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REVIEW ARTICLE

ANTICANCER POTENTIAL OF GREEN SYNTHESIZED SILVER NANOPARTICLES: A REVIEW

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ABSTRACT

Silver nanoparticles synthesis is a vastly growing area of research adding day by day new applications in various fields. There are a number of physical and chemical methods which are energy and capital intensive and employ toxic chemicals. Thus more and more researchers are involved in green synthesis of nanoparticles from plants, fungi and bacteria. Use of silver nanoparticles in the field of biomedical nanotechnology and nanomedicine is rapidly increasing because of their antimicrobial, anticancer, antioxidant property and less toxicity. Cancer is one of the most deadly diseases treated by conventional chemotherapeutic agents exhibiting poor specificity and dose limiting toxicity. The present study is aimed to throw a focus on the potential of green synthesized silver nanoparticles cytotoxicity on skin cancer cell lines and its application as an anticancer agent. Green silver nanoparticles from different origin have the ability to defend against various types of cancers. The data available for comparison are mostly in vitro studies and therefore in the coming days in vivo studies, development of formulation and clinical trials are required for utilizing the anticancer potential of silver nanoparticles in practice.

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INTRODUCTION

Nanoparticles have smaller size (1 to 100nm) and a larger surface area which increases their efficiency. Silver nanoparticles are one of the most commonly used nanomaterials both in everyday life, and in research laboratories (Pantic, 2014). These nanoparticles are a solution to many technological and environmental challenges in the area of medicine (Zhang *et al*., 2008), water treatment (Dharmendra, 2008), as catalysts (Hvolbaek, 2007) and in energy conservation (Teesetsopon *et al*., 2012). So the synthesis of metal nanoparticles for these fields is an area of interest (Hussain *et al*., 2003). Recently silver nanoparticles have found a wide range of applications in biolabelling (Xu *et al*., 2004), cancer treatment (Gopinath *et al*., 2010), as antimicrobial (Patil *et al*., 2015) and anti-inflammatory agents (Aparna *et al*., 2015). Therefor it is necessary to develop clean, nontoxic and an eco friendly method for its synthesis. There are many methods to synthesize silver nanoparticles such as a chemical reaction, co-precipitation, sol gel method, etc. The problem with most of these methods is that they are very expensive and also involve the use of toxic and hazardous chemicals, which may pose potential environmental and biological risks. In recent years, green synthesis of nanoparticles have had several advantages as this technique eliminates the use of energy, high pressure, temperature, and toxic chemicals. There are many reports on green synthesis of silver nanoparticles using plants (Rajesh *et al.,* 2010), bacteria (Priyaragini *et al.,* 2012) and fungi (Selvi and Sivakumar, 2014). Cancer continues to be a worldwide deadly disease, despite the enormous amount of research and rapid developments seen during the past decade (Anand *et al.,* 2008).

It is a complex genetic disease that is caused primarily by environmental and genetic factors (Malcolm and Alisonand, 2001). Usually cancer is treated with combination of radiation, surgery, chemotherapy and targeted therapy, but all these therapies have some drawbacks, so it is needed to investigate more desirable therapy for cancer treatment. Silver nanoparticles may be employed as an effective treatment agent against various types of cancers. As per the recent works, green synthesized silver nanoparticles have anticancer activity and are proved more effective and safe. In this article there is a brief review on anticancer effect of green synthesized silver nanoparticles.

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Green synthesis of silver nanoparticle

The reducing potential of the biological systems from higher plants and microbes have been used for biogenic silver nanoparticle synthesis and is currently under wide expansion.

The particles were characterize the synthesized particles by various techniques like, UV- Vis spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), X-ray Diffraction microscopy (XRD) and Energy Dispersive X-ray spectroscopy (EDX) (Erick and Nalini, 2014).

