the dose of 50 mg/kg body weight. However, the standard 5-Fluorouracil at the dose of 20 mg/kg body weight showed high activity when compared to the biosynthesized silver nanoparticle from *Halymeniaporphyroides*.

Effect of Silver Nanoparticles on Biochemical Parameters

The inoculation of DLA in experimental animal cell lines caused a significant increase in the level of total cholesterol (140.95 ± 4.60 mg/dL), aspartate aminotransferase (AST) (87.80 ± 2.80 U/L), alanine aminotransferase (ALT) (62.35 ± 2.80 U/L), triglycerides (TGL) (215.25 ± 4.75 mg/dL) and alkaline phosphatase (ALP) (240.35 ± 4.32 U/L) in the tumor control animals (G2), when compared to the normal group (G1). The treatment with biosynthesized silver nanoparticles from *Halymeniaporphyroides* at a dose of 50 mg/kg body weight exhibited a normal level of total cholesterol (123.35 ± 3.30 mg/dL), aspartate aminotransferase (66.55 ± 2.25 U/L), alanine aminotransferase (46.65 ± 1.58 U/L), triglycerides (163.60 ± 2.42 mg/dL) and alkaline phosphatase (183.30 ± 2.52 U/L). The results indicate the biosynthesized silver nanoparticles from the marine seaweed *Halymeniaporphyroides* reversed the biochemical levels to normal (Table. 4).

Histopathological Studies of Liver

Histopathological investigation revealed the hepatic necrosis caused by Dalton's Lymphoma Ascites (DLA). Theformation of malignant hepatocytes was malformed in the portal tract and central vein (Figure 1). Tumour induced using Dalton's Lymphoma Ascites treated with 5- Fluorouracil at a concentrated dose of 20 mg/kg had shown a reduction in malignancy to some extent. However, liver of tumor-induced animals treated with AgNps biosynthesized from *Halymeniaporphyroides* at a concentrated dose of 50 mg/kg exhibited a high extent of malignancy and alsofewer hepatocytes, which diffuse necrosis and mononuclear infiltrate when were compared to the 5-fluorouracil treatment.

TABLE 2

Effect of biosynthesized silver nanoparticles from *Halymeniapor-phyroides*on the life span, Body weight and cancer cell count of tumour induced mice.

S. No.	Treatment	No of animals	% ILS Life span	Body weight (mg/kg)	Cancer Cell Count
1	Normal control(G_1)	6	>>30 days	2.20 ± 0.55	Nil
2	Cancer control(G_2)	6	48	$7.70 \pm 0.92^{a**}$	$2.75 \pm 0.40^{a**}$
3	Positive control (G_3)	6	90	$3.80 \pm 0.60^{b**}$	$1.45 \pm 0.35^{b**}$
4	*H. porphyroides* Control(G_4)	6	82	$4.65 \pm 0.84^{b**}$	$1.68 \pm 0.45^{b**}$

G_1–Normal Control,G_2–Cancer Control,G_3–Positive control (5-Fluorouracil at 20 mg/kg),
G_4–Treatment control (AgNps biosynthesized from *Halymeniaporphyroides*at 50mg/kg)
All values are expressed as mean ± SEM for 6 animals in each group.
**a – Values are significantly different from control (G_1) at $p<0.001$
**b – Values are significantly different from cancer control (G_2) at $p<0.01$

TABLE 3

Effect of silver nanoparticles biosynthesized from *Halymeniaporphyroides*on haematological Parameters.

S.No.	Treatment	Total WBC Cells /mLx10^3	RBC Count Mill/cumm	Hb Gm/dL	PCV %	Platelets Lakhs/cumm
1	Normal control($G_{1)}$	10.90 ± 1.40	4.30 ± 0.95	12.35± 1.26	14.85 ± 1.42	3.35 ± 0.80
2	Cancer control(G_2)	13.30 ± 2.42^{a**}	2.40 ± 0.40^{a**}	7.05 ± 0.75^{a**}	30.05 ± 3.45^{a**}	1.60 ± 0.32^{a**}
3	Positive control (G_3)	11.35 ± 1.66^{b**}	3.83 ± 0.85^{b**}	11.6 ± 1.06^{b**}	18.50 ± 1.55^{b**}	2.62 ± 0.76^{b**}
4	*H. porphyroides* control(G_4)	12.05 ± 1.82^{b**}	3.22 ± 0.52^{b**}	9.68 ± 0.86^{b**}	22.30 ± 1.80^{b**}	2.08 ± 0.32^{b**}

