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# Pharmaceutical Efficacy of Artemisinin from *Artemisia Pallens* with Reference to Antitumor Potential

G.Renuga<sup>1</sup>, P. Latha Brindha<sup>2</sup>, Dr. G. Renuga M.Sc. Ph. D<sup>3</sup>

<sup>1</sup>Principal & Research Co-ordinator, Dept of Biochemistry, Sri Adi Chunchanagiri women's College,

<sup>2</sup>Research scholar Dept of Biochemistry, Sri Adi Chunchanagiri women's College, Cumbum, Theni (Dt), Affiliated to Mother Teresa Women's University, Kodaikanal, Tamil Nadu, India.

<sup>3</sup>Principal & Research Co-ordinator, Sri Adi Chunchanagiri women's College, Cumbum-625516, Theni (Dt), Tamil Nadu, India.

**Abstract:** *This rapidly developing field of nanoscience has raised the possibility of using therapeutic nanoparticles in the diagnosis and treatment of human cancers. The research demonstrates the efficiency of artemisinin is a sesquiterpene lactone isolated from the leaves of Artemisia pallens by using classical biochemical techniques and the purified molecules analyzed by HPLC. The artemisinin concentration has been reported to be higher at the top of the leaves Artemisia pallens plants, shown to be effective against Dalton's lymphoma ascites (DLA) cancer. Carboplatin, a platinum-containing anticancer drug treatment caused a significant increase in the life span of ascites Dalton's lymphoma tumor bearing mice. However, as compared to carboplatin, artemisinin and combination treatment with artemisinin plus carboplatin resulted in better therapeutic efficacy against Dalton's lymphoma tumor. Artemisinin loaded silver nanoparticles were administrated to ascites Dalton's lymphoma tumor induced mice 100µg/kg/day IP injection for 15 days at two days intervals, the antitumor efficiency of AgNPs significantly increased the survival time in the tumor mouse model in comparison with tumor controls also decreased the volume of ascitic fluid in tumor-bearing mice by 75%, thereby returning body weight closer to normal animals. Artemisinin may be especially effective in treating drug resistant cancers. These findings confirm the antitumor properties of AgNPs, and suggest that bioactive substance Artemisinin may be a cost-effective alternative in the treatment of cancer.*

**Keywords:** *Artemisia pallens, Artemisinin, antitumor, silver nanoparticles, Dalton's lymphoma ascites.*

## I. INTRODUCTION

Medicinal plants have been used by man for centuries. They are still important especially in many developing communities where traditional remedies are commonly used in the treatment of numerous ailments. A medicinal plant is any plant which in one or more of its parts contains potent chemical compounds that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. Plants have been used as drugs by humans since thousands of years ago. Today, all the world's cultures have an extensive knowledge of herbal medicine. Traditional medicine is based on beliefs and practices that existed before the development of so-called "modern medicine" or "scientific drug therapy". Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times. *Artemisia L.* is a genus of small herbs and shrubs found in northern temperate regions. It belongs to the important family Compositae (Asteraceae), one of the most numerous plant groupings, which comprises about 1,000 genera and over 20,000 species [1].

Many compounds used in today's medicine have a complex structure and synthesizing these bioactive compounds chemically at a low price is not easy [2]. The increasing awareness about side effects of drugs had made the western pharmaceutical industries to turn towards the plant based Indian and Chinese medicine [3].

The genus *Artemisia* is known to contain many bioactive compounds, artemisinin exerts not only anti malarial activity but also profound cytotoxicity against tumor cells [4]. *Artemisia* species are popular plants which are used for the treatment of diseases such as hepatitis, cancer, inflammation and infections by fungi, bacteria and viruses [5]. *A. Pallens* is a small and aromatic herbaceous plant which is native to the southern part of India, Its leaves and flowers are highly valued in the making of floral decorations and oils. Leaves are very small, bluish green with yellow flowers and inconspicuous. It is utilized in traditional Ayurvedic medicinal formulations. Oral administration of the methanol extract of the aerial parts of *Artemisia pallens* Wall (Used in Indian folk medicine for the treatment of diabetes mellitus) led to significant blood glucose lowering effect in glucose-fed hyperglycemic and alloxan-induced diabetic rats.

