Evaluation of Chemopreventive Potential and Regulated Toxicity of a Phyto-fabricated Silver Nanoformulation using Bark Extract of *Saraca asoca* in Acute Myeloid Leukemia *in vivo* and *in vitro*

**Pratyusha Banerjee, Puspendu Roy, Debjani Nath**

Department of Zoology, University of Kalyani, West Bengal, India

*Corresponding author:* Debjani Nath, Department of Zoology, University Of Kalyani, Kalyani Nadia, West Bengal 741235, India


**Received Date:** 28 December, 2021; **Accepted Date:** 06 January, 2022; **Published Date:** 11 January, 2022

**Abstract**

**Background:** Acute myeloid leukemia (AML) results impaired production of myeloid blood cell which leads to the reduction of erythrocytes, lymphocytes, and platelets. In contrast to the conventional treatment like chemotherapy, radiotherapy and stem cell transplantation, plant phytochemicals can be used as nontoxic chemopreventive agents to provide long-term therapeutic effect. In this study, we have analyzed the efficacy of the phyto-fabricated silver nanoparticle using the bark extract of *Saraca asoca* as a reducing agent against the secondary AML mice model both *in vivo* and *in vitro* in comparison to common anticancer drug doxorubicin.

**Methods:** The efficacy of silver nanoformulation was tested *in vivo* using secondary model of AML by survivability assay in comparison with control and standard chemotherapeutic drug doxorubicin. The cytotoxic and genotoxic potential of the nanoparticle *in vitro* was studied in leukemic cells in comparison to normal lymphocytes. The level of cytotoxicity was measured using cell viability assay, generation of percentage of nitric oxide, reactive oxygen species and mitochondrial membrane potential. The genotoxic potential was evaluated by using DAPI staining, comet assay, and DNA fragmentation assay and quantification of percentage of apoptosis and necrosis. The damage of leukemic cells was evaluated by transmission electron microscopy.

**Results:** The percent survivability was increased maximum by 50% in comparison to that of untreated group of AML mice whereas mice treated with standard drug doxorubicin showed 83% survivability. But AgNPs were selectively cytotoxic towards leukemic lymphocyte cells through oxidative damage unlike the standard chemotherapeutic drug as doxorubicin induce general impact of DNA damage inhibiting cell proliferation, impairing mitochondrial function and cell death in normal cellular system too.

**Conclusions:** This finding opens a new avenue for the biomedical application of phyto-fabricated AgNPs as nontoxic agents in chemoprevention of AML.

**Keywords:** Acute myeloid leukemia; Silver nanoparticle; Chemoprevention; Genotoxicity; Cytotoxicity; Doxorubicin; Regulated toxicity; Sustainable therapy

**Background**

Acute myeloid leukemia (AML) is one of the most complex types of leukemia that needs new and advanced treatment. The impaired production of myeloid blood cells or pancytopenia of AML leads to the reduction of erythrocytes, lymphocytes, and platelets. AML occurs due to exposure to agents like smoking, certain chemicals like alkylating agents, platinum and benzene,

Methods

Chemicals

Osmium tetroxide, Glutaraldehyde (electron microscopy grade), 4',6-diamidino-2-phenylindole dihydrochloride (DAPI), 2,7-dichlorofluorescein di-acetate (DCFH-DA) dye, Histopaque, doxorubicin (purchased from Sigma Aldrich Chemical Company, Mumbai, India). Annexin V- FITC apoptosis detection kit was purchased from Invitrogen, Thermo Fisher Scientific (Mumbai, India).

Animals

All the animal experimentations were performed strictly adhering to the recommendation of Institutional animal ethics committee of the institution (Registration no: 892/GO/Re/S/01/CPCSEA). A total of 30 Swiss albino male mice (Mus musculus) aged 6-8 weeks from specified strain were purchased from a registered supplier. All the animals were maintained in stainless steel wire cages (Tarsons, India) under 12-hour light-dark cycle. Pellet diet (West Bengal Diary and Poultry Development Corp. Ltd., Kalyani Industrial Area, Kalyani, WB India) was provided and water was supplied ad libitum automatically through the tubing during the study period.

