

In-Vitro Anticancer Activity of Silver Nanoparticle in Terpenoid for *Andrographis Paniculata* (Ag-Nps TAP) by MTT Assay Method against HeLa & Hep-2

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Abstract: *The huge number of most important active compounds which includes Flavonoids, Flavones, Flavones glycosides, Chalcones, Chalcones glycosides, Xanthones, Diterpenoids, , Dimeric diterpenes and Sterols have been presented in the various parts of *Andrographis Paniculata* (Kalmegh) of family Acanthaceae. Though in traditional siddha and ayurvedic systems of medicine and tribal medicine, in India and some other countries, multiple clinical applications like anti-inflammatory, antiproliferatory, antihepatic, antithrombogenic, antsnake venom, antipyretic activities, has been indicated for this plant. The compounds that are present in groups in plants produce adverse side effects like gastric upset, headache, bitter taste, and fatigue. But a very few of them isolated the compounds and tested experimently for the above clinical activity. This gave us an impetus to isolate not only that but also produce silver nanoparticles in terpenoid (Ag-NPsTAP) for A.Paniculata and tested for anticancer activity. The anticancer activity of TAP Ag-NPs have been examined over human pathogens such as **HeLa**, **Hep-2** and were examined in different concentrations by MTT assay method. The TAP Ag-NPs showed a maximum activity against **HeLa** (Human cervical cancer cells) and **Hep-2** (Human liver cancer cells) and it was detected to be **59.01%** and **48.79%** at 250µg/ml respectively. The synthesized Ag-NPs can be used for various applications due to its eco-friendliness, non-toxic and compact ability for pharmaceutical and other applications.*

Keywords: TAP, silver nitrate, silver nano particle, Ag-NPsTAP, anti cancer, MTT assay, **HeLa**, **Hep-2**

1. INTRODUCTION

In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. More than 13,000 plants have been studied during the last five year period [1, 2]. Plants have been used as remedies and botanical literature has described the usage of plant extracts. The World Health Organization (WHO) estimates that 80 percent of the populations of some Asian and African countries presently use herbal medicine for some aspect of primary health care (Fabricant and Farmsworth., 2001) [3].

Cancer [4, 5] is the third leading cause of death worldwide, preceded by cardiovascular and infectious diseases. It is a generic term for a group of more than 100 diseases that can affect any part of the body.

Plants have been sources of the well known anticancer drugs such as camptothecin, podophyllotoxin and paclitaxel [6, 7]. The potential of natural products as anticancer agents was

recognized for the first time in the 1950s by the U.S. National Cancer Institute (NCI), and after that many investigations have been performed to the discovery of new natural anticancer agents. Different methods are used for screening of anticancer agents. One of the techniques is MTT [3-(4, 5-dimethylthiazol-2yl)-2, 4-diphenyltetrazolium bromide] assay which is a simple and reliable method for preliminary evaluation of anti-cancer agents [8].

Nanoparticles are being considered as fundamental building blocks in nanotechnology. The most important and distinct property of nanoparticles is that they exhibit larger surface area to volume ratio. Metal nanoparticles have tremendous applications in science and technology. Synthesis of metal nanoparticles and their characterisation has been an emerging field of nano technology since the past few decades because of their unique properties and potential application in the fields of Physics, Chemistry, biology and medicine [9]. Widely, nanoparticles are synthesised by different routes. However, the synthesis of nanoparticles by chemical methods is not environment friendly. Therefore, the synthesis of nanoparticles by biological route (using microorganisms, enzymes and plant extract) is suggested alternative to non eco friendly methods [10]. The recent reports include the synthesis of nanoparticles using medicinal plants (Mukunthan KS et al., 2011. Prasad TNVKV et. al., 2011) [11]. This stands as a great application in the field of nano medicine. Medicinal property of the extract and nano silver could play vital role in treatment of many diseases (Akhil Gupta et al., 2011; Shreesh Kumar Ojha and Dharamvir Singh Arya 2011). The noble metals (Ag, Au, Pb, Pt and Hg) are widely used for the synthesis of nanoparticles [12]. Among the noble metals, silver is the metal of choice because it is used as a health additive in traditional medicine [13] and shows a strong toxicity over microorganisms. [14,15, 16, 17].