Nanoscale particles and molecules are a potential alternative for treatment of disease because they have unique biologic effects based on their structure and size, which differ from traditional small-molecule drugs. Silver nanoparticles (AgNPs) are among the emerging nanoproducts that have gained increasing interest in the field of nanomedicine due to their unique properties and obvious therapeutic potential in treating a variety of diseases, including retinal neovascularisation [6] and acquired Immunodeficiency syndrome due to human immunodeficiency virus (HIV), AgNPs are also for their antimicrobial potential against several other viruses, including hepatitis B, respiratory syncytial virus, herpes simplex virus type and monkey pox virus. Results of microbiologic studies indicate that the interaction of silver ions with molecules of an extracellular lipoprotein matrix increases the permeability of the plasma membrane of microbial cells and eventually causes their death [7].

This study tends to investigate the chemical constituents that contribute to the medicinal potentials of *Artemisia pallens*, in an attempt to provide a scientific backing to the claim of their wide application in traditional medicine. Interest is booming in biomedical applications for use outside the body, such as diagnostic sensors techniques, which are suitable for analyzing blood and other samples, and for inclusion in analytical instruments for R&D on new drugs. For inside the body, many companies are developing nanotechnology applications for anticancer drugs. Single high dose or repeated doses of carboplatin chemotherapy have been shown to produce ototoxicity as a side effect in cancer patients [8]. Carboplatin induced ototoxicity has also been demonstrated in experimental animals such as guinea pigs and chinchillas.

The active compound might be inserted in a nanotube or bonded to a particle's surface. Other types of nanopowders or biomolecules are also useful and are closer to the marketplace. Present study was designed in order to evaluate the effects of synthesized AgNPs on Dalton's lymphoma ascites (DLA) tumorigenesis in animal model.

## II. MATERIALS AND METHODS

### A. Plant Material

*Artemisia pallens* were collected from in and around Thavaram, belonging to the district Theni of Tamil Nadu state. The plant was identified and authenticated by Medicinal Plants Survey and Collection Unit, Ootacamund, Tamil Nadu, India.

### B. Plant Extraction

The samples were cleaned and put in an oven at a temperature of 60°C for 6 hours. After drying, the leaf samples were ground into a fine powder with electric blender prior to use for further analysis. The dried powdered materials were stored in air-tight bottles until required for analysis.

Soxhlet method of extraction 10gms of powdered plant material was exhaustively extracted with 200ml ethanol by continuous hot percolation method. After three hours the extract was concentrated to 3/4th of volume to get a concentrate. All extracts were preserved in refrigerated condition till further use.

### C. Preparation of Artemisinin

Accurately transfer 10 mg of artemisinin working standard into a 10 ml volumetric flask and dissolve in methanol. Make the final volume with methanol to give 1.0mg/ml solution of artemisinin. Label and store the solution in a refrigerator below 2-8°C. Artemisinin extracts 10 mg was accurately transferred into a 10 ml volumetric flask and dissolve in methanol. Final volume was made up with methanol to give 1.0mg/ml solution of artemisinin.

### D. Silver Nanoparticles Synthesis (agnps)

For the synthesis of AgNPs, 5 mL aqueous extract of *Artemisia pallens* leaves was taken and diluted to 25 ml with double distilled water. This diluted extract then added drop wise in 25 mL of silver nitrate solution (1 mM) at room temperature with constant stirring for 3 hours. The resulting brown solution was centrifuged at 15,000 rpm for 20 minutes and then washed with distilled water (3x10 mL). The solid obtained was dried under vacuum and used for further study.

### E. Characterization of Silver Nanoparticles

Characterization of the synthesized and purified AgNPs was used for drug (artemisinin) loaded and the resulted particles/ samples were subjected for transmission electron microscopy (TEM) analysis. TEM measurements were performed on a JEOL model 1200EX instrument operated at an accelerating voltage of 120 kV.



#### F. Experimental Animal Maintenance and Tumour Transplantation

In vivo studies were conducted on female Swiss albino mice aged 5–6 weeks, weighing cages (five mice per cage) at an ambient temperature of  $25 \pm 2^\circ\text{C}$  with a 12-hour light and 12-hour dark cycle. The mice were fed with commercially obtained rodent chow and water *ad libitum*.

