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RESEARCH ARTICLE

Silver Nanoparticles Bio-genesis from *Colpomenia sinuosa* and its *in-vivo* Anti-tumor Efficacy on DLA Inoculated tumor in albino mice

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ABSTRACT

Objective: The anti-tumor activity of biosynthesized silver nanoparticles from marine brown seaweed *Colpomenia sinuosa* against DLA (Dalton's lymphoma ascites) induced tumor was investigated.

Methods:

The biosynthesis of silver nanoparticles from marine macroscopic red seaweed *C. sinuosa* was synthesized by the green synthesis method and characterized by UV–Vis spectroscopy, Fourier transform infrared (FT-IR) spectroscopy, X-ray diffraction, Thermogravimetric analysis (TGA), scanning electron microscope (SEM), and transmission electron microscopy (TEM). The complete study was done by purchasing 20 to 25 g of male Swiss albino mice from KMCP College of Pharmacy animal experimental laboratory. The Daltons' lymphoma ascites cell line induced tumor in albino mice was evaluated for anti-tumor activity with the biogenic silver nanoparticles from marine brown seaweed *Colpomenia sinuosa* and was estimated for tumor cell count, body heaviness, Life expectancy, haematological and biochemical factors, histologic analysis of liver using H&E and PAS staining.

Results: The oral administration of the biosynthesized silver nanoparticles from marine brown seaweed Colpomenia sinuosa at 50 mg per kg body weight albino mice were given daily for 14 days. The haematological and biochemical factors along with bodyweight of the animal, cell count (tumor), and cell volume (packed) were analyzed and compared with Dalton's lymphoma control group of mice. The treatment control group mice with biosynthesized silver nanoparticles exhibited an increase in haematological factors, a decrease in white blood cells, and normalcy of biochemical factors compared to Dalton's lymphoma group mice. The reduction in body weight of mice, cell count (tumor), and cell volume (packed) were also observed in treatment group mice with biosynthesized silver nanoparticles as compared to Dalton's lymphoma group mice.

Conclusion: The eco-friendly and green synthesis methodology of biosynthesized silver nanoparticles from *Colpomenia sinuosa* reversed the haematological, and biochemical factors to near normal range against the DLA control group proving the efficacy of the studies. The improvement in the body weight and the life expectancy

of the animals also confirmed the anti-tumor efficacy of the biogenic silver nanoparticles.

Keywords
Anti-tumor, *Colpomenia sinuosa*, AgNps, Dalton's lymphoma

Introduction

Nano-biotechnology and nanoscience enable the process to deal at nanometer scales ranging from 1 to 100 nm. The current modern material sciences currently focus on active research areas in the field of nanotechnology due to their specificity and selectivity which are widely used in biological, therapeutics, and pharmaceutical industries (Alivisatos, 2004; Daniel and Astruc, 2004; Feynman, 1991; Love et al., 2005; Mirkin and Taton, 2006; Nie et al., 2007; Wang et al., 2007). The biosynthesis of nanoparticles plays an important role especially silver nanoparticles due to their ease, eco-friendly, and accepted environmentally with minimum toxicity (Iravani et al., 2014). The focus on silver nanoparticles is increased manifold in recent years due to their large surface area, minimal toxicity, and specificity which are widely employed in therapeutics, drug delivery systems, and gene therapy (Ivanova et al., 2018; Gopinath et al., 2008; Jain et al., 2009). Silver nanoparticles are also known to possess excellent antibacterial, antidiabetic, antioxidant, anticancer, and anti-inflammatory properties (Vishnu Kiran and Murugesan, 2014; Vishnu Kiran and Murugesan, 2014; Vishnu Kiran and Murugesan, 2014; Vishnu Kiran and Murugesan, 2014; Hebeish et al., 2014). The current research involving the biosynthesis of silver nanoparticles via the green synthesis method includes plant-mediated, seaweed-mediated, and microbial-mediated synthesis (Li et al., 2007; Ahmed et al., 2016; Vishnu Kiran and Murugesan, 2020; Feroze et al., 2020). The change in the cellular environment due to metabolic and pathological variations leads to a group of diseases known as cancer that leads to angiogenesis, metastasis, abnormal cell proliferation, cell outgrowth, or tumor via diverse signal mechanisms (Jason and Raymond, 2004; Seigneuric et al., 2010). The rise in cancer in the modern materialistic world sets an alarm for the alternative treatment methods with current chemotherapy and conventional drugs seem to be an option. More research areas are required for finding an alternative in the treatment of cancer and drug delivery systems, one such promising research focus enables silver nanoparticles as an alternative due to their vast special attributes. Enormous efforts have been put forth in recent decades to study the anticancer activity of silver nanoparticles (Igaz et al., 2016; Souza et al., 2016; Buttacavoli et al., 2018; Gomathi et al., 2020). The current study focuses on the investigation of *in vivo* anti-tumor efficacy of the biogenic silver nanoparticles from marine brown seaweed Colpomenia *sinuosa* against DLA-induced tumors in mice.

