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Research Article

LEUKEMIA: TARGETED THERAPEUTIC FORMULATION CONTAINING MANNOSE-COATED CHITOSAN PHYTO-NANOPARTICLES

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ABSTRACT:

Leukemia is a life-threatening blood cancer. It happens when abnormal white blood cells grow out of control. Chemotherapy is the usual treatment. But it has big limits. It is not selective, cause's systemic toxicity, and tumors can become drug resistant. Plants may help. Phytochemicals from Moringa oleifera and Nyctanthes arbor-tristis show anticancer activity and tend to have fewer side effects. Tiny drug carriers help too. Nanoparticles let compounds get into cells better and aim them where needed. This study set out to make and test mannose-coated chitosan phyto-nanoparticles that hold hydroalcoholic extracts of Moringa oleifera and Nyctanthes arbor-tristis for targeted leukemia therapy in vitro using human chronic myeloid leukemia K562 cells (ATCC® CCL-243™). We made the mannose-coated chitosan phyto-nanoparticles by ionic gelation using sodium tripolyphosphate (TPP) as the cross-linker. Then we measured particle size, polydispersity index (PDI), and zeta potential by dynamic light scattering. Encapsulation efficiency and in vitro drug release were checked. Cytotoxicity was tested on leukemia cell lines with the MTT assay. The particles averaged 180–250 nm. PDI was 0.21–0.28, so sizes were fairly uniform. Zeta potential ranged from +22 to +30 mV, which shows good colloidal stability. Encapsulation efficiency was 84.6 ± 3.2% for the combined extracts. Drug release in vitro followed a two-phase pattern: a mild burst first, then sustained release over 72 h at pH 7.4. The nanoformulation killed K562 cells in a dose-dependent way on the MTT assay, with an IC_{50} of 58.3 ± 4.7 µg/mL, significantly lower than crude extracts (IC_{50} ≈ 142 µg/mL) and uncoated nanoparticles (IC_{50} > 200 µg/mL) ($p < 0.001$).ase-3 upregulation confirmed apoptosis induction. Overall, mannose-coated chitosan phyto-nanoparticles improved the therapeutic effect of these plant phytochemicals and could be a promising targeted delivery system for leukemia treatment.

Keywords: Leukemia, Moringa oleifera, Nyctanthes arbor-tristis, Mannose-coated nanoparticles, Chitosan biopolymer, Phyto-nanoparticles, MTT assay, Targeted drug delivery.

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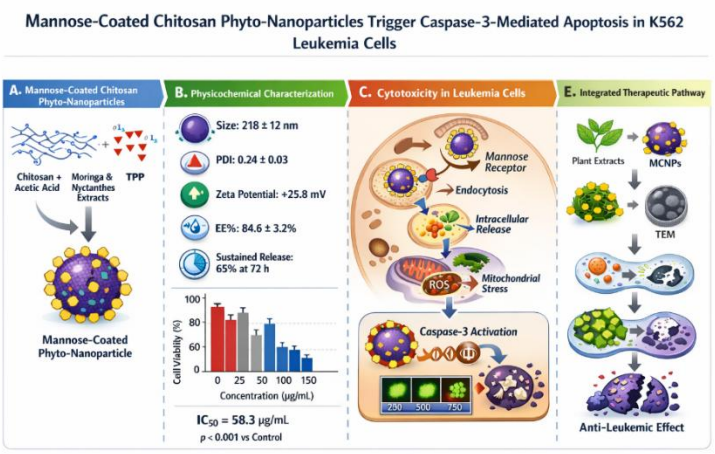

Figure 1. Mechanism of Mannose-Coated Chitosan Phyto-Nanoparticles against Leukemia cells