Secondary AML mice model

The secondary AML mice model was established following the methodology of Saha et al. [2012] [28]. Liquid benzene (HPLC grade) (MERCK, India) was vaporized by heating at 16°C and then the vapor was channeled into the inhalation chamber (1.3 m²). A total of 24 mice were exposed to 300 ppm benzene (in vapor form) for 6 h/day, 5 days/week for 2 weeks. Cumulative exposure= ppm × Number of hours × Number of days. Temperature and humidity were maintained automatically at (24±1)°C and (55±10)%, respectively inside the chambers.

Green phyto-fabricated silver nanoparticle

Green phyto-fabricated silver nanoparticles were synthesized using aqueous bark extract of Saraca asoca as the reducing agent [29]. The taxonomic identification of plant material was confirmed by Dr. GG Maity, Professor of Taxonomy, Taxonomy, and Plant
Systematic Unit, Department of Botany, University Of Kalyani. The voucher specimens (Deb.kly-60) was deposited and preserved in the Department of Botany. The LD50 of the synthesized silver nanoparticle was 2.165 mg/kg body weight (Table 1). Two selected doses (for in vivo) were 43 mg/kg b.w and 86 mg/kg b.w. which were actually the 1/50th and 1/25th fraction of the determined LD50 of the green silver nanoparticle. Doses were selected as per the guidelines of OECD (2001) [30].

**Table 1.** LD5 to LD90 values of synthesized silver nanoparticle

<table>
<thead>
<tr>
<th>Percentile</th>
<th>Probit</th>
<th>Dose (mg/kg) Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>3.36</td>
<td>720.02 ± 330.51</td>
</tr>
<tr>
<td>10</td>
<td>3.72</td>
<td>908.05 ± 358.37</td>
</tr>
<tr>
<td>20</td>
<td>4.16</td>
<td>1142.45 ± 398.48</td>
</tr>
<tr>
<td>30</td>
<td>4.48</td>
<td>1403.09 ± 412.11</td>
</tr>
<tr>
<td>40</td>
<td>4.75</td>
<td>180936 ± 421.15</td>
</tr>
<tr>
<td>50</td>
<td>5.00</td>
<td>2165.26 ± 452.17</td>
</tr>
<tr>
<td>60</td>
<td>5.25</td>
<td>2673.28 ± 491.44</td>
</tr>
<tr>
<td>70</td>
<td>5.52</td>
<td>3113.51 ± 615.91</td>
</tr>
<tr>
<td>80</td>
<td>5.84</td>
<td>3768.40 ± 903.93</td>
</tr>
<tr>
<td>90</td>
<td>6.28</td>
<td>5322.89 ± 1647.36</td>
</tr>
</tbody>
</table>

Study of anti-leukemic activity of phyto-fabricated silver nanoparticle in secondaryAML mice model

**Experimental design**

The treatments (the synthesized silver nanoparticle and the standard drug) were given by injection i.p. every second day up to 30 days. Overall survival was recorded on the 35th day of the study. The groups included Group I : the control group normal healthy mice without any disease and received no treatment; Group II : AML mice received no treatment; Group III: AML mice treated with phyto-fabricated AgNPs at 43 µg/ml b.w.; Group IV: AML mice treated with phyto-fabricated AgNPs at 86 µg/ml b.w.; Group V : AML mice treated with doxorubicin at 2 mg/kg b.w.

**Survivability assay**

The study was conducted for a period of 35 days. Kaplan-Meier [1958] [31] analysis was performed to determine the in vivo survival distributions. The log-rank method was used to compare the survival curves among groups.

Cytotoxicity and genotoxicity of the phyto-fabricated silver nanoparticle

**Cell sample preparation**

Lymphocytes were isolated from the bone marrow of the secondary AML mice using Histopaque1077 (Sigma–Aldrich, St. Louis, MO, USA) following the methodology of Thorsby [32] with modifications. The isolated leukemic lymphocytes were incubated with different concentrations of the phyto-fabricated AgNPs (40 µg/mL and 80 µg/mL) and standard drug doxorubicin (at a concentration of 1 µM) in RPMI-1640 media for 3 hours at 37°C. Normal lymphocyte cells treated with phyto-fabricated AgNPs at the highest concentration were the control system Concentration at 10g/mL and 20 g/mL of silver nanoparticle when tested in vivo (survivability assay) showed insignificant improvement in comparison with the standard drug Doxorubicin. Thus for the in vitro experiments with AML cells 40µg/mL and 80µg/mL concentration of AgNPs were tested.