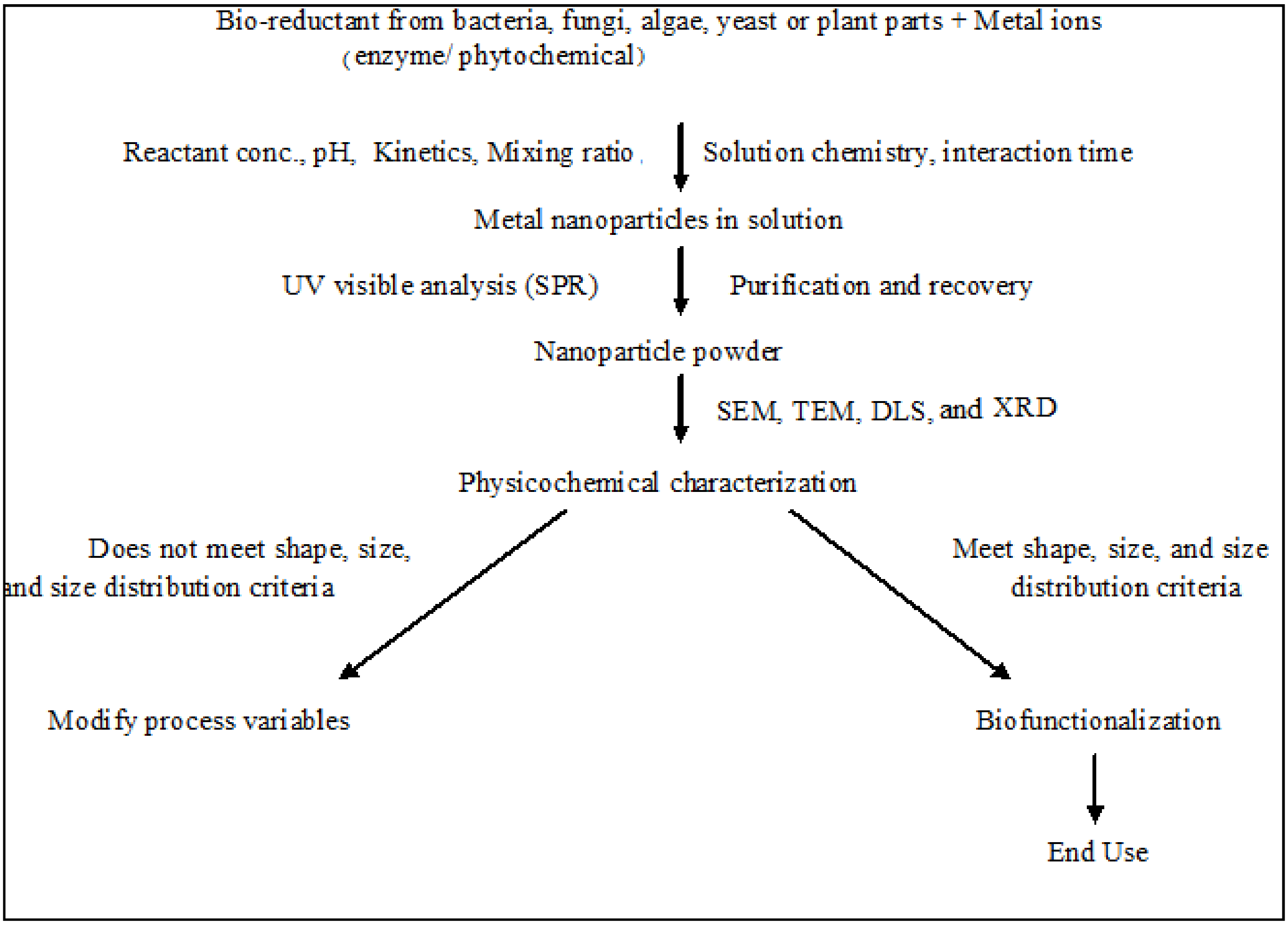

Fig. 1. Generalized flow chart for biosynthesis of nanoparticles (Ratnika, 2012)

Fabrication of silver nanoparticles using bacterias like *Pseudomonas stutzeri* (Joerger *et al*., 2000), *Lactobacillus* (Nair and Pradeep, 2002), *Klebsiella pneumonia* (Mokhtari, 2009), *Escherichia coli,* and *Enterobacter cioacae* (Shahverdi *et al*., 2007) have been done successfully. Govindaraju *et al.* (2009) reported the synthesis of silver nanoparticles by using a brown seaweed alga, *Sargassum wightii*.

