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**Cytotoxic and genotoxic properties of silver nanoparticles synthesized by ethanolic extract  
of *Salacia chinensis***

Madhuranthakam Reddi Nagesh<sup>a</sup>, Natesan Vijayakumar<sup>a,\*</sup>, Ramaswamy Anandan<sup>b</sup>, Mani Renuka<sup>c</sup>, Venkatesan Amalan<sup>a</sup>, R. Kavitha<sup>c</sup>, Samuelraj Babu Arulmani<sup>d</sup>, Mohammad Z. Ahmed<sup>e</sup>, Ali S. Alqahtani<sup>e</sup>, Fahd A. Nasr<sup>e</sup>, Abdulaziz M. Alqahtani<sup>e</sup>, Omar M Noman<sup>e</sup>, Abdullah A. Al-Mishari<sup>e</sup>

<sup>a</sup>*Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar 608002, Tamil Nadu, India*

<sup>b</sup>*Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Annamalainagar 608002, Tamil Nadu, India*

<sup>c</sup>*Department of Biotechnology, School of Bioscience, Periyar University, Salem 636011, Tamil Nadu, India*

<sup>d</sup>*University of Rennes, CNRS, ISCR (Institut des Sciences Chimiques de Rennes), Campus de Beaulieu, 35000 Rennes, France*

<sup>e</sup>*Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia.*

**\* Address for Corresponding author:**

Dr. Natesan Vijayakumar,  
Research Supervisor & Mentor, Assistant Professor,  
Department of Biochemistry and Biotechnology,  
Faculty of Science, Annamalai University,  
Annamalainagar 608002, Tamil Nadu, India  
E-mail ID: nvkbiochem@yahoo.co.in

## ABSTRACT

In this study, *in vitro* and *in vivo* methods were used to evaluate the cytotoxicity and genotoxicity properties of silver nanoparticles (Ag-NPs) made from a crude ethanolic extract of *Salacia chinensis*. The test Ag-NPs had no cytotoxicity on the fibroblast cell line at a concentration of 100 µg/mL, according to the MTT assay results. The Chinese hamster ovary (CHO) cell line treated with varied concentrations of test Ag-NPs, with a maximum concentration of 200 µg/mL, did not exhibit any appreciable genotoxic activity, either by comparing the results with positive controls of genotoxicity caused by Methyl methane sulfonate and Benzo (a) pyrene at the concentration of 20 µg/mL, the lack of genotoxicity was established. An *in vivo* study in Swiss albino mice using various concentrations (250, 500, and 1000 mg/kg) of test Ag-NPs, which were compared with positive controls, further confirmed this *in vitro* result pattern. Contrary to the genotoxicity caused by the positive control, mouse bone marrow micronucleus testing findings revealed the absence of genotoxicity. These findings imply that at the measured doses, the Ag-NPs produced from the crude ethanolic extract of *Salacia chinensis* do not exhibit any cytotoxicity or genotoxicity.

**Keywords:** Ag-NPs; Cytotoxicity; Fibroblast cell line; Genotoxicity; CHO cell line

## 1 Introduction

A critical enabling technology that forms the basis for upcoming advancements and technical products, nanotechnology has emerged as one of the most desired discoveries across a variety of study fields [1, 2]. Nanoparticles range in size from 1-100 nm and contain 20-15,000 Ag atoms. They can form naturally or be created artificially using physical, chemical, or biological processes [3, 4]. Due to their small size, nanoparticles might have characteristics that are very different from those of their larger counterparts. In particular commercial applications, these modified qualities may impart highly desirable traits, including being more robust, less expensive, and generally more effective [5, 6]. As the use of nanoparticles grows, exposure to them is anticipated to rise. According to this, nanoparticles are being used in industrial and commercial applications at an increasing rate, which is causing worry about their possible adverse effects on biological systems globally [7, 8]. The number of variables that may affect how different nanoparticles interact with biomolecules has increased significantly, although these variables are still unresolved [9, 10]. The toxicity and safety of nanoparticles have developed far more slowly than their rate of production. This appears to be caused by a lack of regulations, disagreements between researchers regarding scientific methods or upcoming studies in this field, and the distinctive properties of nanostructured particles, which present difficulties during the toxicological assessment of novel nanoparticles [11]. The antibacterial properties of silver nanoparticles (Ag-NPs), which come in different sizes, have been widely employed in recent years [3]. The European Commission estimated that about 20 metric tons of Ag-NPs have been marketed annually [12-15], and at the present rate of production, it might reach 800 metric tons by 2025 [1]. Globally, the market for AgNPs is anticipated to grow quickly. However, whether exposure to AgNPs through commercial or household waste will have a negative

biological impact on people, animals, or plants is debatable [16, 17]. AgNPs are continually exposed to people through a variety of ways, including oral consumption, skin contact, and inhalation. AgNPs are consumed in diets at a rate of 70 to 90  $\mu\text{g}$  per day [18-21].