**Cell viability assay**

Cell viability was checked by the trypan blue exclusion method described by Tennant [1964] [33]. Trypan blue at 0.4% solution in a buffered isotonic salt solution, pH 7.2 to 7.3 (i.e., phosphate-buffered saline) was added to 1 mL of cell suspension (10⁶ cells/mL). Cell viability should be at least 95% for a healthy log-phase. The percent of viable cells was calculated using the formula: % of viable cells = (1.00 × Number of blue cells/ Number of total cells) × 100 and percent Viability reduction = [Total Viable cells before treatment (Unstained) - Total Viable cells after treatment (Unstained)] / Total cells (Viable +Dead)] X 100.

**Determination of nitric oxide (NO)**

Nitric oxide basically is generated from sodium nitroprusside and it is measured by the Griess reaction [34]. The incubation time was five hours so that the part of the generated nitric oxide was scavenged. The amount of leftover nitric oxide was calculated. The absorbance of the chromophore was then measured at 546 nm. Percentage of scavenging activity = (OD of control / OD of the test) × 100

**Reactive oxygen species (ROS) generation by DCFDA assay**

The generation of ROS was detected by DCFH-DA fluorescence. The cell suspension containing approximately 10⁶ cells per tube was taken for the flow cytometric analysis. The generation of ROS was detected following the methodology of Roy et al.[2008] [35]. The samples were normal cells treated with highest concentration of AgNPs (80µg/ml); Leukemic lymphocytes treated with two different concentrations of AgNPs...
(40 μg/ml and 80 μg/ml); leukemic lymphocytes treated with standard drug (1 μM). DCF was excited by the 488 nm laser and detected at 535 nm (typically FL1). Ideally, 10,000 cells were analyzed per experimental condition. Mean fluorescent intensity change was tested among the treated normal control, positive control and treated diseased cells samples.

### Determination of mitochondrial membrane potential (MMP)

Change in the MMP was determined following the methodology of Dash et al. [2013] [36] by flow cytometry at the single-cell layer. Four groups of animals were tested for MMP. **Group 1:** Normal lymphocytes treated with phyto-fabricated AgNPs at 80 μg/mL concentration (control). **Group 2:** AML cells treated with phyto-fabricated AgNPs at a concentration of 40 μg/ml. **Group 3:** AML cells treated with phyto-fabricated silver nanoparticle at a concentration of 80 μg/mL. **Group 4:** AML cells treated with standard drug doxorubicin at a concentration of 1 μM.

The cells were stained with the dye DiOC6 by incubating for 15 min at 37°C in the complete dark in phosphate buffer saline. Loss of DiOC6 fluorescence indicates the disruption of the mitochondrial inner transmembrane potential. The fluorescence was detected at an excitation wavelength of 488 nm and an emission wavelength of 530 nm. The fluorescence intensity was measured on a FACS Calibur (Becton Dickinson), and data were analyzed using WINMDI 2.9 software, representing the mean fluorescence intensity.

### DAPI

Analysis of the nuclear membrane integrity of four groups of cells as mentioned above was performed by DAPI following the methodology of Mollick et al. [2014] [37]. Cells were exposed to 300 nM DAPI stain solution. Cells were imaged under a fluorescence microscope at 340 nm.

### Analysis of apoptotic and necrotic cells

Three groups of cells were treated for testing Apoptosis. **Group 1:** Normal lymphocytes treated with phyto-fabricated AgNPs at 80 μg/mL concentration. **Group 2:** AML cells treated with phyto-fabricated silver nanoparticle at a concentration of 80 μg/mL. **Group 3:** AML cells treated with standard drug doxorubicin at a concentration of 1 μM.

Alexa Fluor® 488 annexin V and propidium iodide (PI) (Invitrogen) assay was performed according to the manufacturer’s protocol to quantify the number of apoptotic and necrotic cells. After staining with Alexa Fluor® 488 annexin V and PI the apoptotic cells show green fluorescence, dead cells show red and green fluorescence, and live cells show little or no fluorescence.