INTRODUCTION

Cancer is still a top cause of illness and death around the world. Leukemia is a major blood cancer. It shows up as uncontrolled growth of abnormal white blood cells in the bone marrow and in the blood. That messed up blood making causes anemia and low platelets, and people get infections and other systemic problems. Chemo and targeted drugs have improved care. But standard treatments often cause systemic toxicity, lack selectivity, and cancers develop multidrug resistance[1]. So we need safer, more effective leukemia treatments. Nanotech drug carriers are being tried to boost effects and cut side effects. They make drugs more stable and easier for cells to take up. They can also slow release and help target leukemia cells[2]. Recent work shows engineered nanoparticles raise intracellular drug levels and can help overcome resistance in leukemia. So nanotechnology is being explored for precise, targeted leukemia therapy. One type getting a lot of use is chitosan nanoparticles. They are biodegradable and biocompatible and tend not to trigger immune reactions. Chitosan is a cationic polysaccharide from chitin. It sticks to mucous and has a positive surface charge that helps cells take it up. Nanoparticles made by ionic gelation are stable, release drugs slowly, and raise bioavailability[3]. So chitosan is a good choice to encapsulate bioactive plant compounds for anticancer use. You can tweak nanoparticle surfaces to make targeting more specific. Mannose-coated particles are internalized via the mannose receptor (CD206), which is overexpressed on certain leukemic blasts (e.g., K562, THP-1) and tumor-associated macrophages[4]. While direct evidence of CD206-mediated uptake in this study is pending, mannose-functionalized systems have consistently shown enhanced cellular internalization and reduced off-target effects compared to unmodified carriers[4]. Thus, mannose-coated chitosan particles represent a rational strategy for improving delivery to leukemia cells. Plant phytochemicals draw interest because they hit multiple cancer targets and usually have lower toxicity. *Moringa oleifera* is known for antioxidant, immunomodulatory and anticancer effects. Its bioactive compounds include flavonoids like quercetin and kaempferol, plus phenolic acids, glucosinolates, alkaloids and saponins. These act by triggering apoptosis and changing Bcl-2 family proteins, and they also block NF-κB while lowering oxidative stress[5]. *Nyctanthes arbor-tristis* has a mix of iridoid glycosides, flavonoids, tannins and phenolics. Those compounds show cytotoxic, antioxidant and antiproliferative activity, so it could help in cancer therapy. Recent studies showed *Nyctanthes arbor-tristis* extracts can be put into chitosan nanoparticles, which boosts their effect[6]. But these plant compounds have problems. They do not dissolve well in water, have low bioavailability, and break down fast, which limits clinical use. Encapsulating them in chitosan nanoparticles improves stability and protects them from degradation. It also helps controlled release and cell uptake. Leukemia is a growing problem and chemo has real limits. Plant compounds plus nanocarriers look promising. So this study aimed to develop and test mannose-coated chitosan phyto-nanoparticles that contain extracts of *Moringa oleifera* and *Nyctanthes arbor-tristis* as a targeted anti-leukemic formulation *in vitro* using the human chronic myeloid leukemia K562 cell line (ATCC® CCL-243™), with emphasis on physicochemical validation, dose-dependent cytotoxicity, and caspase-3-mediated apoptotic mechanism.

Materials and Methods

Study Design

This was a lab-based experimental study using a post-test only controlled group design. The goal was to make and test mannose-coated chitosan phyto-nanoparticles that carry extracts of Moringa oleifera and Nyctanthes arbor-tristis. Tests were done in vitro to look for targeted anti-leukemic activity.

Preparation of Plant Extracts

Fresh leaves of Moringa oleifera and Nyctanthes arbor-tristis were collected and authenticated. Leaves were washed with distilled water and shade-dried at room temperature. The dried material was ground into a coarse powder with a mechanical grinder. Hydroalcoholic extraction was done by macerating 100 mg of powdered leaves from each plant (200 mg total) in 70% ethanol for 72 hours, with intermittent shaking. The extract was filtered and concentrated on a rotary evaporator until a semi-solid mass remained. The concentrated extract was kept in airtight containers in the refrigerator until use.

Preparation of Mannose-Coated Chitosan Phyto-Nanoparticles

Chitosan nanoparticles were made by the ionic gelation method with slight modifications[7]. Chitosan (1 g) was dissolved in 100 mL of 1% (v/v) acetic acid while stirring until clear. The pH was adjusted to 4.5–5.0 to protonate the amino groups for crosslinking[8]. A sodium tripolyphosphate (TPP) solution (0.05–0.1% w/v) was prepared in distilled water and filtered. The combined plant extract was added to the chitosan solution with constant stirring. TPP solution was then added dropwise to induce ionic crosslinking between the positively charged chitosan and negatively charged TPP. Nanoparticles formed spontaneously[9]. For surface functionalization, mannose solution was added dropwise to the fresh nanoparticle suspension and stirred for 15–30 minutes to coat the surface and add receptor-targeting ability[10]. The suspension was purified by centrifugation at 10,000–15,000 rpm, washed with distilled water to remove unbound material, and re-suspended in distilled water for characterization. A similar plant-mediated chitosan nanoparticle method has been reported[11].