There have been numerous studies published on the genotoxicity and cytotoxicity of AgNPs in mammals and human cell lines, as well as a few discoveries in plants [22, 23]. The majority of studies on nanotoxicity have used tissue culture methods, which are thought to be the most accurate way to measure nanotoxicity. Additionally, genotoxicity plays a significant role in this safety assessment [24, 25]. Through major DNA lesions, genetic abnormalities, chromosomal aberrations, or genetic recombination, potential mutagens or carcinogens can be identified [1, 26]. Guidelines on genotoxicity diagnostics and data interpretation for active pharmaceutical ingredients approved for human usage have been published by the International Council for in this study [27, 28].

## **2 Materials and methods**

### **2.1 Brief profile about test AgNPs**

The antibacterial, antioxidant, and antiproliferation potentials of the AgNPs produced in our prior investigation were discovered [29]. The particle was also measured to be 8 nm in size and spherical in form. This AgNPs was chosen and employed in this investigation to assess their potential for cytotoxicity and genotoxicity on standard, corresponding cell lines to grasp the possibilities to use and create medicines for commercialization.

### **2.2 *In vitro* cytotoxic activity**

The National Centre for Cell Sciences in Pune, India, was where the oral fibroblast cell line was purchased. To determine if the tested AgNPs were cytotoxic, the conventional MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay methodology was used. In a nutshell, the 96-well plate was seeded with approximately 100 microliters of viable fibroblast cells (5000 cells/well) and cultured at 37 °C for 24 h in a CO<sub>2</sub> (5%) incubator. To get test AgNP concentrations of 10-100 µg/mL, the medium was then replaced with 200 microliters of complete medium and cultured for 3 days at 37 °C. After treatment, the cells of the fibroblasts were rinsed with phosphate buffered saline (PBS), the wells were once again filled with fresh medium supplemented with 0.5 mg/mL of MTT, and they were cultured for 3 h in the dark before the medium was withdrawn. Each well received 100 microliters of DMSO to dissolve the MTT formazan crystals. An ELISA plate reader was used to measure the optical density of treated wells at 570 nm. As a positive control, puromycin (10-100 µg/mL) was utilized [29].

## **2.3 *In vitro* genotoxicity study**

### **2.3.1 Chromosome aberration analysis**

With a few minor adjustments, the genotoxicity and chromosomal aberration analysis procedures were used in this work. In a nutshell, the National Centre for Cell Sciences in Pune, India, provided the Chinese Hamster Ovary (CHO) cell line. With a density of  $2 \times 10^5$  cells per T25 cm<sup>2</sup> tissue culture plate and 5 mL of complete media, the acquired cells were seeded (RPMI 1640 medium). First, the conventional cytotoxicity experiment was carried out using different concentrations (50, 100, and 200 µg/mL) of test AgNPs, and the relative increase in cell counts (RICC) was calculated using the following equation to determine the percentage of cytotoxicity (1).

$$\text{RICC (\%)} = \frac{(\text{Increase in number of cells in treated cultures (final - starting)})}{(\text{Increase in number of cells in control cultures (final - starting)})} \times 100$$

The genotoxicity and chromosome aberration traits of test AgNPs were studied in three phases with and/or without the S9 metabolic activation system mix as follows:

### **2.3.2 Preparation of the metabolic activation system:**

The S9 metabolic activation (homogenate) was isolated from Aroclor 1254-I-induced rat liver microsomal enzymes as a 40% S9 homogenate mix (contains: 1 mL of glucose-6-phosphate (180 mg/mL), 1 mL of NADP (25 mg/mL), 1 mL of potassium chloride (150 mM), and 2 mL of S9 fraction) and diluted into a 1% S9 (dilution: adding 2.5 mL of 40% S9 mix in 100 of culture medium).

**Phase I:** Ag-NPs were examined in a brief treatment (4 h) without S9 mix in medium (RPMI 1640 medium) containing CHO cells ( $2 \times 10^5$  cells) that were subjected to 50, 100, and 200  $\mu\text{g/mL}$  of various doses. Positive controls were benzo(a)pyrene and methyl methane sulfonate concentrations of 20  $\mu\text{g/mL}$ , respectively. Distilled water served as the negative control and DMSO served as a vehicle control. The sample taken at this time was equivalent to 1.5 normal cell cycles.

