



INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

***IN VIVO* EVALUATION OF GENOTOXICTY OF *Acorus calamus* MEDIATED SILVER NANOPARTICLES**

BETTY LINCOLN¹, AKHIL MENON¹, V DEEPA PARVATHI², SUMITHA RAVINDRAN¹

1. Department of Biomedical Sciences, Sri Ramachandra University, Chennai.
2. Department of Human Genetics, Sri Ramachandra University, Chennai.

Accepted Date: 03/04/2014; Published Date: 27/04/2014

Abstract: Metal nanomaterials are found to have a potential application in catalysis, photonics, optics and biomedicine. Silver nanoparticles have gained immense interest and have been exploited over the years to expand their applications. Green synthesis is one of the recently developed methods for synthesizing silver nanoparticles using different organisms such as plant, bacteria and fungi. The present study is aimed to synthesize and characterize the silver nanoparticles using *Acorus calamus* plant extract and to check their genotoxicity using *Drosophila* as the model organism. *Acorus calamus* green silver nanoparticles were synthesized and characterized using High-Resolution Scanning Electron Microscopy (HRSEM) and ultraviolet and visible (UV-VIS) spectrum. The *in vivo* toxicity of the synthesised green silver nanoparticles was tested on flies. Three defined concentrations of the NPs(10mg/L, 50mg/L and 100mg/L) was chosen; and the flies were exposed to food mixed with the above defined concentrations for 24 hours along with a negative control. Post 24 hours, phenotypic changes in the flies were observed under the stereo zoom microscope and documented. Also, the DNA from flies (post exposure) was isolated using phenol chloroform method and subjected to DNA fragmentation assay. Marked phenotypic changes in terms of discoloration were observed. DNA fragmentation assay revealed distinct shearing in all three concentrations on comparison with the control. Novelty of this present study is that the evaluation of green nanoparticles with *Drosophila* as the model organism.

Keywords: *Acorus Calamus*, Silver Nanoparticles, *Drosophila melanogaster*, Genotoxicity



PAPER-QR CODE

Corresponding Author: MS. BETTY LINCOLN

Access Online On:

www.ijprbs.com

How to Cite This Article:

Betty Lincoln, IJPRBS, 2014; Volume 3(2): 126-132

INTRODUCTION

In recent years nanomaterials are found to have a wide range of applications because of its customized physiochemical properties [1, 2]. Out of all nanomaterials, the metal nanoparticles have kindled much interest owing to their potential applications in catalysis, photonics, optics and biomedicine [3-5]. Nanotechnology has proven to open newer synthesis methods that can produce size controlled nanoparticles that can fight and stop the spread of disease. Metallic nanoparticles have large surface to volume ratios and crystallographic surface structure that make them excellent antibacterial materials [6]. The most commonly synthesized metallic nanoparticles are prepared from metals such as Gold, Silver, Platinum and Lead. Among these silver (Ag) has been used for ages in the field of biology and medicine for its antibacterial potency [7]. Silver nanoparticles are silver particles of size ranging from 1 nm and 100 nm. These particles get attached to cell wall thus disconcerting cell-wall permeability and cell respiration. These attached nanoparticles damage the DNA and protein by interacting with phosphate and sulphur present in them.

In view of the wide spread applications of silver nanoparticles, several easy and effective methods were adopted to synthesize them. Chemical synthesis method is one of the oldest methods but the resultant silver nanoparticles may show undesirable genotoxicity effect. Green synthesis is one of the recently developed methods for synthesizing nanoparticles using different organisms such as plant, bacteria and fungi. This method of synthesis is eco friendly and proven to give better outcome in terms of controlled particles size, stability and cost. Although much data is not available on their carcinogenic property, they possess excellent antibacterial potential to resist microbes but less attention has been given to their toxicity.

Acorus calamus is a medicinal plant, commonly known as Sweet flag and used for the treatment of various diseases [8, 9]. Its active constituents are known to possess antibacterial, antipsychotic and hepatoprotective activities. Owing to smooth muscle stimulation and relaxation properties of *Acorus calamus*, it is used as an antiepileptic drug. The wide range of pharmacological activities of *Acorus calamus* kindled our interest to narrow down our choice of plant extract to *Acorus calamus*. Hence, the present study is aimed to synthesize and characterize the silver nanoparticles using *Acorus calamus* plant extract and to check their genotoxicity using *Drosophila* as the model organism.