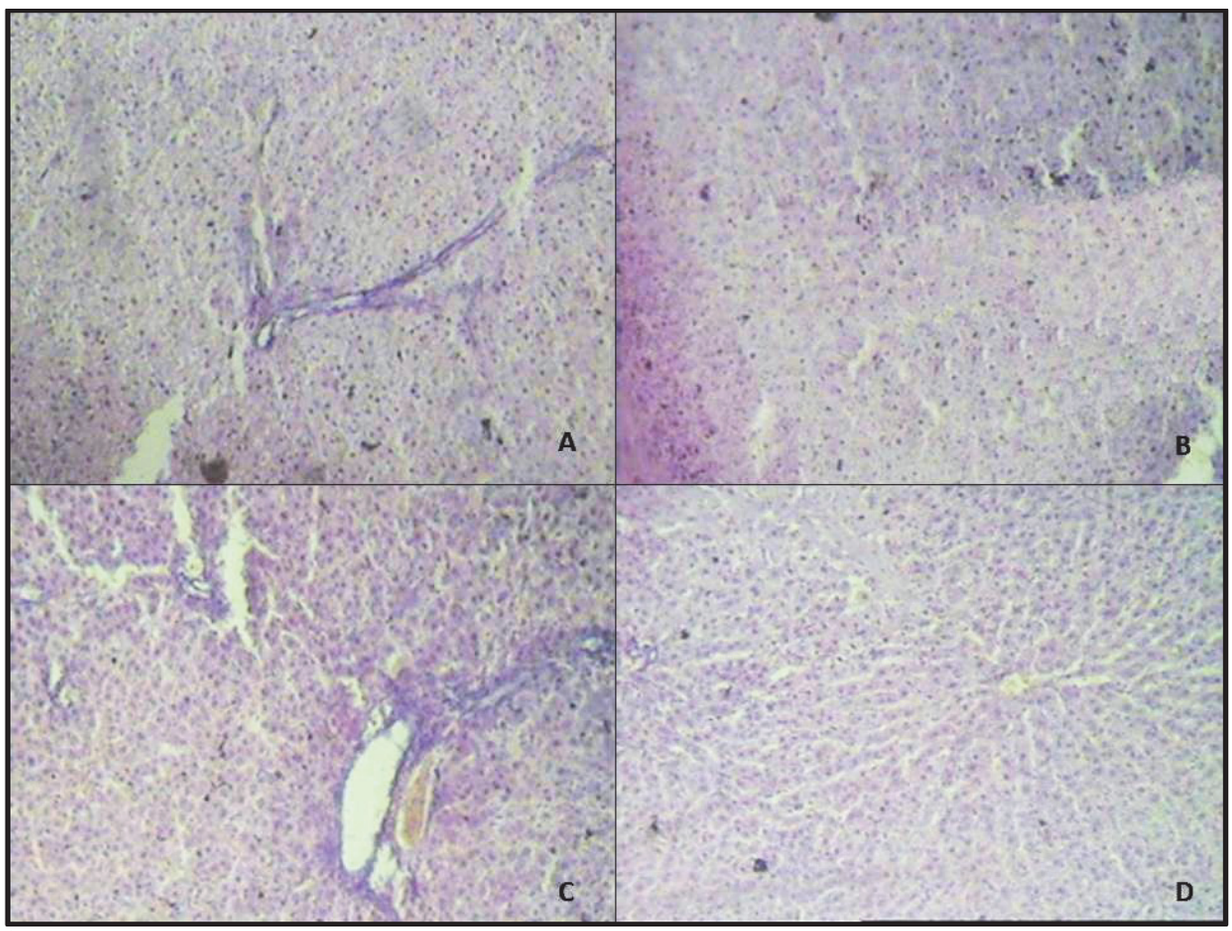
G_1–Normal Control, G_2–Cancer Control, G_3–Positive control (5-Fluorouracil at 20 mg/kg),
G_4–Treatment control (AgNps biosynthesized from *Halymeniaporphyroides*at 50mg/kg)
All values are expressed as mean ± SEM for 6 animals in each group.
**a – Values are significantly different from control (G_1) at p<0.001
**b – Values are significantly different from cancer control (G_2) at p<0.01

TABLE 4

Effect of biosynthesized silver nanoparticles from *Halymeniaporphyroides*on serum enzymes and lipid proteins.