The animals were allowed to acclimatize to the laboratory environment and were then randomly subjected to the experiment. All the experiments were carried out as per the guidelines of the institutional animal ethics committee and had prior approval from the same committee. Ascites Dalton's lymphoma tumor was maintained in vivo by intraperitoneal (ip) transplantation of  $1 \times 10^6$  tumor cells per animal (0.25 volumes, in phosphate - buffered saline, PBS). PBS was prepared by adding 0.15 M NaCl to 0.01 M sodium phosphate buffer, pH 7.4. The DLA cell lines were maintained in mice models by aseptic serial transplantation in Swiss albino mice from tumor-bearing mice after the 10<sup>th</sup> day of ascites induction. Dalton's Lymphoma ascites was obtained from Amala Cancer Institute, Trissshur, Kerala Dt.

#### G. Experimental Scheduled Given Below

The mice were divided into four groups, with six animals in each group: Group 1: blank non tumor mice (nontumor, untreated); Group 2: tumor control mice (tumor induced, untreated);

Group 3: animals received a single dose of drug carboplatin was administrated to tumor bearing mice on the 8<sup>th</sup> day of tumor growth (Preparation of doses: Carboplatin Dose (10 mg /ml was dissolved in 5% dextrose in water and use immediately). Group 4: animals received a combination dose of drug carboplatin and artemisinin loaded nanoparticle (100 $\mu\text{g}/\text{kg}/\text{day}$ ). Group 5: animals received a combination dose of artemisinin loaded nanoparticle (100 $\mu\text{g}/\text{kg}/\text{day}$ ) was administered to tumor bearing mice 10  $\mu\text{l}$  in aqueous solution via intraperitoneal (IP) injection for 15 days at two days intervals; Treatment was started 7<sup>th</sup> day after the tumor inoculation and continued for 16 days, body weight of animals were noted daily in all groups during treatment period.

On the 17th day of mice in all the groups mice were subjected for experimental studies, fasting blood samples were collected from 3 animals from each batch and the rest of the animal were kept to check the survival time of DLA bearing mice, subsequently animals were sacrificed by anesthesia and organs tissue were isolated (blood, ascites tumor) frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$  until biochemical analysis could be completed.

If any death, of the animals in different groups were recorded daily and the survival pattern of the animals were determined for different group. The parameters such as survival time, packed cell volume, body weight, haematological parameters like RBC count, WBC count, were studied during the period of experiment (data not given).

#### H. Measure of Viability

This procedure can be performed along with the cell counting procedure but cell density may require adjustment in order to obtain approximately  $10^6$  cells/ ml. Mix 1 drop of trypan blue with one drop of the cell suspension and allow 1 - 2 minutes for absorption. Prepare haemocytometer and load chambers as described in "Cell Quantitation". Count both the total number of cells and the number of stained (dark) cells.

Calculation: percent viability=  $(\text{Total cell counted} - \text{Stained cells}) \times 100 / \text{Total cells counted}$ . The ascites tumor cells are obtained by aspirate with Phosphate buffer saline in the peritoneal Cavity of DLA bearing mice. The cells were then mixed with 0.4% Trypan blue in the ratio of 1:1 and the cells were counted using the Haemocytometer. (Live cells do not take stain whereas the dead cells get stained).

#### I. Effect of silver Nanoparticles on Ascitic Tumor

The minimum inhibitory concentration (IC<sub>50</sub>) determined by MTT assay was used for the in vivo experiments. The AgNP delivered to the mice was carried out using the IC<sub>50</sub> obtained. Tumor mice in Group 3 were treated with AgNPs at a concentration of 500 nM for a period of 15 days, and their ability to reduce tumor volume and the number of cells was compared with Group 2 tumor control mice.

### III. RESULTS AND DISCUSSION

Artemisia species invariably found as small fragrant shrubs or herbs and most yield essential oils. Phytochemical analysis of plant extracts for active components showed the presences of Artemisinin. The ethanol extract of Artemisia pallens showed the presence of Artemisinin and whereas in the aqueous extract, tannins and phenols were also found.