The cells were washed in cold phosphate-buffered saline. Finally, 5 μL Alexa Fluor® 488 annexin V and 1 μL 100 μg/mL of PI working solution was added to each 100 μL of cell suspension. The stained cells were analyzed by flow cytometry at an excitation wavelength of 488 nm, the fluorescence was measured at the emission wavelength of 530 nm and 575 nm (or equivalent).

### Comet assay

Analysis of the extent of DNA damage was assessed in three groups as mentioned above by comet assay following the methodology of Sing et al. [1988] [38]. Following unwinding, the DNA is electrophoresed and stained with a fluorescent dye. DNA migration was analyzed by fluorescence microscopy (excitation filter 515–560 nm and a barrier filter of 590 nm) attached with a CCD camera. The images were analyzed by the Komet version 5.5 auto image analysis software. Quantitative and qualitative analysis of DNA damage was determined by measuring the length of DNA migration and the percentage of migrated DNA in the cells to calculate the tail length.

### DNA fragmentation assay

DNA was extracted from both the leukemic lymphocyte cells and control cells in three groups of cells as mentioned above following the protocol suggested by Paul et al. [2011] [39]. After the treatment, the cells were lysed and DNA was extracted with a mixture of phenol, chloroform, and isoamyl alcohol (25:24:1) and electrophoretically separated on a 2% agarose gel containing 1 μg/mL ethidium bromide and visualized under ultraviolet transillumination.

### Study of cellular uptake and accumulation by transmission electron microscope (TEM)

A modified cytotoxicity test with AML cells treated with phyto-fabricated AgNPs was carried out by TEM imaging following the methodology of Asharani et al. [2009] [40] for the qualitative assessment. AML cells incubated with phyto-fabricated AgNPs (80 μg/mL) were evaluated for structural alterations and phyto-fabricated AgNPs accumulation. Embedded samples were fixed with 2% glutaraldehyde and then post fixed with 2% osmium tetroxide. Ultrathin sections were cut with glass knives and examined in a Zeiss EM910 transmission electron microscope at an acceleration voltage of 80 kV.

### Statistical analysis

The log-rank method was used to compare the survival curves among groups. All data are expressed as means ± SD (Standard deviation of the Mean) and were compared with the ANOVA (n=5), p < 0.05 was taken as the level of significance after doing T test.
Results

Survivability assay

Figure 1A represents the survivability percentage of different groups. At the end of 35 days of the study period, the survivability was significantly increased ($P<0.05$) in the group treated with 43 mg/kg b.w and 86 mg/kg b.w. of phyto-fabricated AgNPs. The normal control group showed 100% survivability whereas the untreated AML group showed only 17% survivability. Mice treated with phyto-fabricated AgNPs at 43 mg/kg b.w. revealed an increase of 33% and 50% at 86 mg/kg b.w. in comparison to that of the untreated group. Mice treated with standard drug at 2 mg/kg b.w. showed the highest survivability (Figure 1A).

![Survivability assay](image)

Figure 1: Survivability time. A: Survivability percentages of different groups of animals. B: Survival curve.

Cytotoxicity and genotoxicity of phyto-fabricated silver nanoparticles in leukemic lymphocytes

Cell viability test by trypan blue exclusion method

Figure 2A shows the cell viability of the leukemic lymphocyte cells treated with different concentrations (10 µg/mL, 20 µg/mL, 40 µg/mL and 80 µg/mL) of phyto-fabricated AgNPs and treated with standard drug doxorubicin at 1 µM. The viability of leukemic lymphocyte cells was reduced by 89.14% in the treatment of phyto-fabricated AgNPs at 80 µg/mL, and by 91.23% in doxorubicin treated leukemic lymphocytes. The viability of leukemic lymphocytes was significantly reduced when treated with different concentrations of phyto-fabricated AgNPs in a dose-dependent manner ($P<0.05$).
NO release level

Phyto-fabricated AgNPs at 80 µg/mL significantly ($P<0.05$) increase NO level to 63.4% in the leukemic lymphocyte cells as compared to the control and the value was close to the level of NO (68.7%) in the leukemic lymphocytes treated with doxorubicin (Figure 2B). No significant increase in the level of NO was found in the normal cells treated with the highest concentration of phyto-fabricated AgNPs.