**Phase II:** As similar to Phase I protocol except for presence of 50  $\mu\text{L}$  of 1% S9 mix in the cells containing medium.

**Phase III:** As similar to Phase I protocol except for continuous treatment for 24 h.

Cells treated with Giemsa stain were used to analyze the chromosomal aberrations after the treatment. The chromosome-fixed slides were prepared according to the normal slide preparation methodology in order to examine the chromosomal aberration patterns. For this, the incidence of structural abnormalities in about 300 widely distributed metaphases per concentration (single culture) was examined. A score was assigned to cells that had structural chromosomal abnormalities, including and excluding gaps. Chromatid and chromosome-type aberrations were logged and categorized individually (breaks and exchanges).

## **2.4 *In vivo* genotoxicity analysis**

### **2.4.1 Bone marrow micronucleus test of *Sc*-AgNPs in Swiss albino mice**

In accordance with OECD No. 474 criteria for evaluating the harmful effects of test compounds, this micronucleus study was conducted to evaluate the potential for genotoxicity of test Ag-NPs. The Institutional Animal Ethical Committee gave its clearance for the animal study (IAEC approval number for Nandha College of Pharmacy, Erode: NCP/IAEC/2020-21/43). Swiss albino mice that were 7 weeks old (25 males and 25 females) were utilized in this investigation. They were divided into five groups, each of which contained five males and five females, and they were raised in a polycarbonate cage according to conventional procedure.

For the genotoxicity research, group I (control) - mice once orally administered with distilled water only (5 mL/kg b.w.), group II- mice once orally administered with test Ag-NPs (250 mg/kg b.w.) in distilled water, group III- mice once orally administered with test Ag-NPs (500 mg/kg b.w.) in distilled water, group IV- mice once orally administered with test Ag-NPs (1000 mg/kg b.w.) in distilled water, group V (positive control)-mice treated once with



cyclophosphamide monohydrate (50 mg/kg b.w.) in distilled water by intraperitoneally (group V). All the group mice had received a single dose. After the 24 hours of dose administration, all the mice were killed by cervical displacement, and the remaining mice from each group had their femur bones harvested for their bone marrow. The bone marrow was flushed using fetal bovine serum. After applying and letting bone marrow cells air dry on the specimens, they were fixed with methanol in the slide, stained with a 4% Giemsa stain and acridine orange (40 mg/mL), and covered with mountable coverslips. Hayashi et al. [24] examined bone marrow slides under a light microscope for micronuclei and calculated the proportion of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE). Additionally, 500 erythrocytes and 4000 PCE per animal were scored in order to assess the ratio of PCE/NCE and the prevalence of micronucleated polychromatic erythrocytes (MNPCE) [31].

### **3 Results**

#### **3.1 *In vitro* cytotoxicity traits of tested Ag-NPs**

In a 96-well plate approach, the normal fibroblast cells were exposed to different concentrations (10–100 µg/mL) of test Ag-NPs. The findings of this investigation demonstrated that there was no detectable cytotoxicity activity, even at a test Ag-NP concentration of 100 µg/mL. The maximal cytotoxicity of the test Ag-NPs reached >16% at 100 µg/mL concentration (Fig. 1A), whereas the cytotoxicity of puromycin (a positive control) reached 98.7% at the same concentration (Fig. 1B).

### 3.2 *In vitro* genotoxicity traits of test Ag-NPs

The MTT experiment clearly demonstrated that oral fibroblast cells are not significantly cytotoxic to the Ag-NPs produced by the ethanolic extract of *Salacia chinensis* (measured as a 40% relative increase in cell count [RICC] over the concurrent negative control data). The chromosomal or chromatid abnormalities of treated cells were assessed using an *in vitro* genotoxicity assay on the CHO cell line in the presence and/or absence of exogenous S9 metabolisms activating homogenate mixes containing microsomal enzymes. Furthermore, it was compared with positive control and vehicle control. Phase I (short-term exposure without metabolic activation), Phase II (short-term exposure with metabolic activation), and Phase III (continuous exposure without metabolic activation) were performed, with negative, vehicle, and concurrent positive controls (20 µg/mL) included.