S.No.	Treatment	Cholesterol (mg/dL)	Triglycerides (mg /dL)	Aspartate amino transferase (U/L)	Alanine amino transferase (U/L)	Alkaline phosphatase (U/L)
1	Normal control ($G_{1)}$	102.30 ± 3.55	132.80 ± 2.45	40.60 ± 1.35	34.40 ±1.40	125.35 ± 2.40
2	Cancer control (G_2)	140.95 ± 4.60^{a**}	215.25 ± 4.75^{a**}	87.80 ± 2.80^{a**}	62.35 ± 2.80^{a**}	240.35 ± 4.32^{a**}
3	Positive control (G_3)	116.52 ± 3.35^{b**}	154.45 ± 2.62^{b**}	59.40 ± 1.75^{b**}	42.65 ± 1.66^{b**}	162.40 ± 2.40^{b**}
5	*H. porphyroides* control (G_4)	123.35 ± 3.30^{b**}	163.60 ± 2.42^{b**}	66.55 ± 2.25^{b**}	46.65 ± 1.58^{b**}	183.30 ± 2.52^{b**}

G_1–Normal Control, G_2–Cancer Control, G_3–Positive control (5-Fluorouracil at 20 mg/kg),
G_4–Treatment control (AgNps biosynthesized from *Halymeniaporphyroides*at (50mg/kg)
All values are expressed as mean ± SEM for 6 animals in each group.
**a – Values are significantly different from control (G_1) at p<0.001
**b – Values are significantly different from cancer control (G_2) at p<0.01



(A) Normal control [Section of liver parenchyma with hepatocytes which appear normal, and central vein & portal tract are normal].
(B) Cancer control [Section of liver parenchyma with focal area of necrosis of hepatocytes].
(C) Positive control (5- Fluorouracil) [Section of liver parenchyma with hepatocytes which appear normal, and central vein & portal tract are normal].
(D)Treatment control (AgNps from *Halymeniaporphyroides* at 50 mg/kg) [Section of liver parenchyma with hepatocytes which appear normal, and central vein & portal tract are normal].

Fig. 1. Cross section of liver stained with Haematoxylin and eosin (Magnification 400 ×).

The present study clearly exhibited the significant improvement in the antitumor cell suppression activity of the biosynthesized nanoparticles from the experimental seaweed in the liver tissue samples of the male Swiss albino mice. The results were compared with that of standard 5-fluorouracil and the treatment with AgNps biosynthesized from experimental seaweed *Halymeniaporphyroides* significantly increases the haemoglobin content, RBC, platelets, and decreasing the WBC count to about normal level examined through the blood samples of the Swiss albino mice.

Discussion

In the present study, biosynthesized silver nanoparticles from the experimental red seaweed *Halymeniaporphyroides* were analyzed for their *in vivo* tumor cell suppression activity using Dalton's Lymphoma Ascites (DLA) cell line in male Swiss albino mice at 50 mg/kg body weight. The reliable criteria for judging the antitumor activity of the biosynthesized silver nanoparticle is the prolongation of the life span of animals (Clarkson and Burchenal, 1965).

Effect of Silver Nanoparticles on Tumor Cell Count, Life Span, and Body Weight

Significant inhibition of tumor viable cell count and the retention of haematological factors and body weight to near normal levels were observed in mice treated with biosynthesized silver nanoparticles from the experimental seaweed *Halymeniaporphyroides* when compared to the mice bearing DLA tumor. 5-Fluorouracil at 20 mg/kg was used as the positive control. The increase in cell count after 14 days confirmed the proliferation of cells in the mice treated with biosynthesized silver nanoparticles from the experimental red seaweed *Halymeniaporphyroides*and a decrease in cancer cell count shows confirmatory evidence of protection against DLA cell line as reported by Muthu et al., (2010); Sangiliyandi et al., (2015) (Muthu et al., 2010; Sangiliyandi et al., 2015).