Experimental Methods

Seaweed - collection and extraction

The macroalgae Colpomenia sinuosa (Mertens ex Roth) Derbes and Solier were procured from the inshore zone of Kanyakumari in Southern India. Procured macroalgae were rinsed with saline water for removing impurities. The cleaned macroalgae samples were conserved with five to ten per cent formaldehyde in saline water and kept in slushed ice under laboratory conditions. The seaweed material was fixed and preserved by formalin fumes in the laboratory; post which the seaweed samples were washed with fresh water followed by deionized water to decant the salt and other impurities and dried at 37°C for 12 to 15 days. The processed seaweed samples were made into powder and preserved in the anaerobic container. The processed seaweed sample of about 1 g was mixed with 100 ml of double-distilled water boiled at 70^{0}C for 10 minutes which was then evaporated using a rotary type evaporator and preserved using coloured bottles at 4^{0}C for experimental analysis (Rajesh et al., 2017).

Silver nanoparticles biogenesis

The silver nanoparticle biogenesis was carried out using the crude extract from marine brown seaweed *Colpomenia sinuosa* where silver nitrate ($AgNO_{3-}$) (Merck) was used as the precursor for the biogenic synthesis. The processed crude extract of seaweed sample of about 500 mg was taken and mixed with 10^{-3} M aqueous $AgNO_{3-}$ a mixture which was kept at 37^{0}C and the pH of the medium was maintained at 5.09 during the reaction process. The biogenesis of silver nanoparticles occurred at 24 hrs stirring conditions with 95 % bio-reduction of $AgNO_3$- ions. The characterization of the biogenic silver nanoparticles was done using characterized by Ultraviolet-Visible spectroscopy; the dimensions of the AgNPs by Scanning Electron Microscope (SEM) and the morphology by Transmission Electron Microscopy (TEM). The structural elucidation was studied using the X-ray diffraction (XRD) technique and the thermal stability of AgNPs was determined using Thermogravimetric analysis (TGA). The biomolecules involved in the capping agent of AgNPs were determined from Fourier to transform infrared (FT-IR) spectroscopy (Vishnu Kiran and Murugesan, 2020).

Experimental design and tumor induction

The experimental design was done by procuring mice (Swiss albino) which weighed approximately between 20 to 25 g. The mice (Swiss albino) were acquired from KMCHOP, Coimbatore, India, and maintained under good animal house laboratory conditions with 25°C temperature, 70% humidity, and a twelve hours cycle of

both dark and light. The IAEC protocol was followed for sufficient diet and water consumption for the acquired mice. The protocols for conducting the animal laboratory animal experiments were approved with the reference no IAEC/KMCP/155/2014 by the governing committee IAEC (Institutional Animal Ethics Committee). The standard practice of quarantine of Swiss albino mice was maintained for 15 days in asepsis condition and proper diet before the commencement of the experiment (Unnikrishnan and Kuttan, 1990).

The Daltons' lymphoma ascites cell line induced tumor in albino mice was evaluated for anti-tumor activity with the biogenic silver nanoparticles from marine brown seaweed *Colpomenia sinuosa*. Tumor induction in animals involves techniques like chemicals and cell line induction in animal models. Chemicals such as DMBA or Croton oil (Agarwal et al., 2009), whereas cell lines like EAC, DLA, L929 f fibroblast, Sarcoma (180, ULCA and Jensen) are widely used (Appleman et al., 1950; Chitra et al., 2009). The Dalton's Lymphoma cell for the present study was procured from ACRC (Amala Cancer Research Center) in Kerala, South India. Dalton's lymphoma cells were perpetuated *in vivo* conditions in the experimental animals within the peritoneal cavity. The buffered saline was used to aspirate Dalton's lymphoma cells from the peritoneum of the albino mice and the cells with tumors were transformed into the segregated animals. The cell count was done by the dilution method and a total cell volume of 1 x 10^6 was injected intra-peritoneally into the albino mice and observed for a week for the tumor formation for the experimental studies.