The fungi *Penicillium, Corious versicolor* (Sanghi and Varma, 2013) and *Saccharomyces boulardii* (Kaler *et al*., 2013) have been used in the biosynthesis of silver nanoparticles. Extracts of different plant parts like banana, neem, tulsi leaf extract (Banerjee *et al*., 2014), *Embilica officinalis* fruit extract (Ankamwar *et al*., 2005), leaf and seed extract of *Syzygium cumini* (Kumar *et al*., 2010), Onion extract (Saxena *et al*., 2010), latex of *Jatropa curcas* (Bar *et al*., 2009) etc., have been employed for synthesis of silver nanoparticles. The green method used for synthesis of silver nanoparticles is very simple and fast. The biogenic extract is allowed to react with silver nitrate till the colour of reaction mixture turned yellowish brown.

The generalized method for green synthesis is summarized in Fig. 1

The anticancer potential of green synthesized silver nanoparticles

Silver nanoparticles are cytotoxic to cancer cells and have potential as an antitumor agent. Varieties of nanoparticles have shown novel biological activity to induce autophagy and promote cell death (Lin *et al*., 2014). Green synthesized nanoparticles fabricated from bacteria, fungi, algae, yeast or plant exhibit good anticancer activity which is shown in Table 1. Sriram *et al.* demonstrated the efficacy of biologically synthesized silver nanoparticles from bacteria *Bacillus licheniformis*. The antitumor efficacy of this silver nanoparticles have been studied on Dalton's lymphoma ascites (DLA) by *in vivo* and *in vitro* methods giving IC_{50} at 500 nM. Observation had shown the inhibition of tumor progression and thereby effectively controlling disease progression without causing toxicity to normal cells (Asha Rani *et al*., 2009).

Table 1. IC_{50} Values of green synthesized silver nanoparticles on various cancer cell lines

Source	Biogenic agent used for synthesis of silver nanoparticles	Size in nm	Cell line	IC_{50} value	References
Bacteria	*Bacillus licheniformis*	50	DLA	500nM	(Muthu *et al*., 2010)
			G292	3.42 µg/ml	
	Anabaena doliolum	10-50	DLA	20 µg/ml	(Singh *et al*., 2014)
			Colo205	30 µg/ml	
Fungi	*F.oxysporum*	--	Hep2	12.5µg/ml	(Selvi and Sivakumar, 2014)
			MCF7	37µg/ml	
			HT 29	49µg/ml	
	Penicillium spp.	149-397	HT-29	30 µg/ml	(Verma *et al*., 2013)
	Aspergillus ochraceus	13.88	HCT-116	1.4 µg/ml	(Magdi *et al*., 2014)
			MCF-7	2.1 µg/ml	
			Hep-G2	1.2 µg/ml	
	Aspergillus flavus	33.5	HL-60	-	(Sulaiman *et al*. 2015)
	Ganoderma neo-japonicum		MDA-MB-231	6 µg/ml	(Gurunathan *et al*., 2013)
Algae	*Ulva lactuca*	20-56	Hep-2	12.5 µg /ml	(Devi Valentin, 2012)
			MCF-7	37µg/ml	
			HT- 29	49µg/ml	
	Gelidiella sp.	40-50	Hep-2	31.25 µg/ml	(Saraniya *et al*., 2012)
Yeast	*Saccharomyces boulardii*	3-10	MC -7	10µg/ml	(Kaler *et al*., 2013)
Plant	*Phyllanthus emblica*	188	Hep-2	30 µg/ml	(Fathima *et al*., 2012)
	Indigofera aspalathoids	--	Hep-3B	194.65ng/ml	(Krishnasamy *et al*., 2014)
	Eucalyptus chapmaniana	60	HL-60 cells	1mmol/ml	(Ghassan *et al*., 2013)
	Cynodon dactylon	--	Hep-G2	45.6 µg/ml	(Supraja *et al*., 2015)
	Taxus baccata	75	MCF-7	0.25 µg/ml	(Kajani *et al*., 2014)
	Sargassum polycystum	5-7	MCF-7	135 µg/ml	(Nallamuthu *et al*., 2012)
	Andrographis Paniculata	--	Hela	59 µg/ml	(Dhamodaran and Kavitha, 2015)
			Hep-2	49 µg/ml	
	Dodonaea viscose	60-90	MCF-7	98.03 µg/ml	(Giridharan *et al*., 2014)
	Capparis deciduas		MCF-7	108.69 µg/ml	(Giridharan *et al*., 2014)
	Sesbania grandiflora	22	MCF-7	20 µg/ml	(Jeyara *et al*., 2013)
	Plumbago indica	50-60	DLA	600nM	(Sujin, 2013)
	Citrullus colocynthis	7-19	Hep-G2	42µg/ml	(Shawkey *et al*., 2013)
			HCT-115	41µg/ml	
	Cauliflower	40-50	MCF-7	190.5µg/ml	(Ranjitham *et al*., 2013)
	Olax scandens	20-60	A549	--	(Mukherjee *et al*., 2014)
			B16	--	
			MCF-7	--	
	Olive leaves		MCF-7	0.024 µg/ml	(Rashidipour and Rouhollah, 2014)
	O*riganum heracleoticum l*	30-40	MCF-7	-	(Rajendran *et al*. 2015)