In Phase I, cultures were exposed for 4 h (short-term exposure) to a negative, vehicle, Ag-NPs, and a positive control without metabolic activation. The RICC values ranged from 96% (vehicle control) to 81.35% (at 50 µg/mL), 66.67% (at 100 µg/mL), and 47.92% (at 200 µg/mL). When compared to the vehicle control (0.33%), there was no significant increase in mean % aberrant cells (mean % aberrant cells of 0.33, 0.33, and 0.67 at 50, 100, and 200 µg/mL, respectively) (Table 1). The RICC values for phase II (with 1% v/v S9 mix) were 97.00% (vehicle control), 95.88% (at 50 µg/mL), 80.41% (at 100 µg/mL), and 44.33% (at 200 µg/mL). When compared to the vehicle control (0.33%), there was no significant increase in the mean percent aberrant cells (mean percent aberrant cells of 0.33, 0.67, and 0.67 at 50, 100, and 200 µg/mL, respectively) (Table 1). RICC values for phase III range from 92.58% (vehicle control) to 81.53% (at 50 µg/mL), 67.94% (at 100 µg/mL), and 44.95% (at 200 µg/mL). When compared to the vehicle control (0.33%), there was no significant increase in the mean percent aberrant

cells (mean percent aberrant cells of 0.33, 0.67, and 0.67 at 50, 100, and 200  $\mu\text{g/mL}$ , respectively) (Table 1).

**Figure 2** depicts the pattern of chromosomal abnormalities caused by exposure to test *Sc*-AgNPs at varied doses in CHO cell lines. It demonstrates that CHO cell lines are free of chromosomal abnormalities. The table 2 represents, the chromosomal aberrations such as chromatid gap, break, chromosome gap, break, and dicentric nature of positive control and it was significantly no aberration found. These findings strongly imply that there is no detectable genotoxicity of the crude ethanol extract of *Salacia chinensis* or the manufactured Ag-NPs in CHO cell lines.

### **3.3 Bone marrow micronucleus test**

No mortality or clinical indications were observed in any of the mice in the vehicle control group, the various doses of 250, 500, and 1000 mg/kg body weight, or the positive control (cyclophosphamide monohydrate, 50 mg/kg) groups. The slides were coded before scoring, and MNPCE (micronucleated polychromatic (immature) erythrocytes) were looked for under a microscope (Figure 3). The P/E ratios from polychromatic to total (polychromatic + normochromatic) red cells were calculated when a minimum of 500 normochromatic (mature) erythrocytes (NCE) were compared to their equivalent polychromatic erythrocytes. Micronucleated polychromatic erythrocytes were detected in a minimum of 4000 polychromatic red cells per mouse, and the proportion of these cells was measured (Supplementary Data 1 and Figure 3). Male or female mice given dosages of 250, 500, or 1000 mg/kg body weight did not exhibit toxic effects on the bone marrow (decrease in P/E ratio). Mice in the positive control group that received cyclophosphamide monohydrate at a dose of 50 mg/kg body weight have shown harmful effects on the bone marrow. According to the statistical analysis ( $P < 0.05$ ) of the

studies, mice from the dosing groups (both male and female) at concentrations of 250, 500, and 1000 mg/kg body weight did not significantly differ from the vehicle control group in terms of the percentage of micronucleated polychromatic (immature) erythrocytes ( $P < 0.05$ ). Mice of both the gender administered the Cyclophosphamide monohydrate showed a significant increase in MNPCE. Cyclophosphamide monohydrate (50 mg/kg body weight) demonstrated the sensitivity of an experimental setup, the suitability of the procedures, and the efficacy of the testing circumstances (Fig. 4).

#### 4. Discussion

Nanomaterials and nanoparticles have received a lot of attention recently due to their unique properties and possible applications in several biotechnology and life science assays. It is currently too early to tell whether nanobiotechnology may harm people, non-human biota, or ecosystems, despite its rapid progress and early acceptance. Furthermore, forecasts show that nanomaterials will harm the environment much more adversely in the future.

Synthesized NPs were revealed to have great biological promise, and several excellent organic sources have been disclosed as having excellent NPs manufacturing potential [32–34]. However, the study on biosynthesized NPs has not adequately examined their toxicity (cytotoxicity and genotoxicity) or adverse effects. Nevertheless, it must be taken into account as the most important component in studies pertaining to commercialization [35]. Therefore, in this investigation, pre-synthesized and characterized Ag-NPs were submitted to an assessment of their cytotoxicity and genotoxicity using an *in vitro* and *in vivo* approach, which was derived from an ethanolic extract of *Salacia chinensis*. Additionally, figure 4 illustrates the essential idea of this study.