Alteration in MMP

In this study, the MMP of the leukemic lymphocytes treated with phyto-fabricated AgNPs was depleted significantly in comparison to the control lymphocytes; similar depletion was also found in the doxorubicin treated leukemic lymphocyte cells. The percentage of MMP was decreased in a dose-dependent manner. Phyto-fabricated AgNPs at 80 µg/mL induced a significantly higher decrease in MMP by 72.7% ($P<0.05$) in comparison to the control (Figure 2C).

ROS generation

The intracellular concentration of ROS (Figure 3) was significantly ($P<0.05$) higher in the leukemic lymphocytes treated with phyto-fabricated AgNPs at 80 µg/mL when compared to the control. In phyto-fabricated AgNPs at 80 µg/mL treated cells, the ROS was elevated by 53.7% (2.26 fold compared to control or normal cells treated with AgNPs at 80 µg/mL) and in doxorubicin treated cells the ROS was elevated by 66.76% (2.81 fold compared to control).
Figure 3. Flow cytometric analysis (A) and fold change (B) of ROS generation. (a): control cells with AgNP at 80 µg/mL; (b) leukemic lymphocytes treated with AgNPs (40 µg/mL); (c) leukemic lymphocytes treated with AgNP (80 µg/mL); (d) leukemic lymphocytes treated with standard drug doxorubicin (1 µM).

Fluorescence study by DAPI staining

Imaging of cells after staining with DAPI revealed chromatin condensation and fragmentation in leukemic lymphocytes treated with phyto-fabricated AgNPs at 80 µg/mL (Figure 4B). A similar change in the nuclear material was also observed in leukemic cells treated with doxorubicin (Figure 4C). But normal lymphocytes treated with the highest concentration of phyto-fabricated AgNPs showed no typical characteristics of apoptosis such as nuclear condensation or cell blebbing (Figure 4A).

We also found a significant ($P<0.05$) increase in both apoptosis and necrosis in the leukemic lymphocyte cells treated with phyto-fabricated AgNPs (Figure 5A). The result was similar in the case of the doxorubicin treated leukemic lymphocyte cell population (Figure 5A). A significant increase in the number of necrotic cells was observed at the highest concentration of phyto-fabricated AgNPs (Figure 5B).

Figure 4. Tests of cytotoxicity by staining with DAPI. A. Control cells treated with phyto-fabricated AgNPs; B: leukemic lymphocytes treated with phyto-fabricated AgNPs; C: leukemic lymphocytes treated with doxorubicin.
Estimation of DNA damage by Comet and DNA fragmentation assay

After the treatment with phyto-fabricated AgNPs, the damaged DNA of the leukemic lymphocytes was observed by comet assay (Figure 6A&B). The quantification of the damaged DNA was determined by comparing the tail length of the comet between the leukemic lymphocyte cells treated with phyto-fabricated AgNPs with the control cell population. A significant ($P < 0.05$) increase in DNA damage was observed in both phyto-fabricated AgNPs at 80µg/mL and doxorubicin treated leukemic lymphocytes (Figure 6C). In the phyto-fabricated AgNPs treated AML cells, the tail length of DNA was approximately 11 fold higher whereas in doxorubicin treated cells it was 14 fold higher than the value of comet obtained in the control cells (normal lymphocytes treated with phyto-fabricated AgNPs) (Figure 6C).

![Figure 5](image_url)

**Figure 5.** Assessment of apoptosis and necrosis. A: Flow cytometric analysis (a) Control cell (with AgNPs); (b) after treatment with AgNPs(80µg/ml); (c) leukemic lymphocytes treated with standard chemotherapeutic drug doxorubicin. B: Comparison among the percentage of leukemiclymphocytes at different phages of cell death.
Uptake and structural alterations of AML cells by TEM

AML cells incubated with phyto-fabricated AgNPs were characterized by different degrees of deformities like damaged cell membranes, extensive vacuolation, and vesicular degeneration. Features like loss of nuclear organization with the ruptured plasma membrane and shrinkage of the protoplast (Figure 7A) were observed AML cells treated with phyto-fabricated AgNPs. The localization of nanoparticles was observed in the vesicles (Figure 7E) of the cells. Extensive vacuole formation (Figure 7D) and lysosomes with partly degraded content (Figure 7C) were also observed.
Discussion