Table:1 Estimation Of Minerals In The Leaves Of *Artemisia Pollens* By Aas

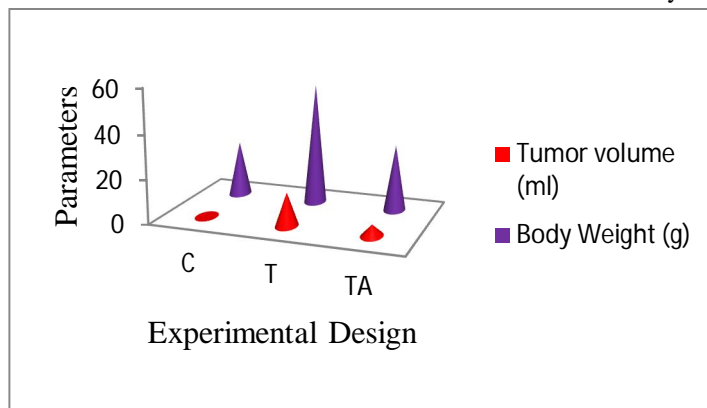
Major mineral elements to %		Trace mineral elements to %	
K	0.764	Ni	9.54
Na	0.252	Zn	37.18
P	0.462	Cu	8.93
Ca	1.750	Cd	0.32
Mg	0.867	Pb	9.04
N	1.980		

Macro and microelements present in *Artemisia pollens* plant leaves were analyzed by Atomic Absorbance Spectrophotometer (table 1) revealed essential nutrients were found in high level particularly K, N, P and also trace elements also showed its presence which supported healthy plants has been selected for extraction of Artemisinin preparation, characterization of these bioactive material and experimental confirmation for its therapeutic potential against cancer.

The results clearly show that the study *Artemisia pollens* plants are rich sources of mineral elements that are highly essential for normal body growth and development. The results obtained for sodium and potassium are within the range for the management of hypertension. Phosphorous is necessary for energy producing reactions in the cell as well as the structural component of the skeleton [9].

The major mineral elements have calcium as the most predominant while zinc tops the list of the trace elements. Calcium and phosphorus are needed for blood coagulation. Deficiency of magnesium causes muscular cramps, rigidity and spasm. The presence in these plants of calcium, phosphorus and magnesium that form the skeleton makes them useful for those suffering from bone demineralization and justifies the use of these plants to cure rheumatism [10]. Copper is a micronutrient that acts as a biocatalyst which is required for body pigmentation and in addition to iron, maintains a healthy central nervous system and prevents anemia [11]. Zinc is the most important metal for normal body growth and development in humans. It provides a protective mechanism against virus and its deficiency leads to the weakening of the immune system, diarrhea and mental depression [12]. Selenium is an antioxidant that stimulates the immune system and contributes to the formation of antibodies against infectious agents. Nickel activates some enzyme systems in the body [13]. The presence of these minerals reinforces the medicinal potentials of these study plants.

Figure:1 Effect of Artemisinin on ascitic tumor volume and body weight



Legend: X axis represented treatment groups in which C- indicates control (nontumor mice); T-indicates tumor mice (tumor induced, untreated); TS-indicates tumor-induced mice treated with Artemisinin at a concentration of 100 µl IP injections for 15 days at two days intervals. Each value represents the mean n=6.

Animals were injected with DLA tumor cells and treated with different combinations of drug carboplatin maintained the experimental setup then subjected for all experimental studies such as host survival and bodyweight differences, haematological parameters. The efficiency of the drug administration was studied and the results shown that the Artemisinin in nanoparticle treated group was found to be more effective and in exhibiting the antitumor activity against DLA cells. The body weight differences after

treatment was calculated by observing the weight gain on 17<sup>th</sup> day after cancer induction. Whereas, the tumor inhibition was calculated by estimating the packed cell volume of DLA, treated groups showed better tumor inhibition [figure1]. Since artemisinin is a novel molecule by its chemical structure and mode of action, it is thus a new lead compound, which can be exploited for further drug development.