Bacteria *Anabaena doliolum* mediated synthesized silver nanoparticles induced loss of survival of HLA and colo205 cell lines (Singh *et al*., 2014). Silver nanopartices from *Penicillium* species has shown more efficacy than *F.oxysporum* on HT-29 cell line(Selvi and Sivakumar, 2014; Verma *et al*., 2013). The result of Selvi and Sivakumar showed Hep-2 cells as more sensitive to *F. oxysporum* mediated silver nanopaticles than MCF-7 and HT-29. Potential of silver nanoparticles from *Aspergillus ochraceus* are more promising because of its cytotoxicity at very low concentrations on HCT-116, MCF-7, Hep-G2 cell lines. Magdi *et al*. also compared the normal vero cell lines cytotoxicity by using same concentrations used for cancerous cells. The results obtained by him indicated that the sensitivity of human cancer cell lines is much higher than that of vero cell lines (Magdi *et al*., 2014). These silver nanoparticles have been further explored as novel leads to cancer chemotherapy. The reports for silver nanoparticles from *Ganoderma neo-japonicum* suggest that cytotoxicity was induced through ROS generation, activation of caspase 3and DNA fragmentation (Gurunathan *et al*., 2013). By using marine microalgae *Ulva lactuca* silver nanoparticles were synthesized and its cytotoxicity has been assessed against HCT-116, MCF-7, Hep-G2 cell lines (Devi and Valentin, 2012), and the anticancer activity same as like *F.oxysporum* synthesized silver nanoparticles.

Here requires more detail investigation for selecting better alternative therapeutic measures against cancer. Devi and Valentin suggested the cytotoxic effect is inversely proportional to the size of bioactive silver nanoparticles synthesized from *Ulva lactuca.* Use of cell free extract of *Saccharomyces boulardii* for synthesis of silver nanoparticles has been done by Kaler *et al*.. The anticancer activity of silver nanoparticles was evaluated on MCF-7 cells in comparison to ionic silver salt.

Comparative study has shown that silver ions have less efficiency than silver nanoparticles, which are having IC_{50} value less than 10µg/ml. This promising result may be valuable for future application of silver nanoparticles in breast cancer treatment (Kaler *et al*., 2013). There are more data available on the anticancer activity of silver nanoparticles synthesized from plant source. These nanoparticles have shown good potential against various types of cell lines. Among these study silver nanoparticles synthesized from *Taxus baccata* extract was found to be more potent with IC_{50} value of 0.25µg/ml against MCF-7 cell lines (Kajani *et al*., 2014).