Our findings were somewhat consistent with the observation that 100 µg/mL concentrations of fungal-derived test nanoparticles exhibited 27% cytotoxicity on a fibroblast cell line. However, they noted that the fungal-mediated Ag-NPs displayed about 65% of cytotoxicity at a higher concentration (512 µg/mL) [36–38]. Ag-NPs cytotoxicity studies are frequently carried out *in vitro* due to their ease of use, control of the chemical and physical environment, relative affordability, specificity, higher throughput, and also because the test results closely resemble those found *in vivo* [39]. Various descriptive and analytical procedures could be used to examine cytotoxicity *in vitro*; however, quantification experiments are preferred since they quantify the number of live cells [40]. This MTT assay, a colorimetric method, quantifies the mitochondrial succinate dehydrogenase's conversion of yellow MTT. Because MTT reduction can only occur in metabolically active cells, it is possible to estimate the proportion of living cells in a solution. Living cells have the ability to catalyze cell reductions [41]. The cytotoxicity was evaluated using a variety of cell lines, with oral fibroblasts serving as more biologically applicable test subjects than persistent fibroblasts [42]. We discovered that intracellular oxidative stress, which generates reactive oxygen species and harms cellular components like lipids, DNA, and proteins before finally leading to cell death, could be the source of cytotoxic effects. As a result, the current research reveals that Ag-NPs manufactured using green technologies have good cytotoxic effects and can be used as a source of chemotherapy for cancer.

In this examination, structural chromosome anomalies, including chromatid and chromosomal breaks, gaps, rings, fragments, and a dicentric chromosome, were found. Gaps were not included in the total aberration frequency because they were recorded and reported separately. These findings were notably novel because, according to a previous study, CHO-K1

exposed to Ag-NPs with 22 nm-sized particles at a concentration of 500 µg/mL for 24 hours exhibited genotoxicity [43]. This may be due to the Ag-NPs size and form, which may be to blame for their genotoxicity and ability to cause chromosomal abnormalities. In this investigation, Souza et al. [44] found that Ag-NPs with a size range of 10 nm had much lower cytotoxicity and genotoxicity than those with a size range of 100 nm. Previous research has demonstrated that Ag-NPs are cytotoxic to a variety of cells, including peripheral blood mononuclear cells, alveolar endothelial cells, neurohormonal cells, osteocytes, and many others, seeking to obstruct their cell activities and resulting in DNA damage to cells [45]. The processes behind these detrimental cytotoxic and genotoxic effects are characterized in terms of the impact of Ag-NPs on the formation of reactive oxygen species [46]. According to a previous report, oxidative stress is caused by the charged particles released from Ag-NPs, which were added in a Trojan horse-like manner [47].

To assess the genotoxicity characteristics of test Ag-NPs, this bone marrow micronucleus test is most frequently applied to mouse bone marrow cells. The OECD standards strongly recommend this bone marrow micronucleus test to evaluate the same [42]. To assess the genotoxicity damage (DNA lesions) and oxidative stress induction of nanomaterials, the EFSA has recommended the comet assay [1–48]. Unless one of the *in vitro* assays indicates mutagenicity or some of the *in vitro* experiments isn't suitable for evaluating NPs, an *in vivo* micronucleus analysis (OECD TG 474) [50] and/or comet assay (OECD TG 489) [51] and/or a genetically modified mouse germ cell mutation assay (OECD TG 488) [52] should be performed. In this investigation, we discovered that due to Ag-small NP's size, it may easily diffuse into the nucleus through the pores. Ag-NPs or certain Ag<sup>+</sup> ions inside the cell nucleus may bind to the DNA and enhance the DNA damage brought on by reactive oxygen species. According to a

recent discovery, Ag-NPs accumulation within an organism can be size- and dose-dependent. As a result, they might have a more profound effect on the bone marrow cells. Several writers who observed the accumulation of Ag-NPs in several organs of laboratory animals [53] and a dose-dependent rise in Ag-NPs in the blood hypothesized that Ag-NPs were dispersed systematically by circulating blood. Micronuclei can develop as a result of chromosomal fragments or lagging chromosomes during mitosis (MN). The correlation of MI with an increased frequency of mitotic abnormalities and chromosomal aberrations that is considerably less frequent at high concentrations than control reflects the cytotoxic and genotoxic capability of the Ag-NPs photosynthesized in the plant extract.