The bioreduction of Ag⁺ to Ag-NPs involves plant extracts and microorganisms [18, 19]. It has been shown that variety of plant extracts served as green reactants in Ag-NPs synthesis [20, 21, 22,23]. A recent study has demonstrated that the synthesised Ag-NPs using leaf extract of *A. paniculata* displayed good antiplasmodial activity. However, no reports demonstrate in individual components for leaf extract of *A. paniculata* derived silver nanoparticles and its effects on some characterisation.

Andrographis paniculata is an herbaceous plant in the Acanthaceae family, native to India and Sri Lanka. It is commonly known as “*king of Bitters*”. It is widely cultivated in Southern and southern Asia, where it is used to treat infections and some diseases, often being used before antibiotics were created. Mostly the leaves and roots were used for medicinal purposes. **Terpenoids present in all parts of the plant, maximally in the leaves (>2%) was reported by Kanokwan Jarukamjorn and Nonuo Nemoto et al-2008.** Therefore, the study assumed that preparation of TAP Ag-NPs using *A. paniculata* would be useful to develop new anticancer drugs with increased efficiency. The study of synthesised TAP Ag-NPs using aqueous extract of *A. paniculata* and was confirmed by colour transformation and UV-visible spectrophotometry. The size of nanoparticles were observed by particle size, Zeta potential, SEM and the stability of nanoparticles was studied by FTIR. The synthesised TAP Ag-NPs over human pathogens such as **HeLa, Hep-2** were studied in the anticancer activity.

2. MATERIALS AND METHODS

2.1. Collection of Plant & Chemical Materials

The plant *Andrographis paniculata* was collected from the campus of A.V.C. Arts & Science College, Mayiladuthurai. Silver nitrate and other chemicals were purchased from (supreme chemicals) Ponmani & Co, Trichy. The microbial cultures were obtained from Microbial Culture Maintaining Laboratory, Department of Medical Microbiology, Rajah Muthaiah Medical College, Annamalai University, Tamil Nadu, India.

2.2. Preparation of the Extract

Andrographis paniculata leaves were collected and washed with tap water then rinsed with distilled water, dried, cut into fine pieces and were crushed into fine powder and stored at 37° C.

2.3. Preparation of Solvent Extract

10 grams of sample powder was placed in 100ml of chloroform and kept at room temperature for 7 days. The extract was filtered through a sterile funnel containing sterile Whatmann filter paper No.1 and filtered. It contains preservatives and stored in a brown bottle at 4° C.

2.4. Synthesis of Silver Nanoparticles (AG-NPS)

0.1N aqueous solution of Silver nitrate (AgNO₃) was prepared and used for the synthesis of silver nanoparticle. 10ml of isolated bioactive compound terpenoid was added into 90ml of aqueous solution of 0.1N Silver nitrate for reduction into Ag⁺ ions and kept at room temperature for 5 hours. After five hours of incubation, the change of green colour to brown colour indicates the synthesis of silver nanoparticle. The reduction of pure Ag⁺ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 hours after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer.

2.5. Anticancer Activity

2.5.1. In Vitro Anti-Cancer Activity

The anticancer activities of the Ag-NPs against HeLa, Hep2, cell lines by using MTT assay was carried out (Xie *et al.*, 2013) [18]. MTT assay method is widely used method for the detection of cell survival and growth. It is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. HeLa and Hep2 cells were seeded at a density of 4×10^4 cells/ml in a volume of 0.1 ml in 96-well plates, respectively. After 24 hrs, the silver nanoparticle (50–250 mg/ml) which was dissolved in a medium was added to each well and incubated for 48 hrs at 37°C in a CO₂ incubator. After the incubation, MTT solution (100 µl/well, 1 mg/ml) was added to each well and incubated again for 4 hrs. The culture media were then removed and 100 µl of DMSO was added to each well for 1 hr. Absorbance at 570 nm was detected by microplate ELISA reader (Spectra MAX 190, Molecular Devices Corporation, USA). The inhibition ratio of the cancer cells proliferation was determined as follows

Calculation

$$\text{Inhibition rate (\%)} = (1 - \text{Abs}_{\text{sample}} / \text{Abs}_{\text{control}}) \times 100$$

2.5.2. Cytotoxicity of Ag-NPs

The silver nanoparticle from TAP was initially evaluated for their effects on cell viability through cytotoxic test. Cytotoxic effect of silver nanoparticles was observed the IC₅₀ value was calculated as 300 µg/ml.