The experimental animals were separated into four groups with six no's of albino mice in each group taken as the protocol for treatment studies. The first three groups of albino mice were injected with Dalton's lymphoma ascites and phosphate buffer saline intraperitoneally at 1 x 10^6 cells per mouse and the fourth group was left normal and treated as the control group (Table 1). The positive control mice group was injected with the chemical 5- Fluorouracil (20 mg per kg body weight). In the present investigation, the tumor-formed albino mice were treated with silver nanoparticles biosynthesized from *Colpomenia sinuosa* post 24 hrs of inoculation into the peritoneal cavity and the trials were conducted for 14 days post which the animals were sacrificed from each group and cardiac puncture blood withdrawing method was adopted to evaluate the haematological, biochemical and histopathological studies using COBASMIRA PLUS-S, Auto analyzer, Roche, Switzerland and Haematoxylin, and Eosin staining respectively.

Table 1: Treatment protocol of the experimental animals [Swiss albino mice]

Groups	Description	Experimental Protocol
I	Normal control	Habitual diet and sufficient water given
II	Tumor control	Habitual diet and sufficient water given
III	Positive control	Injected with 5-fluorouracil [20 mg per kg body]
IV	Treatment control	Administered with biosynthesized AgNps from *Colpomenia sinuosa* [50 mg/kg] orally [LD_{50} OECD Guidelines].

Estimation of tumor cell count, body heaviness, and Life expectancy

The live cell count was estimated by taking the fluid of 0.1 ml from the peritoneum of the albino mice after the experimental trials using an aseptic syringe and diluting with PBS and trypan blue of 0.8 ml and 0.1ml respectively (Mary et al., 1994). The count was done using a bright-line Haemocytometer as per the formula described below

$$\text{Cell counts} = \frac{\text{Cell dilution number}}{\text{Liquid film thickness} \times \text{area}}$$

Similarly, the albino mice were weighed from the 15th day onwards to determine the body heaviness and the standard body weight was calculated in percentage as described below (Santhosh et al., 2007).

$$\text{ILS\%} = \frac{\text{Treated group life span}}{\text{Control group life span}} - 1 \times 100$$

Estimation of haematological and biochemical factors

The haematological and biochemical factors were analyzed from the blood sample of the albino mice after the experimental studies. The factors such as RBC, WBC, haemoglobin, platelet, triglycerides, cholesterol, AST, ALT, and ALP were analyzed.

The cholesterol estimation from the experimental group of mice was done as per (Zlatkis et al., 1953) methodology. The procedure for cholesterol estimation involves the mixing of serum sample (0.1 ml), glacial acetic acid (6.0 ml), and colouring reagent (0.4 ml). The standard was maintained with cholesterol solution (0.01 ml of 2 mg /ml) along with water (0.01 ml) as blank. Finally, the solutions were kept at 37^0C and allowed to cool post which the optical density was determined and recorded at 540 nm using a Shimadzu-UV-visible spectrophotometer. Similarly, the triglycerides were determined by intravenous blood collection from the experimental group of mice. The blood from the experimental group of mice was centrifuged at 2000 rotations per minute for 15 minutes at 4°C using a cooling centrifugal machine (REMI-NEYA) and the triglycerides were evaluated using the serum. The standard curve was plotted using triglycerides assay with 50 µl of serum replacing triolein. The number of triglycerides between triolein concentrations in the range of 0.5 to 14.0 mM /A_{510} was inferred using the standard curve. AST (Aspartate aminotransferase) and ALT (Alanine aminotransferase) and ALP (alanine phosphatase) levels of the experimental group of albino mice were determined as per the method described by (Reitman and Frankel, 1957).

Histologic analysis of liver

The 10% neutral buffered formalin solution was used to prepare and fix the cross-sections of the cleaned liver. The fixed liver was then dehydrated and graded using ethanol and toluene, and finally impacted in paraffin. Haematoxylin and Eosin (H.E.) staining methods were adopted for sectioning the tissues of 3–5 µm thickness

whereas Periodic acid staining (PAS) staining was used for other sections examined for histologic analysis.

Statistical analysis

The mean ± SD triplicates of data were analyzed using MS Excel 2010. Mono-factorial variance analysis (ANOVA) was utilized to analyze the major difference among the groups. The Student-Newman- Keuls Method was utilized for all pairwise multiple comparisons. The statistically crucial difference was obtained at $p<0.05$ for all the accepted data.