Subsequently silver nanoparticles from Sesbania grandiflora *leaf extract have shown their potential with* IC_{50} value at 20µg/ml (Jeyara *et al*., 2013). Silver nanoparticles from

Phyllanthus emblica and *Gelidiella sp.* have shown good anticancer activity against Hep-2 cell lines and proved good anticancer agent against liver cancers (Fathima *et al*., 2012; Saraniya *et al*., 2012). Common Dogwood Berries extract mediated green synthesized silver nanoparticles shown cytotoxicity against epidermal carcinoma cell lines and this nanoparticles may useful for preventive as well as treatment of skin cancers. *Gymnema sylvestre* fabricated silver nanoparticles have shown good activity against HT -29 human colon cancer cell lines (Kantha *et al*., 2015). Mukherjee *et al.* (2014) has done a comparative study of chemically synthesized silver nanoparticles and biologically synthesized silver nanoparticles. This demonstration gives a very significant result that biologically synthesized silver nanoparticles from plant leaf extract showed significant inhibition of proliferation by 40-70% in A-549, 35-90% in B-16 and 25-55% in MCF-7 cell lines with increasing concentration. However mild or none cytotoxicity has been observed with chemically synthesized nanoparticles, a reported by Sierrra-Rivera *et al.* (2013) who compared the *in vivo* anticancer activity of colloidal silver and silver nanoparticles on B16F10 melanoma in which he found that silver nanoparticles are better than colloidal silver in reducing tumor volume and weight.

Likewise, other plant mediated silver nanoparticles have shown anticancer activity as listed in Table 1. These results report the potentiality of silver nanoparticles as an anticancer agent. Among all these studies silver nanoparticles from Capparis deciduas **(**Giridharan *et al*., 2014**)** and Cauliflower (Ranjitham *et al*., 2013) were found to have very less anticancer efficiency with IC_{50} value more than 190µg/ml. Thus, these nanoparticles are not employed for the treatment of cancer. If we compare IC_{50} values of all green synthesized silver nanoparticles there are more variations in their cytotoxicity. These variations may be because of capping agents attached to the surface of nanoparticles from biogenic extract. If we correlate the size of silver nanoparticles and activity, wide variations are observed. The results may be comparable with activity and the size of the silver nanoparticles synthesized from same biogenic extract.

The battle against cancer - role of green silver nanoparticles

Although the mechanism of antitumor action of silver nanoparticles not properly understood, three proposed mechanisms has been reported. First mechanism reports that the silver nanoparticles induces loss of survival of cancerous cell, may be due to reactive oxygen species generation which leads to apoptic morphological changes, DNA fragmentation, oxidative stress resulting in apoptosis (Singh *et al*., 2014; Fathima *et al*., 2012; Krishnasamy *et al*., 2014) The second mechanism depends on interference, proper functioning of proteins which results in changes in cellular chemistry (Rogers *et al*., 2008). Silver nanoparticles are likely to interact with thiol rich enzymes (Morones *et al*., 2005), which may result in partial unfolding of proteins. Silver nanoparticles provide a relative hydrophobicity inside bovine hemoglobin, which results in a transition to the alpha helix to beta sheets which also leads to partial unfolding and aggregation of proteins (Zolghadri *et al*., 2009). These changes in protein may lead to cytoxicity. In the third proposed mechanism, silver nanoparticle treatment makes changes in cell permeability which leads to entry of Ca ions there by activation of enzymes like protease and endonuclease which results in mitochondrial membrane dysfunction and reactive oxygen species generation, subsequent oxidative stress, DNA damage, errors in chromosome segregation and production of micronuclei leads to cell death (AshaRani *et al*., 2009).

Conclusion

Several researchers have reported the biological synthesis of silver nanoparticles and its potential against various types of cancers. These studies conducted on *in vitro* cell lines further needs *in vivo* studies to red to find out its safety and efficacy. The obtained results showed drastic variations in potentiality as per source of biogenic agent. The factors which may change this potentiality are, capping agent from biogenic extract, the size of silver nanoparticles, and stability of nanoparticles. Proper understanding of the exact mechanism of anticancer activity should be required for increasing efficiency. Also, it is needed to find out molecular level changes in cells. Level of toxicity and safety should be checked for its effective application. If it is proved safe biogenic silver nanoparticles will give tremendous breakthrough in the field of nanomedicines and make this agent as an effective alternative in tumor and angiogenesis like diseases.

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