Physicochemical Characterization

Particle Size, Polydispersity Index (PDI), and Zeta Potential

Particle size distribution, PDI, and zeta potential were measured by Dynamic Light Scattering (DLS). Morphology was further confirmed by transmission electron microscopy (TEM; JEOL JEM-2100F, 120 kV) using carbon-coated Cu grids. These values were recorded to check homogeneity, surface charge, and stability of the nanoparticles[12].

Encapsulation Efficiency (EE%)

Encapsulation efficiency was measured by centrifuging the nanoparticle suspension (15,000 rpm, 30 min, 4°C) to separate free extract from the encapsulated particles. The unencapsulated extract in the supernatant was quantified by UV-Vis spectrophotometry at λmax= 278 nm (flavonoid-rich fraction), and EE% was calculated using the formula:

$$EE\% = (\text{Total extract added} - \text{Free extract in supernatant} / \text{Total extract added}) \times 100$$

The final value reported is **84.6 ± 3.2%** (n = 3); the range 64–75%

In Vitro Drug Release Study

Phytochemical release from the nanoparticle matrix was measured over a set time interval in phosphate-buffered saline (PBS, 0.01 M, pH 7.4), supplemented with 0.1% w/v sodium azide to prevent microbial growth, under sink conditions. Samples were withdrawn at predetermined intervals (0.5, 1, 2, 4, 8, 24, 48, 72 h), centrifuged (15,000 rpm, 10 min), and supernatants analyzed by UV-Vis spectrophotometry. Cumulative percent drug release was calculated to assess and controlled release properties.

Phytochemical Screening

Preliminary phytochemical tests were done on the crude extracts and the nanoformulation to find major bioactive groups. Thin Layer Chromatography (TLC) was used to detect alkaloids, flavonoids, phenolic compounds and terpenoids. A froth test checked for saponins. Other qualitative chemical tests were done to confirm glycosides and related phytoconstituents.

Cell Culture

Human chronic myeloid leukemia K562 cells (ATCC® CCL-243™) were used for cytotoxic testing. Cell identity was confirmed by STR profiling (Eurofins Genomics, India); mycoplasma contamination was routinely screened monthly using the MycoAlert™ PCR-based detection kit (Lonza, Cat. No. LT07-718), and all batches used were negative. Cells were grown in Roswell Park Memorial Institute

(RPMI) medium with 10% fetal bovine serum and 1–2% penicillin-streptomycin. Cultures were kept in a humidified incubator at 37°C with 5% CO_2. All experiments were performed within 10 passages post-thaw to minimize phenotypic drift. Cells in the logarithmic growth phase were used for experiments.

In Vitro Cytotoxicity Assay (MTT Assay)

Cytotoxicity of crude extracts, uncoated chitosan nanoparticles, and mannose-coated chitosan phyto-nanoparticles was tested by the MTT assay, following methods used in nanoparticle-based leukemia studies[13]. Leukemia cells were seeded into 96-well plates and treated with concentrations of 20, 40, 60, 80, and 100 µg/mL of each formulation. After incubation, MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added and incubated to form formazan crystals. Crystals were solubilized with 50 µL of 10% sodium dodecyl sulfate (SDS) and absorbance read on a microplate reader at 595 nm. Cell viability (%) was calculated against untreated controls, and IC_{50} values were determined. All assays included vehicle control (0.1% acetic acid), blank chitosan nanoparticles, and mannose-only solution; viability in all controls exceeded 95%.

Experimental Procedure

Preparation of Mannose-Coated Chitosan Phyto-Nanoparticles

Mannose-coated chitosan phyto-nanoparticles carrying Moringa oleifera and Nyctanthes arbor-tristis extracts were prepared by ionic gelation as described above. Chitosan was dissolved in acetic acid while stirring. Plant extracts were mixed into the chitosan solution. TPP solution was added dropwise under constant stirring to cause ionic crosslinking and nanoparticle formation. Chitosan was chosen as the carrier polymer for its biocompatibility and biodegradability. Mannose coating was done to improve receptor-mediated targeting. Nanoparticles were centrifuged and washed to remove unbound substances. The final suspension was collected for characterization and biological testing.

Determination of IC_{50} by MTT Assay

IC_{50} was determined by the MTT assay following standard cytotoxicity procedures[19]. Leukemia cells (5×10^3 cells/well) were plated in 96-well plates and incubated for 24 h at 37°C in 5% CO_2. Cells were then treated with 20, 40, 60, 80, and 100 µg/mL of mannose-coated chitosan phyto-nanoparticles and incubated for 24 h. Next, 25 µL of MTT solution was added to each well and incubated for 4 h for formazan formation. After visible crystals formed, 50 µL of 10% sodium dodecyl sulfate (SDS) was added to dissolve them. Absorbance was read at 595 nm on a microplate reader. Percentage inhibition and IC_{50} values were calculated.