Very few reports are available on the efficacy of green silver nanomaterials in the treatment of AML cells [26, 41]. We have developed eco-friendly, phyto-fabricated silver nanoparticles using the aqueous bark extract of the plant Saraca asoca following the green chemistry method [29]. The average range of particle size calculated using dynamic light scattering measurements was 3-10 nm [29]. AFM analysis showed the presence of almost spherical shaped particles within the size range of <5 nm. FTIR analysis indicated the involvement of carboxyl (-C=O), hydroxyl (-OH) and amine (-NH) functional groups of the phytochemicals in capping and stabilizing silver nanoparticles. The capping and/or stabilizing materials are phyto-compounds like 1, 2, 4-triazole and diethyl acetylenedicarboxylate with relatively higher abundance (data not shown). The rapid electrokinetic behavior of the silver was evaluated using zeta potential (approx -23.2 mV) to confirm its stability [29].

The therapeutic study of the synthesized phyto-fabricated silver nanoparticle [42,43] revealed that the survivability was increased by 50% in comparison to that of untreated AML mice whereas mice treated with doxorubicin (2 mg/kg b.w.) showed 83% survivability. This study demonstrated that phyto-fabricated silver nanoparticles could effectively prolong the survival time in vivo. The cytotoxicity and genotoxicity of the phyto-fabricated silver nanoparticle were compared with the normal lymphocytes and AML cells in vitro to confirm the target-specific toxicity of the capped nanoparticle as reported by Netchareonsirisuk et al. [2016] [44]. We also confirmed the dose-dependent toxicity of the synthesized phyto-fabricated silver nanoparticle in AML cells. Previous evidences were available stating that exposure to AgNPs at different concentrations can cause dose-dependent toxicity by inducing oxidative stress and DNA damage which ultimately leads to cell death [45].

The result of the trypan blue exclusion method also revealed the dose-dependent toxicity of the green AgNPs as the reduction in the viability of leukemic lymphocytes was increased from 50% to 89.14% at 80 µg/mL whereas normal lymphocytes treated at a concentration of 80µg/mL showed no significant reduction. This
finding confirmed the cell-specific cytotoxicity of the synthesized phyto-fabricated silver nanoparticle. Many researchers showed that silver nanoparticles can cause DNA damage and stimulate oxidative stress to those cells with a lack in their capacity to repair damaged DNA or other oxidative stress [45]. In our study, nanosilver with their phytochemical capping on its surface inhibited the viability of the cells by inducing oxidative stress. The presence of triazole is assumed to have induced the cytotoxicity of AgNPs as it has proven anti-cancerous activity against a wide range of cancerous cells [46]. So the potential vulnerability of the AML cells to the cytotoxicity of the synthesized green silver nanoparticle can be exploited to develop these green AgNPs as new therapeutic agents in AML treatment.

The elevated production of NO in the cell after exposure to silver nanoparticles is also crucial in asserting its cytotoxic ability because in reaction with superoxide NO usually produces more toxic peroxynitrite (ONOO\(^-\)) which attributes to severe oxidative damage leading to cell death [36]. In our study, NO level in the leukemic lymphocyte cells was 63.4% which was close to the value of doxorubicin treated AML cells where the value was 68.7%. No significant increase of NO was found in the control cells treated with the green AgNPs (data not shown). Thus the elevated level of NO in the green AgNPs treated leukemic lymphocytes contributed to the severe oxidative injury leading to the death of AML cells.

One possible mechanism to induce cell death is by promoting the generation of ROS. Normal cellular growth and survival are controlled by maintaining homeostasis in cellular ROS [47]. By estimating the amount of intracellular ROS the vulnerability of the cell to oxidative stress can be determined. In our study generation of ROS was elevated by 2.26 fold (53.7%) after exposure to the synthesized silver nanoparticle in comparison to the control where the production of ROS was moderate (23.76%). An excessive amount of ROS attributes to cell death by two pathways either apoptosis or necrosis. In our study, significant (P<0.05) elevation of ROS in AML cells after treatment with AgNPs is due to the cytotoxic efficacy of the synthesized green AgNPs. The toxic effect of nanoparticles is due to its small size (<5 nm) and the power of penetration of biological membrane barriers to reach different organs. On the other hand, surface functionalization by the phyto-components increased the chemical reactivity inside the cell leading to the excess generation of ROS [48].