3. RESULTS AND DISCUSSION

3.1. Bio Synthesis of Silver Nano Particles

Figure 1 shows the colour intensity of aqueous extract of TAP incubated with silver nitrate solution in the beginning (a) and after 24 hrs (b) of reaction. Figure (1b) revealed the bio reduction of Ag⁺ ions to Ag nano particles by ingredients of TAP. The synthesised silver nano particles maximum absorption range was measured using UV-Visible spectrophotometry. The TAP silver nano particles was found to exhibit very strong absorption peaks at 402.5 nm (fig-1). It clearly indicated the presence of silver nano particles.



Fig1a. Silver nitrate solution in the beginning



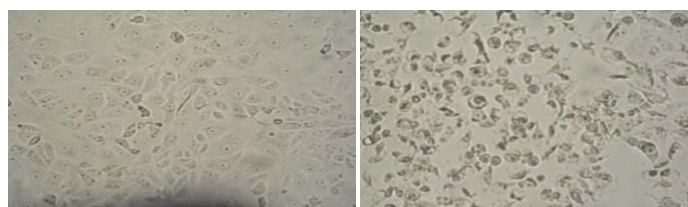
Fig1b. Bio reduction of Ag⁺ ions to Ag

3.2. Anti Cancer Activity

The viability of HeLa and Hep-2 cancer cells with TAP Ag-NPs for 72 hrs was determined using the colorimetric MTT-based assay. The TAP Ag-NPs exhibited a dose-dependent activity within the concentration range of 25 - 250 µg/ml (“Table1”and “Fig.2.a & 2.b”). The TAP Ag-NPs showed a maximum activity against HeLa and Hep-2 and it was recorded as 59.01 and 48.79% at 250µg/ml respectively.

Table1. Anticancer activity of TAP silver nanoparticles

Cell Lines	Name of the sample	(%) Percentage of inhibition/Concentration µg/ml					
		Control	50	100	150	200	250
HeLa	Ag-NPs	99.96	11.18	17.08	28.07	40.07	59.01
Hep2		99.96	10.08	15.73	20.34	31.08	48.79



Control

Ag-NPs treated cells

Fig2a. Cytotoxicity effect of TAP silver nano particles from andrographic paniculata against Vero (African green monkey kidney) cell line

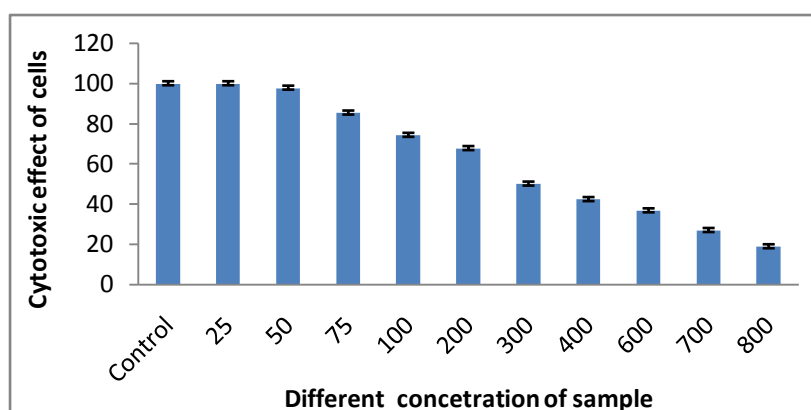


Fig2b. Cytotoxic effect of TAP Ag-NPs from andrographic paniculata

The following “3-a, 3-b & 3-c figure(photos)” are clearly indicate the anticancer activity ant its cytotoxicity in 50 to 250 µg/ml of TAP Ag-NPs from andrographic paniculata against HeLa and Hep2 cell line.