## 5. Conclusion

We have shown in the current investigation that the fibroblast cell line was not harmed by the produced Ag-NPs from the ethanolic extract of *Salacia chinensis*. Even at a concentration of 200 µg/mL of test Ag-NPs, the results of the mutagenic assay on the CHO cell line show that there was no genotoxicity. Ag-NPs at a concentration of 1000 mg/kg did not result in any genotoxicity in experimental animals, according to research on bone marrow micronuclei and *in vivo* genotoxicity in Swiss albino mice. These results suggested that when examined *in vitro* and *in vivo* experiment, the produced Ag-NPs did not significantly cause cytotoxicity or genotoxicity. Consequently, in a range where there are no genotoxic and cytotoxic effects, employing biogenic Ag-NPs might be a possibility.

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### **Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

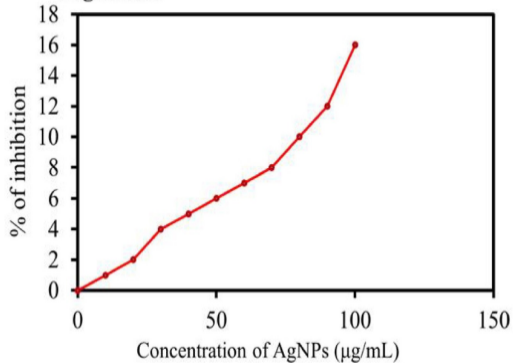
**Figure 1. Cytotoxicity profile oral fibroblast cell lines through MTT assay; Figure 1A. Cytotoxicity profile of test AgNPs on oral fibroblast cell lines through MTT assay; Figure 1B. Cytotoxicity profile of test puromycin on oral fibroblast cell lines through MTT assay**  
The values provided with different superscripts remains significant at  $P < 0.05$ .

**Figure 2. Chromosomal aberration pattern of CHO cells treated with various concentrations of test AgNPs**

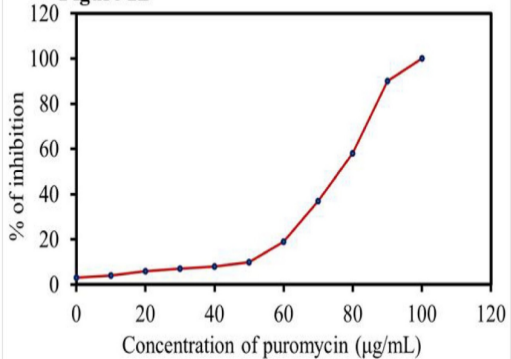
**Figure 3. Bone marrow micronucleus test profiles of albino mice exposed to various dosage of test AgNPs**

**Figure 4. Schematic diagram of this research**

**Figure 1A**

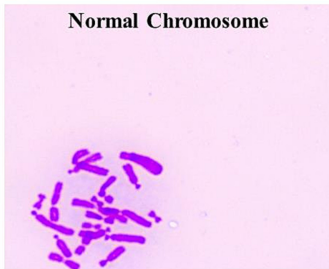


**Figure 1B**



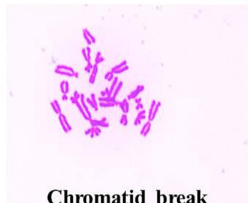
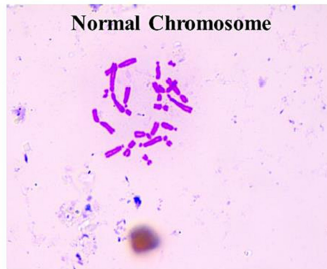
**Negative Control**