The apoptotic cells can be identified by the loss of mitochondrial membrane integrity though in living cells the mitochondrial membrane integrity remains unchanged. The percentage of MMP decreased significantly (P<0.05) with the increase in the concentration of AgNPs. After treatment with the phyto-fabricated silver nanoparticle, the depletion in MMP was 2.4 fold at 40 µg/mL, 2.95 fold at 80 µg/mL and in doxorubicin treated cells it was 3.4 fold. The dissipation in MMP in the AgNPs exposed AML cells are assumed to be disrupted of their mitochondrial membrane.

The quantification of the percent of apoptosis and necrosis also revealed that the phyto- fabricated silver nanoparticles were toxic to the AML cells in a dose-dependent manner, green AgNPs at highest concentration increased the percent of apoptotic cells by 1.5 fold in comparison to control, and the percent of necrosis was higher than apoptosis in this group of cells. After exposure, the increase in ROS and subsequent decrease in MMP might be the reason for the induction of apoptosis and necrosis in the green AgNPs treated cells. DAPI staining and imaging of the AML cells showed condensed and fragmented chromatin as well as cell blebbing in AML cells after treatment with nanoparticles, a similar result was obtained when AML cells were treated with doxorubicin indicating that the synthesized phyto-fabricated silver nanoparticle was also able to induce death of leukemic cells like that of standard chemotherapeutic drug. The disruption in chromatin structure contributes to cell death by turning on the apoptotic process [37]. Comet tail length of the AML cells treated with AgNPs and doxorubicin in comparison with control cells concurrent with this observation. The value of tail DNA was approximately 11 fold higher than the control whereas in doxorubicin treated cells it was approximately 14 fold higher. The presence of comet tail was due to the oxidative attack causing DNA damage which was attributed by the cytotoxic effect of the green silver nanoparticle. The damage of the cellular architecture was shown by TEM images of the AML cells treated with phyto-fabricated silver nanoparticles. The damage was due to morphological alterations, extensive vacuolation, loss of nuclear organization, ruptured plasma membrane, partly degradation of lysosome contents, and shrinkage of the protoplast associated with apoptotic/necrotic cell death induced by the phyto-fabricated AgNPs after internalization into the cellular vesicles. These findings thus confirmed the uptake of green AgNPs by the AML cells and also the induced cytotoxicity due to oxidative damage.

**Conclusions**

We have demonstrated that the synthesized phyto-fabricated silver nanoparticle has a unique toxicity profile. The AML cells were vulnerable to the toxicity of the green silver nanoparticle in a highly selective manner which was in agreement with other published reports (49, 50). Furthermore, we found that the phyto-fabricated AgNPs were selectively cytotoxic to the AML cells at higher concentration but it showed no such effect on the non leukemic control lymphocytes. A recent study demonstrated that the cytotoxicity of AgNPs might be approx.2-fold more in acute myeloid leukemia cells in comparison to the normal bone marrow derived lymphocytes (26) in concurrence with our observations. To develop the phyto-fabricated silver nanoparticle as a new line of treatment of AML, it was necessary to determine the sensitivity
of both leukemic and non-leukemic cells towards the green AgNPs and also its comparative cytotoxic efficacy against standard chemotherapeutic drug. The analysis through the comparative study including multiple criteria like cell viability, release of NO, production of ROS, change in mitochondrial membrane potential, induction of apoptosis and necrosis, and DNA damage revealed that the synthesized phyto-fabricated AgNPs were selectively cytotoxic towards leukemic lymphocyte cells and its anti-leukemic efficacy was comparable to the standard chemotherapeutic drug and simultaneously it increased the percent survivability of secondary AML mice when administered in vivo. These findings definitely open a new avenue directed towards the biomedical application of green phyto-fabricated AgNPs in cancer cells.

**Summary Points**

In summary, we demonstrated that the synthesized phyto-fabricated silver nanoparticle has a unique selective toxicity profile. Furthermore, we found that the phyto-fabrication reduced the toxicity of the nanoparticle itself on normal cells and capping materials imposed the chemopreventive potential on AgNPs in an environmentally sustainable pathway.

**Acknowledgments**

The authors gratefully acknowledge the DST-PURSE programme, RUSA grant, Government of India and personal research grant of University of Kalyani for funding this project.

**Conflict of interest statement**

The authors declare that there is no conflict of interest.

**References**