Results

Tumor cell suppression activity - *in vivo*

Tumor cell suppression *in vivo* efficacy of biogenic AgNPs was investigated using Dalton's Lymphoma inoculated experimental group mice (50 mg per kg body weight). The tumor cells were transformed into the experimental mice by aspirating the DLA cells from the peritoneal cavity with buffered saline. The dilutions were done and the cell count of 1 x 10^6 ml was injected intraperitoneally into the experimental mice and observed for a week for the tumor formation before commencing treatment protocols. Factors such as life expectancy, body heaviness, and the count of tumor cells were analyzed during the treatment. The suppression of tumor cells by silver nanoparticles from Colpomenia sinuosa was evident based on the various studies conducted such as biochemical, haematological, and histopathological in the experimental animals.

Efficacy of AgNPs on life expectancy, body heaviness, tumor cell count

The experimental results showed an increase in 86%. of life expectancy of the animals (50mg/kg body weight) in the treated group with biogenic silver nanoparticles from *Colpomenia sinuosa* as compared to 48%, in Dalton's lymphoma group of albino mice. The positive control group (20 mg/kg body weight) injected with 5-Fluorouracil showed a 90% increase in the life expectancy of the animals. The results indicated potent tumor cell suppression activity. The active tumor cell suppression activity of biosynthesized silver nano-particles was also evident by a considerable increase in the weight of the animal, decrease in packed volume of the cell, and viable cell count (tumor) as compared to Dalton's lymphoma group of experimental mice (Table 2 and Table 3).

Table 2: Efficacy of biosynthesized silver nanoparticles from *Colpomenia sinuosa* on the life Expectancy, body heaviness, and tumor cell count of tumor-induced mice

Experimental Protocol	Animals in Nos	Life Expectancy [ILS%]	Body heaviness (mg/kg)	Tumor Cell Count
Normal control ($G_{1)}$	6	30 days	2.20 ± 0.55	Nil
Cancer control (G_2)	6	48	7.70 ± 0.92^{a**}	2.75 ± 0.40^{a**}
Positive control (G_3)	6	90	3.80 ± 0.60^{b**}	1.45 ± 0.35^{b**}
C. sinuosa control (G_4)	6	86	4.10 ± 0.80^{b**}	2.30 ± 0.46^{b**}

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

Efficacy of AgNPs on haematological factors

The experimental results treated with biogenic silver nanoparticles from *Colpomenia sinuosa* showed a significant increase in haematological factors (Hb content (9.83 ± 0.90 gm/dL), RBC (3.40 ± 0.60 Mill/cumm), platelets (2.35 ± 0.42 Lakhs/cumm)) and decrease in WBC (11.90 ± 1.70 cells/ml × 10^3) to normalcy as compared to that of Dalton's lymphoma group of experimental albino mice (50 mg/kg body weight) (Table 3). The standard 5-Fluorouracil injected in a group of experimental animals (20 mg/kg body weight) exhibited a high activity as compared to the treatment group with biogenic silver nanoparticles from seaweed *Colpomenia sinuosa*. The results revealed the action of biogenic silver nanoparticles in tumor cell suppression activity.

Efficacy of AgNPs on biochemical factors

The Dalton's lymphoma experimental animals showed a considerable increase in cholesterol (140.96 ± 4.60 mg/dL), aspartate aminotransferase (AST) (87.81 ± 2.82 U/L), alanine aminotransferase (ALT) (62.33 ± 2.82 U/L), triglycerides (TGL) (215.23 ± 4.72 mg/dL) and alkaline phosphatase (ALP) (240.33 ± 4.35 U/L) levels compared with a standard control group of albino mice (Table 4). On the contrary, the treatment group animals with biosynthesized silver nanoparticles from *Colpomenia sinuosa* showed normal levels of cholesterol (122.60 ± 3.88 mg/dL), aspartate aminotransferase (64.85 ± 2.35 U/L), alanine aminotransferase (47.54 ± 1.90 U/L), triglycerides (168.75 ± 2.86 mg/dL) and alkaline phosphatase (185.32 ± 2.60 U/L) (Table 4). The results indicate the biosynthesized silver nanoparticles from the marine brown seaweed *Colpomenia sinuosa* reversed the biochemical levels to normalcy and hence the efficacy was visible.