MATERIALS AND METHODS

Preparation of aqueous extract of *Acorus calamus*:

The rhizome parts of *Acorus calamus* (AC) were procured from authenticated source and dried under shade. The coarsely powdered rhizome was subjected to extraction with mixture of

chloroform: water (1:9, 5%) at room temperature on a shaker for two days. After completion of extraction, the solvent was completely evaporated by heating in a water bath and stored for further use.

Synthesis of *Acorus calamus* derived Silver Nanoparticles:

Acorus calamus mediated silver nanoparticles were synthesized according to the green synthesis method described elsewhere [10]. Briefly, 5ml of the *Acorus calamus* extract was added to 95 ml of AgNO₃ solution and the mixture was observed periodically for color change. Development of brown colour indicates the formation silver nanoparticles. The solution was filtered and centrifuged for 10 min at 4000rpm and the pellet was used for further characterization and DNA fragmentation assay.

Characterization of Silver Nanoparticles:

The development of brown colour on addition of the aqueous extract of *Acorus calamus* to silver nitrate solution indicates formation of silver nanoparticles due to reduction of silver ions and is the visual confirmation for the formation of silver nanoparticles. Further, the morphology and reduction of pure Ag⁺ ions were assessed by using FEI Quanta FEG 200 - High Resolution Scanning Electron Microscope (HR-SEM). The SEM image is as shown in figure 1 and UV-Vis spectrum is shown in figure 2.

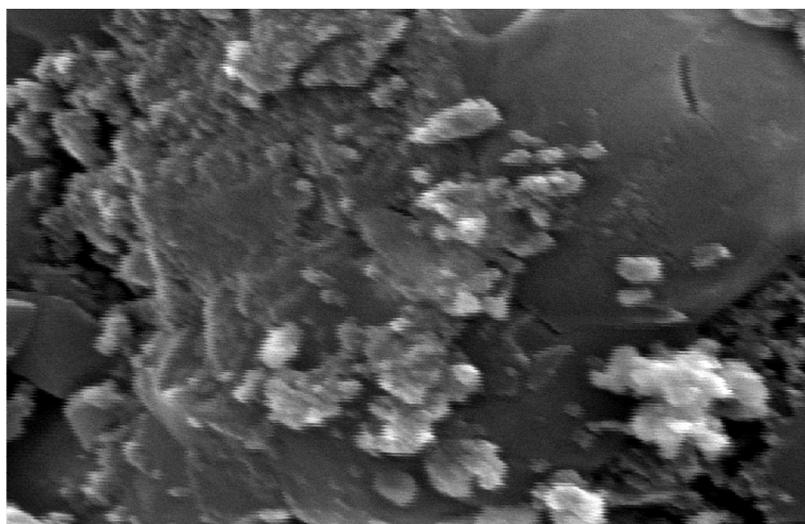


Figure 1: SEM image of *Acorus calamus* derived Silver nanoparticles

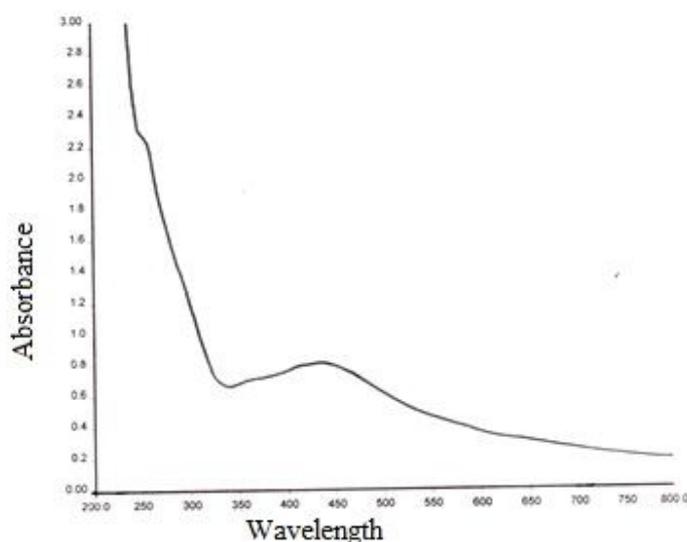


Figure 2: UV spectrum of *Acorus calamus* mediated silver Nanoparticles

Treatment of *Drosophila* with *Acorus calamus* mediated Silver Nanoparticles:

The genotoxicity of *Acorus calamus* silver nanoparticles was tested *in vivo* using the fly model. Wild type (Canton-S) flies of *Drosophila melanogaster* were cultured and maintained in corn meal agar. The green silver nanoparticles were introduced in the food at concentrations of 10mg/L, 50mg/L and 100mg/L. The flies were exposed for 24 h in the food containing different concentrations of *Acorus calamus* silver nanoparticles along with a negative control. Phenotypic variations in the exposed flies were observed under the stereo zoom microscope. Genomic DNA from 25 to 30 flies from each concentration were isolated using phenol-chloroform method [11]. Concentration of DNA from each sample was determined using NanoDrop ND-1000 Spectrophotometer (Thermo Scientific NanoDrop Technologies, Wilmington, DE). DNA fragmentation assay was performed on 1% agarose gel. 40 ng of genomic DNA was loaded into the slots of 1% agarose gel containing 1 $\mu\text{g}/\text{mL}$ Ethidium bromide, at a constant power supply of 80 volts for one hour. The gel was visualized under UV transilluminator and documented.

RESULTS

The formation of silver nanoparticles from *Acorus calamus* extract showed evident color change with the solution turning brown from initially being colorless. The average particle size of the synthesized nanoparticles were estimated using HRSEM was approximately 50nm. The UV absorption spectrum showed the characteristic peaks at 420nm which is also the characteristic peak region of metallic silver. Exposure of *Drosophila melanogaster* to three different

concentrations of green silver nanoparticles (10mg/L, 50mg/L and 100mg/L) showed clear phenotypic changes in the flies (Figure 3).

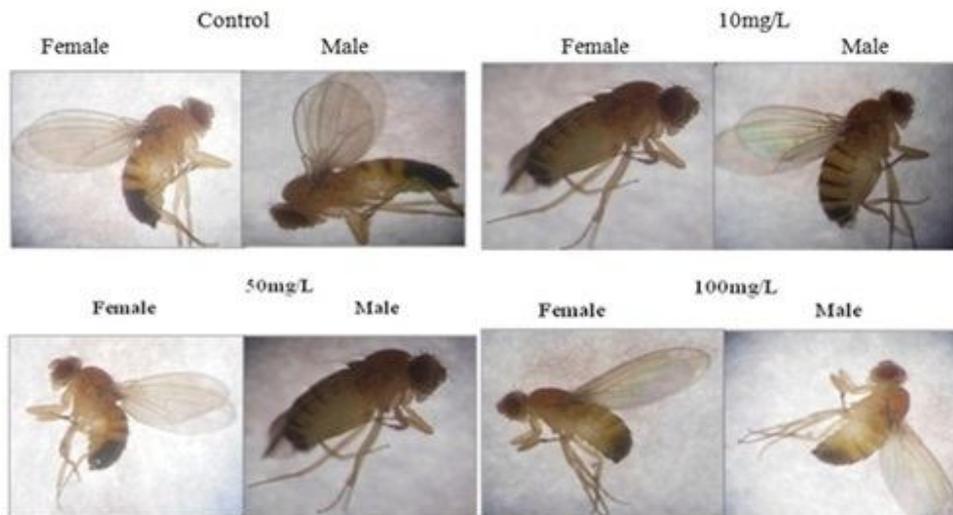


Fig. 3: Phenotypic changes observed in flies exposed to Silver nanoparticles (*Acorus calamus* derived)

When compared with the flies of control group, the flies exposed to 10mg/L concentrations showed mild discoloration and broadened thorax region in female flies and globular coating on the surface of male flies. The flies exposed to 50 mg/L exhibited discoloration in the head and thorax region of both male and female flies. The female flies were long and stout compared to the male flies. Flies in 100mg/L concentration showed deep discoloration in both male and female flies. Agarose electrophoresis of genomic DNA from flies exposed to *Acorus calamus* mediated silver nanoparticles revealed fragmented DNA compared to control (Figure 4).