**Normal Chromosome**



**Vehicle Control**

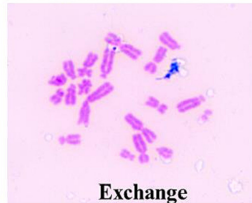
**Normal Chromosome**



**Chromatid break**



**Dicentric**



**Exchange**



**Minutes**

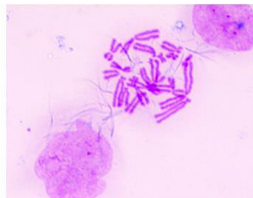
**Positive Control-Benzo[a]pyrene**

**Positive Control-Methyl methanesulfoante**

**Test AgNPs**



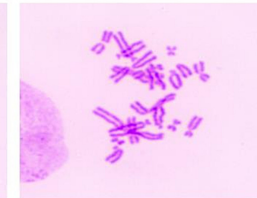
**0 µg/mL**



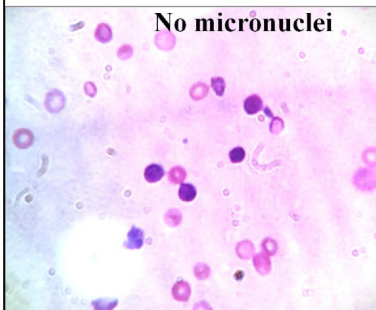
**50 µg/mL**



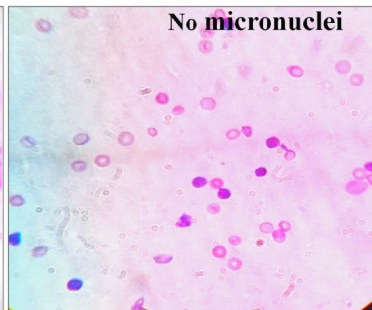
**100 µg/mL**



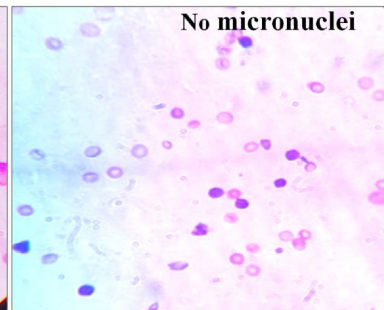
**200 µg/mL**



**No micronuclei**



**No micronuclei**

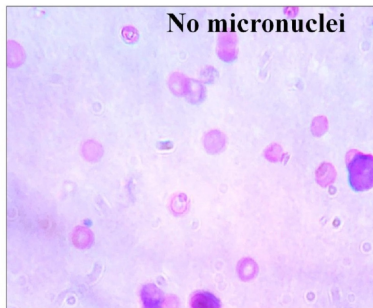


**No micronuclei**

**Vehicle Control – Distilled water**

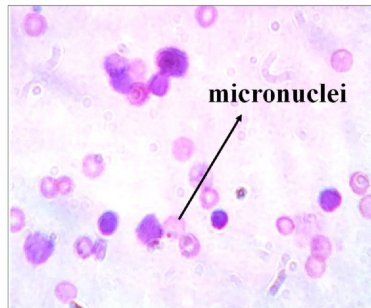
**250 mg/kg body weight**

**500 mg/kg body weight**



**No micronuclei**

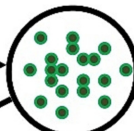
**1000 mg/kg body weight**



**micronuclei**

**Positive Control cyclophosphamide monohydrate (50 mg/kg)**

*Salacia chinensis*



Test AgNPs

Animal study

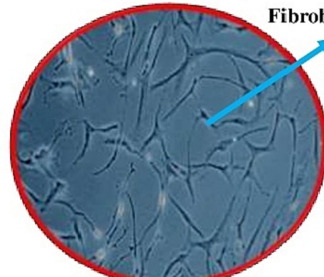
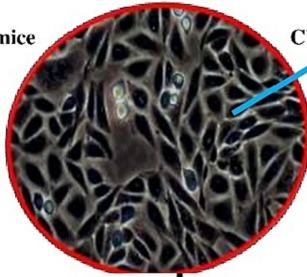
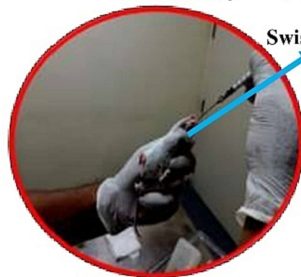
Genotoxicity study

Cytotoxicity study

Swiss Albino mice

CHO cells

Fibroblast cells



Bone marrow micronucleus test

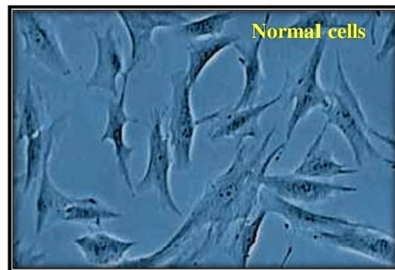
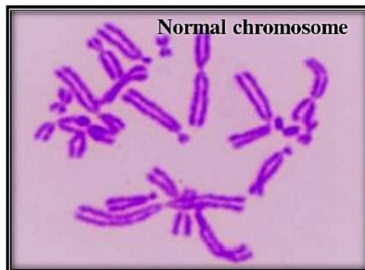
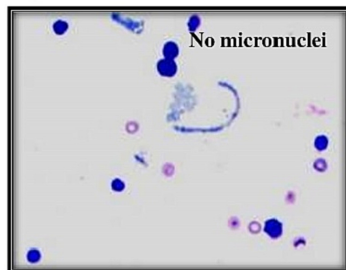
Chromosome aberrations test