Effect of Silver Nanoparticles on Haematological Factors and Ascetic Fluid

In the haematological factors, red blood cells, haemoglobin, platelets were decreased and the white blood cell count got significantly increased in the DLA control group as compared to the normal control group. Retention of normalcy of red blood cells, haemoglobin, and platelets were observed when treated with biosynthesized silver nanoparticles of the experimental seaweed, which shows the protective action on the Haematopoietic system (Hogland, 1982). Ascitic fluid is the direct nutritional source of tumor cells. The rapid increase in ascitic fluid with tumor growth was observed in tumor bearing mice injected with DLA cell line (Sangiliyandi et al., 2015; Sathiyanarayanan et al., 2006). The mice treated with biosynthesized silver nanoparticles from *Halymeniaporyphyroides* at a dose of 50 mg/kg body weight inhibited the tumor volume by decreasing the nutritional fluid volume required for tumor growth, thereby increasing the life span of the mice as compared to mice bearing DLA tumor and the positive control mice which were injected with 5-Fluorouracil at 20 mg/kg (Muthu et al., 2010).

Effect of Silver Nanoparticles on Serum Enzymes and Lipid Proteins

The increase in serum enzymes and lipid proteins like total cholesterol (TC), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and triglycerides (TGL) of DLA tumor bearing mice were observed. The mice treated with biosynthesized silver nanoparticles from experimental seaweed*Halymeniaporphyroides* showed the retention of serum enzymes and lipid proteins to more or less normal as compared to positive control mice with 5-Fluorouracil and normal mice (Rutberg et al., 2008; Bhuvaneswari and Murugesan, 2012).

Effect of Silver Nanoparticles on Survival Rate in DLA Tumor-bearing Mice

Biosynthesized silver nanoparticles from the experimental seaweed*Halymeniaporphyroides* at 50 mg/kg body weight have been confirmed to be endotoxin-free and have exhibited remarkable antitumor activity. Administration of silver nanoparticles intraperitoneally for 14 days resulted in complete protection in five out of six DLA-challenged mice. Furthermore, the AgNPs from *Halymeniaporphyroides* prolonged the life span of treated tumor-bearing mice, which survived for 86 and 82 days as compared with the untreated tumor controls which survived for nearly 30 days from the first day of tumor induction (Muthu et al., 2010).

Histopathological Analysis of Liver and Histologic Analysis of DLA Cells

The structure of the liver parenchyma section from normal male Swiss albino mice with hepatocytes appeared to be normal along with the central vein and portal tract, while a section of liver parenchyma from tumor induced mice using DLA cell lines shows a focal area of necrosis in hepatocytes. The section of liver parenchyma of mice injected with 5-Fluorouracil at 20 mg/kg body weight also appeared to be normal, the central vein, and portal tract was normal with the absence of necrosis in the hepatocytes. The section of the liver parenchyma of the mice treated with biosynthesized silver nanoparticles from the experimental seaweed *Halymeniaporphyroides* at 50 mg/kg body weight showed retention of the normalcy of central vein and portal tract in the hepatocytes and also an absence of necrosis was observed.

Histologic analysis of DLA cells of peritoneal fluid revealed that the tumor-bearing mice treated with the biosynthesized nanoparticles led to a significant reduction in the number of malignant cell clumps for the treated group when compared with the control group, reflecting the potentiality of silver nanoparticles to have cytotoxic effects on tumor cells, without affecting normal cells (Muthu et al., 2010).

Conclusion

The anti-tumor activity was evident against Dalton's lymphoma ascites (DLA)-induced tumor in the treatment group with biosynthesized silver nanoparticles from marine red seaweed *Halymeniaporphyroides.* The reversion of haematological, biochemical parameters to near normal range in the treatment group against the DLA control group showed the efficacy of the studies. Similarly, the improvement in the body weight, the life span of the animals also showed the anti-tumor effect of the biosynthesized silver nanoparticles. The attempted *in vivo* study and approach may pave a possible efficacy and value in further research in cancer therapeutics with minimal toxicity due to the eco-friendly and green synthesis methodology of silver nanoparticles.

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