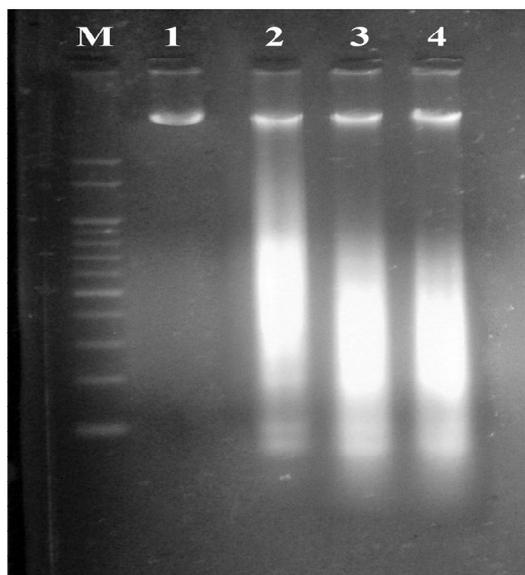


Figure 4: DNA Fragmentation Assay

M: Ladder 100bp; 1: Control; 2:10 mg/L; 3: 50 mg/L; 4:100 mg/L

Distinct dose dependent DNA shearing observed in flies exposed to all three concentrations indicate their genotoxic effect.

DISCUSSION

Although the green synthesis route of synthesizing silver nanoparticles is a much explored route many studies have been performed to evaluate its antibacterial, antifungal and antiviral properties yet its genotoxic effects have been overshadowed by its potential properties and have not been given much importance. The present study synthesized and characterized the green silver nanoparticles. It later evaluated the genotoxic effect of synthesized *Acorus calamus* mediated silver nanoparticles on *Drosophila melanogaster*. Exposure of *Drosophila melanogaster* to three different concentrations of green nanoparticles showed clear phenotypic changes in the flies and these changes were specific at 10mg/L, 50mg/L concentrations. DNA fragmentation assay revealed that the *Acorus calamus* mediated Silver nanoparticles showed showed genotoxic effect in a dose dependent manner.

CONCLUSION

The genotoxic potential assessed in *Drosophila* model gives an insight into the possible genotoxic effect of the said concentrations. The phenotypic changes in terms of discoloration may be attributed to gene mutations as a consequence of toxicity. The assay is qualitative and these results lay emphasis on directing the study towards quantitative genotoxic assessment

(PCR based assays, protein profiling, SMART assay) which will demonstrate the mechanisms of mutations and expression of toxicity. It is also important to compare the data with parallel *in vitro* endpoints and docking studies to clearly explore the contribution of the compound and its possible roles and effects in genotoxicity.

ACKNOWLEDGEMENTS

The authors acknowledge the funding from Sri Ramachandra University for awarding Chancellor's Summer Research Fellowship. The authors would also like to acknowledge the valuable contributions of Dr. L.V.K.S. Bhaskar.

REFERENCES

1. Kaur R, Badea I., Nanodiamonds as novel nanomaterials for biomedical applications: drug delivery and imaging systems., *Int J Nanomedicine* 2013; 8: 203-20.
2. Cao YC., Nanomaterials for biomedical applications., *Nanomedicine (Lond)* 2008; 3: 467-9.
3. Krishna KS, Li Y, Li S, Kumar CS., Lab-on-a-chip synthesis of inorganic nanomaterials and quantum dots for biomedical applications., *Adv Drug Deliv Rev* 2013.
4. Hrenovic J, Milenkovic J, Daneu N, Kepcija RM, Rajic N., Antimicrobial activity of metal oxide nanoparticles supported onto natural clinoptilolite., *Chemosphere* 2012; 88: 1103-7.
5. Wiederrecht GP, Wurtz GA, Im JS, Hranisavljevic J., Aggregates on metal nanoparticles characterized through ultrafast spectroscopy and near-field optics., *Scanning* 2004; 26: 12-9.
6. Kalpana D, Lee YS., Synthesis and characterization of bactericidal silver nanoparticles using cultural filtrate of simulated microgravity grown *Klebsiella pneumoniae*., *Enzyme Microb Technol* 2013; 52: 151-6.
7. Marshall CR, Neave EF., The Bactericidal Action of Compounds of Silver., *Br Med J* 1906; 2: 359-63.
8. Nandakumar S, Menon S, Shailajan S., A rapid HPLC-ESI-MS/MS method for determination of beta-asarone, a potential anti-epileptic agent, in plasma after oral administration of *Acorus calamus* extract to rats., *Biomed Chromatogr* 2013; 27: 318-26.
9. Kim H, Han TH, Lee SG., Anti-inflammatory activity of a water extract of *Acorus calamus* L. leaves on keratinocyte HaCaT cells., *J Ethnopharmacol* 2009; 122: 149-56.
10. Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M., Synthesis of gold nanotriangles and silver nanoparticles using *Aloe Vera* plant extract., *Biotechnol Prog* 2006; 22: 577-83.