TABLE 3: Efficacy of silver nanoparticles biosynthesized from *Colpomenia sinuosa* on haematological factors

Experimental Protocol	White Blood Cells (Cells /mlx10^8)	Red Blood Cells (Mill/cumm)	Haemoglobin (Gm/dL)	% of cell volume (Packed)	Platelets (Lakhs/cumm)
Normal control ($G_{1)}$	10.90 ± 1.40	4.30 ± 0.95	12.35 ± 1.26	14.85 ± 1.42	3.35 ± 0.80
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**}
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**}
C. sinuosa control (G_4)	11.90 ± 1.70^{b**}	3.40 ± 0.60^{b**}	9.83 ± 0.90^{b**}	23.45 ± 2.35^{b**}	2.35 ± 0.42^{b**}

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

TABLE 4: Efficacy of biosynthesized silver nanoparticles from *Colpomenia sinuosa* on biochemical factors

Experimental Protocol	Cholesterol (mg/dl)	Triglycerides (mg /dl)	Aspartate aminotransferase (u/l)	Alanine aminotransferase (u/l)	Alkaline phosphatase (u/l)
Normal control (G_1)	102.30 ± 3.55	132.80 ± 2.45	40.60 ± 1.35	34.40 ±1.40	125.35 ± 2.40
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**}
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**}
C. sinuosa control (G_4)	122.60 ± 3.88^{b**}	168.75 ± 2.86^{b**}	64.85 ± 2.35^{b**}	47.54 ± 1.90^{b**}	185.32 ± 2.60^{b**}

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

Histologic analysis of the liver

The necrosis in the hepatic region by Dalton's Lymphoma was evident from the histologic analysis of the liver. The portal tract and central vein showed hepatocytes of malignant nature (Figure 1). Dalton's Lymphoma Ascites (Tumor –inducing) were injected with the chemical 5-Fluorouracil (20 mg per kg body weight) had shown a reduction in malignancy to some extent. However, liver of tumor-induced animals (50 mg/kg body weight) treated with AgNps biosynthesized from *Colpomenia sinuosa* exhibited a high extent of malignancy and also fewer hepatocytes, as compared to the 5-fluorouracil treatment.

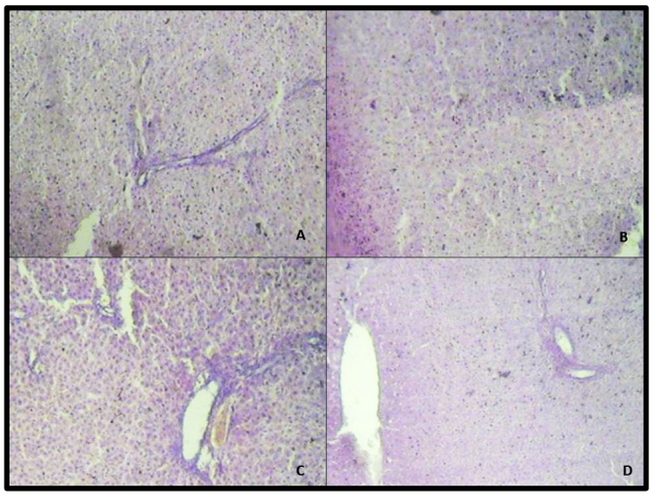

Fig. 1. C.S of liver stained with Haematoxylin and eosin (Magnification 400 ×)

(A) Normal control [Liver parenchyma with hepatocytes and central vein & portal tract appear habitual].

(B) Cancer control [Focal area of necrosis of hepatocytes observed in liver parenchyma].

(C) Positive control (5- Fluorouracil) [Liver parenchyma with hepatocytes and central vein & portal tract appear habitual].

(D)Treatment control (AgNps from *Colpomenia sinuosa* at 50 mg per kg) [Liver parenchyma with hepatocytes and central vein & portal tract appear habitual].

The current analysis confirmed significant progress of the anti-tumor cell suppression in the liver tissue samples of the mice by silver nanoparticles biosynthesized from Colpomenia sinuosa. Furthermore, the treatment group with biosynthesized silver nanoparticles showed an increase in haematological factors, the biochemical factors were retained to the normal levels and decreased white blood cells were evident as compared to that of the standard group with 5-fluorouracil in experimental mice.