Cytotoxicity

No micronuclei

Normal chromosome

Normal cells



Treated with AgNPs

Treated with AgNPs

Treated with AgNPs

**Table 1. Relative Increase in Cell Counts (RICC) of CHO cells exposed to various phases with various concentrations of test *Sc*-AgNPs**

Treatment	Concentration ( $\mu\text{g}/\text{mL}$ )	Cell count		RICC	Cytotoxicity (%)
		Starting	Final		
<b>Phase I -Absence of Metabolic activation (Short term)</b>					
NC	-	1000000	2000000	100.00	0.00
VC	-	1000000	1960000	96.00	4.00
T1	50	1000000	1781000	81.35	18.65
T2	100	1000000	1640000	66.67	33.33
T3	200	1000000	1460000	47.92	52.08
PC	20	1000000	1630000	65.63	34.38
<b>Phase II -Presence of Metabolic activation (Short term)</b>					
NC	-	1000000	2000000	100.00	0.00
VC	-	1000000	1970000	97.00	3.00
T1	50	1000000	1930000	95.88	4.12
T2	100	1000000	1780000	80.41	19.59
T3	200	1000000	1430000	44.33	55.67
PC	20	1000000	1650000	67.01	32.99
<b>Phase III -Absence of Metabolic activation (Continuous)</b>					
NC	-	1000000	4100000	100.00	0.00
VC	-	1000000	3870000	92.58	7.42
T1	50	1000000	3340000	81.53	18.47
T2	100	1000000	2950000	67.94	32.06
T3	200	1000000	2290000	44.95	55.05

PC	20	1000000	2700000	59.23	40.77
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**Legend:** NC - Negative Control, VC - Vehicle Control, PC - Positive Control, RICC = Relative Increase in Cell Counts,  $\mu\text{g}$  – Microgram, mL – Milliliter.

**Table 2. Individual analysis of chromosome aberrations**

Treatment ( $\mu\text{g}/\text{mL}$ )	No. of Metaphases	Frequencies of Aberration	Total No of Aberrant cells	
			with gap	without gap
<b>Phase I: Absence of metabolic activation (short term)</b>				
NC: 0	300	-	0	0
VC: 0	300	1 Ctb	1	1
T1: 50	300	1 Ctb	1	1
T2: 100	300	2 Ctb	1	1
T3: 200	300	2 Ctb, 1 Csb	2	2
PC: 20	300	9 Ctb, 2 ctg, 7 dic, 1 ring, 4 fragment, 6 minutes, 1 exchange	21	20
<b>Phase II: Presence of metabolic activation (short term)</b>				
NC: 0	300	1 Ctb	1	1
VC: 0	300	1 Csb, 1 Ctg	2	1
T1: 50	300	1 Ctb	1	1
T2: 100	300	1 Ctb, 1 dic	2	2
T3: 200	300	2 Ctb	2	2
PC: 20	300	12 Ctb, 1 Csb, 3 dic, 8 fragments, 1 minute,	22	21
<b>Phase III: Absence of metabolic activation (Continuous)</b>				
NC: 0	300	1 Ctb	1	1
VC: 0	300	1 Ctb	1	1
T1: 50	300	1 Ctb	1	1
T2: 100	300	2 Ctb	2	2
T3: 200	300	2 Ctb, 1 Ctg	3	2
PC: 20	300	12 Ctb, 5 dicentric, 1 Ctg, 5 fragments, 1 Csb	23	23



Legend: NC - Negative Control, VC - Vehicle control, PC - Positive Control (methyl methanesulfonate), T1, T2, and T3 - Test concentrations of *Sc*-AgNPs, Ctg - Chromatid gap, Csg - Chromosome gap, Ctb - Chromatid break, Csb - Chromosome break, dic - dicentric.

## Highlights

- The AgNPs not causing cytotoxicity on fibroblast cellline at  $100 \mu\text{g mL}^{-1}$  concentration
- No chromosomal aberration found in CHO cellline at  $200 \mu\text{g mL}^{-1}$  dosage of AgNPs
- The AgNPs not inducing any genotoxicity on both in-vitro and in-vivo studies
- The animal study proved that absence of genotoxicity on bone marrow micronucleus test
- *S. chinensis* synthesized AgNPs not causing cytotoxicity and genotoxicity