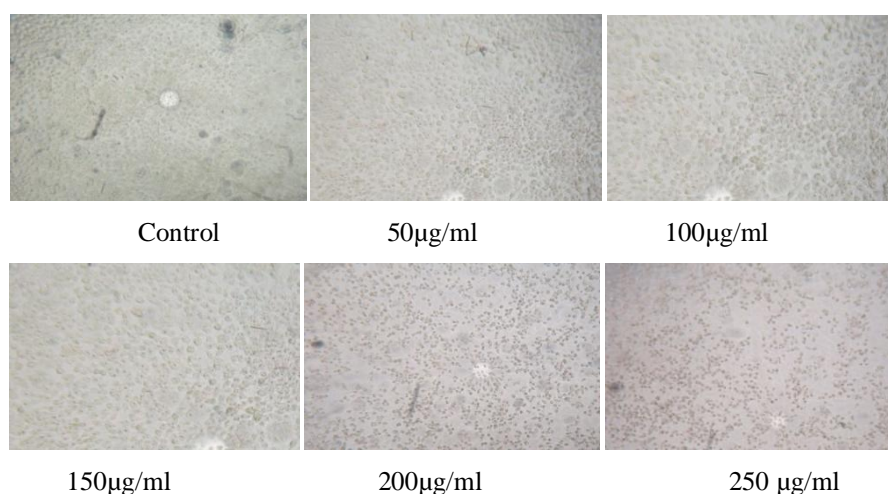


Fig3a. Anticancer activity of TAP Ag-NPs against HeLa cell line

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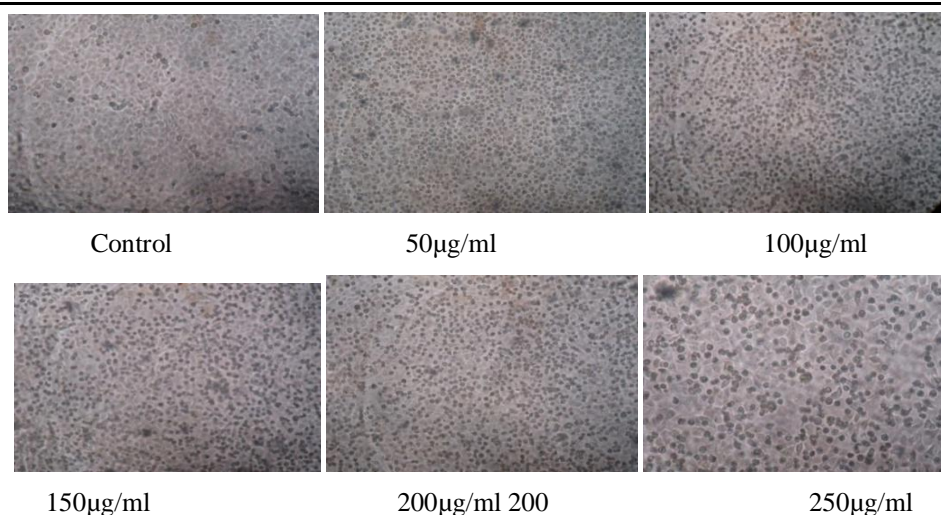


Fig3b. Anticancer activity of TAP Ag-NPs against Hep² cell line

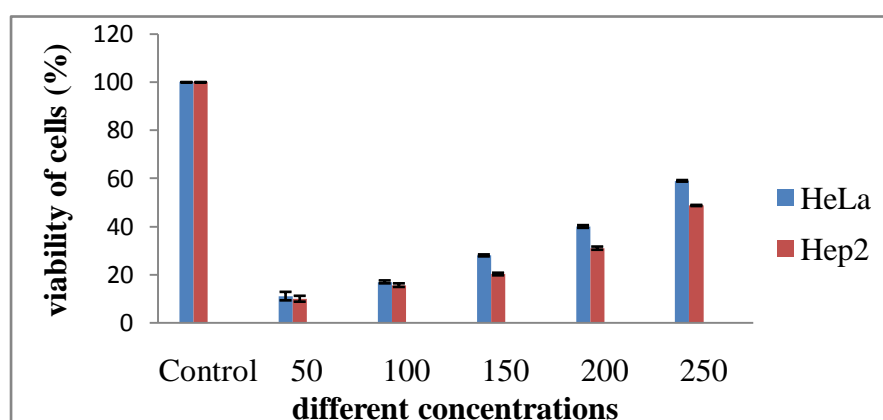


Fig3c. Anticancer activity of TAP agnps from *andrographic paniculata* against hela & Hep2

4. CONCLUSION

The biosynthesised TAP AgNPs displayed good anticancer activity over tested human pathogens HeLa and Hep-2 and it was recorded as 59.01 and 48.79% at 250µg/ml respectively. The obtained TAP AgNPs have potential applications in the medical field and this simple product has several advantages such as low quantity, low cost, high effectiveness, compatibility for medical and pharmaceutical applications as well as large scale production.

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