Discussions

The present study investigates the tumor cell suppression activity in vivo with Dalton's Lymphoma experimental mice (50 mg/kg body weight) by the silver nanoparticles biosynthesized from the macroalgae Colpomenia sinuosa. The criteria for the anticancer activity of the biosynthesized silver nanoparticles were measured and analyzed by factors like life expectancy of the animal, body heaviness, haematological factors, biochemical factors, and histologic studies (Clarkson and Burchenal, 1965).

Efficacy of AgNPs on life expectancy, body heaviness, tumor cell count

The curbing of tumor viable cell count and the retention of haematological factors, and biochemical factors to normalcy were observed in the experimental group of animals treated with biosynthesized AgNps from macroalgae *Colpomenia sinuosa* in comparison with DLA-induced experimental mice and the chemical 5-Fluorouracil (20 mg/kg) was marked positive control group. The significant rise in cell count in the mice treated with biosynthesized silver nanoparticles from the brown seaweed and reduction in tumor cell count shows substantiated evidence of defence against Dalton's lymphoma ascites as reported by (Muthu et al., 2010; Bhuvaneswari and Murugesan, 2012; Sangiliyandi et al., 2015).

Efficacy of AgNPs on haematological analysis

The significant decrease in RBCs, platelets, and haemoglobin with increased WBCs in Dalton's lymphoma experimental group of animals as compared to the standard control group of mice was evident in the haematological analysis. Retention of the normalcy of RBCs, haemoglobin, and platelets with AgNPs treatment from *Colpomenia sinuosa* portrayed the hematogenic system defence mechanism (Hogland, 1982). The tumor-induced mice using a DLA cell line showed a significant increase in the ascitic fluid (Sangiliyandi et al., 2015; Sathiyanarayanan et al., 2006), whereas the treatment group of experimental mice with AgNps from Colpomenia sinuosa showed a decrease in the ascitic fluid meant for the enhancement of tumor cells. Finally, the

enhancement of the life span of the treatment group experimental mice as compared to that of the positive control group mice injected with 5-Fluorouracil (Muthu et al., 2010).

Efficacy of AgNPs on biochemical factors

The increase in biochemical factors was observed with Dalton's lymphoma group of experimental mice whereas, the mice treated with biosynthesized silver nanoparticles from experimental macroalgae *Colpomenia sinuosa* retained the levels of serum enzymes and lipoproteins as compared with mice injected with 5-Fluorouracil and control group mice. (Bhuvaneswari and Murugesan, 2012; Rutberg et al., 2008).

Efficacy of AgNPs on the life expectancy of experimental mice

Biosynthesized silver nanoparticles from the brown seaweed *Colpomenia sinuosa* exhibited remarkable anti-tumor activity free from endotoxins. The treatment control group of mice with biogenic silver nanoparticles from *Colpomenia sinuosa* increased the life expectancy of the experimental animals to 86 days as compared to mice with tumors which survived only 30 days since the inception of tumor formation (Muthu et al., 2010).

Histologic analysis of Liver

The liver parenchyma structure was studied between the normal mice and the DLA cell line-induced tumor mice. The experimental tumor-induced mice revealed necrosis in the hepatocytes of the central vein and portal tract whereas the control group and mice injected with 5-Fluorouracil appeared to be normal with no significant necrosis in hepatocytes. The absence of necrosis and the central vein, and portal tract were retained to normal in the treatment group of mice with AgNPs from *Colpomenia sinuosa* 50 mg per kg body weight.

The peritoneal fluid of Daltons' lymphoma cells showed that the tumor-induced experimental animals treated with the biogenic silver nanoparticles reduced the number of malignant tumor cells as compared to that of the control group of albino mice, confirming the efficacy of the biogenic silver nanoparticles in its cytotoxic effects on tumor cells, without influencing the somatic cells (Muthu et al., 2010).

Conclusions

The anti-tumor efficacy was confirmed against tumor cells inducted with Dalton's lymphoma in the treatment group with biosynthesized silver nanoparticles from marine brown seaweed Colpomenia sinuosa. The eco-friendly and green synthesis methodology of biosynthesized silver nanoparticles from Colpomenia sinuosa reversed the haematological, and biochemical factors to a near-normal range as compared to the tumor-induced experimental group of mice using DLA cell lines which confirmed the efficacy of the AgNPs efficacy in the *in vivo* anti-tumor studies. The efficacy status of biosynthesized silver nanoparticles from the experimental seaweed Colpomenia sinuosa forms a base for further research analysis in cancer therapeutics and drug delivery transmissions.

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Declaration of interest: The authors report no conflicts of interest.

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