Figure 2: Scanning electron microscope

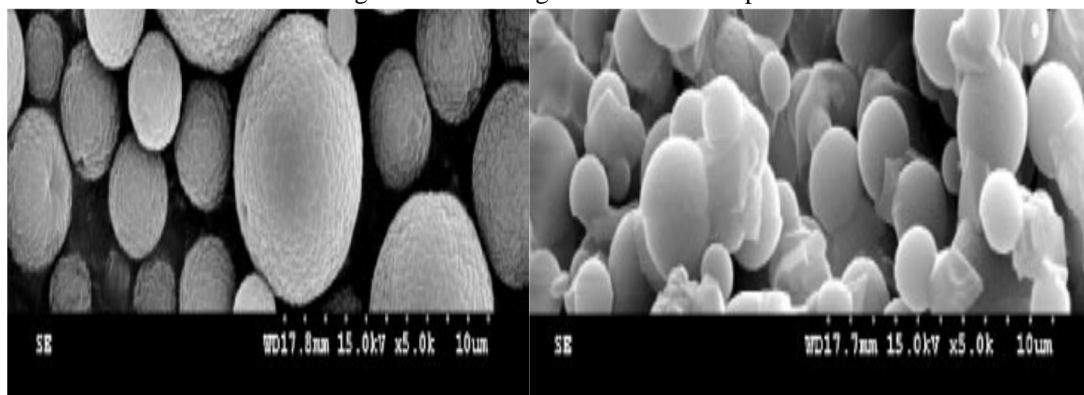
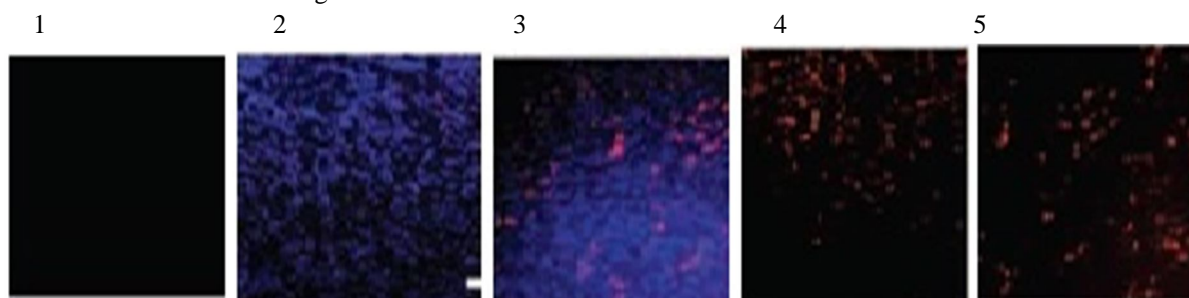


image of nanoparticle

Artemisinin loaded nanoparticle

Treatment with AgNPs for a period of 15 days in DLA tumorbearing mice led to a significant reduction in tumor volume in comparison with tumor controls. Tumor volume in control mice was significantly reduced in the group treated with AgNPs at a concentration of 100 nM for 15 days. Body weight, measured throughout the period of the experiment, was reduced in the treated tumor bearing group when compared with the tumor control group. The group of mice treated with AgNPs alone did not exhibit any abnormalities or reduction in body weight, thereby serving the tumor mice to regain its original weight.

Figure 3: Effect of Artemisinin on ascitic tumor treatment



Legend: Tumor from mice after different treatments indicated. 1-control mice; 2- tumors induced untreated mice; 3- tumor induced carboplatin-treated mice; 4- tumor induced combination of carboplatin and Artemisinin treated mice 5- tumor induced artemisinin nanoparticle treated mice at final stage were undergoing apoptosis. Samples used for experiments were taken from tumor-bearing mice 12 d after initiation of treatment.

To investigate the efficiency of plant based component Artemisinin at a concentration of 100 µl IP injections for 15 days at two days intervals has administrated to tumour induced mice as mention in the methods, tumours were measured and staining data clearly revealed apoptotic cells Figure3. The treatment effect is confirmed by tumor staining that reveals significant apoptotic cells and few proliferation active cells in the treated tumor. Thus, staining results clearly confirmed the treatment efficacy of s artemisinin by inhibiting proliferation and inducing apoptosis of tumor cells. Artemisinin evidenced by its ability of slowing down tumor growth at administrated purified compound of artemisinin in the form of naonparticle delivery even at low dose affords markedly improved treatment efficacy in animal trial. .

Ascitic tumors from mice after different treatments indicated in figure 3 where as tumors from untreated mice DAPI co-staining images of tumor cells from mice after various treatments and few proliferation active cells were observed in the tumor of mice that received treatment of artemisinin loaded particle. Tumors used in this study were taken from ascities Dalton lymphoma-bearing

mice 12 d after initiation of treatment. Staining method has been widely used as a cell proliferation marker to stain proliferation active cells in the G1, G2, and S phases of the cell cycle. Results found that cell proliferation in combination of carboplatin drug and artemisinin treated tumor was as inactive better than carboplatin treated tumor. In the artemisinin loaded particle treated tumor group animals showed 75% of apoptosis cells were noted compared with the number in the untreated tumor ( $P < 0.0001$  versus treated carboplatin alone, results clearly confirmed the treatment efficacy of artemisinin by inhibiting proliferation and inducing apoptosis of tumor cells.

Bioactive material delivered to cancer cells is directly responsible for tumor suppression at a low dose of treatment. Artemisinin are a group of naturally occurring plant glycosides, characterized by their strong foam-forming properties in aqueous solution. The presence of Artemisinin has been reported in more than 100 families of plants out of which at least 150 kinds of natural have been found to possess significant anticancer properties. Chromatography techniques are the most useful and popular tools used for identification of unknown active compounds. The leaf extracts were screened for various phytochemicals

artemisinin ,saponins, glycosides, tannins, flavonoids, phenol, alkaloids and volatile oils) by standard qualitative tests [14]. Recent developments related to the biosynthesis of this important drug, the relevant enzymes and genes and their regulation in the production of artemisinin and other closely related terpenoids in this species, both in vitro and in whole plants.

The plants are rich in alkaloids and saponins which are known to have antimicrobial activity as well as other physiological activity. Flavonoids are known for their vast role in biological activities which include protection against allergies, viruses and tumors, ulcers, inflammation and platelets aggregation. These flavonoids are potent water-soluble super antioxidants and free radical scavengers which provide protection against oxidative cell damage [15]. They also provide antioxidative properties against some certain forms of cancer and protect against all stages of carcinogenesis. AgNPs and ions have been shown to possess intrinsic cytotoxic activity and exhibit an enhanced antimicrobial effect when applied on cells nanoparticles exhibited antitumor properties in transplanted DLA tumor models when administered by ip injection in the form of aqueous dispersions. Drug targeting is an approach to improve the therapeutic index of drugs by manipulating the disposition of the drug in the body. Ascitic fluid plays a crucial role in DLA and is a collection of pleomorphic cells with hyper chromatic nuclei that are clumps of malignant cells. Researchers are focused to develop novel therapeutic and diagnostic modalities for human use.

#### IV. CONCLUSION

Products of nanotechnology are expected to revolutionize modern medicine, as evidenced by recent scientific advances and global initiatives to support nanotechnology and nanomedicine research. The field of drug delivery is a direct beneficiary of these advancements. The major criteria to be taken into consideration for any potential anticancer drug are its efficacy in prolongation of lifespan and decrease of tumor volume and viable tumor cell count. In the present study, IP inoculation of DLA cells in mice produced a marked increase in the cancer cell count which indicated tumor progression in the animals, whereas a substantial decrease in cancer cell numbers in the treated tumor mice. Artemisinin AgNPs had a significant inhibitory effect on tumor cell proliferation and survival.

Due to their versatility in targeting tissues, accessing deep molecular targets, and controlling drug release, nanoparticles are helping address challenges to face the delivery of modern, as well as conventional drugs. Since the majority of drug products employ solids, nanoparticles are expected to have a broad impact on drug product development. In pharmaceuticals, 90% of all medicines, the active ingredient are in the form of solid particles. With the development in nanotechnology, it is now possible to produce drug nanoparticles that can be utilized in a variety of innovative ways. New drug delivery pathways can now be used that can increase drug efficacy and reduce side effects. Experimental results of the present work on isolation, characterize and elucidate the bioactive compounds for industrial drug bases. Artemisinin are potential sources of useful drugs based on successful results alternate strategies which are economically viable for the commercial production of artemisinin which appear promising antitumor drug.

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