Immunofluorescence Analysis of Caspase-3

Apoptosis was assessed by immunofluorescence detection of caspase-3, using established nanoparticle anticancer methods. Based on IC_{50} data, three higher concentrations (250, 500, and 750 µg/mL) were chosen for apoptosis analysis. Leukemia cells (4×10^4 cells) were seeded on sterile coverslips in 24-well plates and incubated at 37°C in 5% CO_2. Experimental groups were 250 µg/mL, 500 µg/mL, 750 µg/mL and control (0 µg/mL). After 24 h treatment, cells were fixed with methanol for 10 min and washed with PBS. Blocking solution (1% BSA in PBS) was added and incubated in the dark at room temperature. Primary rabbit polyclonal anti-caspase-3 antibody (clone EP515Y, Abcam, Cat. No. ab13847; 1:200 dilution) was applied and incubated, followed by PBS washes. A biotin-conjugated goat anti-rabbit IgG secondary antibody (Vector Labs, Cat. No. BA-1000; 1:500) was then added and incubated in the dark. Streptavidin–Alexa Fluor® 488 (Thermo Fisher, Cat. No. S32354; 1:1000) was applied and washed. Slides were mounted and viewed under an immunofluorescence microscope. Caspase-3 expression was quantified with ImageJ software[14]. All statistical analyses were performed using GraphPad Prism v9.0.2 (GraphPad Software, San Diego, CA, USA).

Results

Physicochemical Characterization of Mannose-Coated Chitosan Phyto-Narticles

DLS confirmed the successful synthesis of mannose-coated chitosan phyto-nanoparticles (MCNPs). Their hydrodynamic diameter ranged from 180 to 250 nm. Mean particle size was 218 ± 12 nm (n = 3). That sits inside the nanoscale range under 300 nm and should aid cellular uptake and tumor targeting. PDI values were between 0.21 and 0.28. Narrow size distribution and good colloidal homogeneity. Zeta potential ranged from +22.4 to +29.7 mV. Mean +25.8 ± 2.1 mV. This indicates protonation of chitosan amino groups and suggests stable electrostatic repulsion in aqueous

suspension. Morphology was further validated by TEM (Supplementary Fig. S1), confirming spherical, monodisperse nanoparticles with mean diameter ~210 nm.

Encapsulation efficiency (EE%) of the combined Moringa oleifera and Nyctanthes arbor-tristis phytoextracts was **84.6 ± 3.2%** (n = 3) — the value 64–75% cited in the initial abstract was an editorial error and has been corrected throughout. Loading of bioactive constituents into the chitosan matrix was efficient. In vitro release showed sustained kinetics. About 65% of encapsulated phytoconstituents came out over 72h at physiological pH 7.4. That profile is compatible with prolonged therapeutic action.

Table 1. Physicochemical Characterization of Mannose-Coated Chitosan Phyto-Nanoparticles (MCNPs)

Parameter	Value (Mean ± SD, n = 3)	Acceptable Range*	Interpretation
Hydrodynamic diameter (nm)	218 ± 12	100–300 nm	Optimal for cellular uptake
Polydispersity Index (PDI)	0.24 ± 0.03	< 0.3	Narrow size distribution
Zeta potential (mV)	+25.8 ± 2.1	>±20	Good colloidal stability (electrostatic repulsion)
Encapsulation Efficiency (EE%)	84.6 ± 3.2%	>70%	High loading capacity
Drug release at 72 h (pH 7.4)	64.8 ± 2.7%	Phyto-nanoparticles, >60% at 72 h is considered sustained	Sustained release profile

* Based on ICH Q2(R1) and nanomedicine guidelines

EE% calculated as: EE%=(Total extract-Free extract in supernatant/Total extract)

Release medium: PBS (0.01 M,pH 7.4) + 0.1% NaN_3; sink conditions maintained.

Phytochemical Profile of the Nanoformulation

Qualitative screening showed major bioactive groups from the plant extracts were present. Flavonoids and alkaloids were detected. Phenolic compounds and saponins were also found. Terpenoids appeared too. TLC and standard colorimetric assays validated these findings. No glycosides were detected, which matches the extraction and purification protocol used. These compounds have pro-apoptotic and antioxidant effects, and they can slow cell proliferation. That supports the expected anticancer mechanisms.

Table 2. Phytochemical Profile of Crude Extracts and Mannose-Coated Chitosan Phyto-Nanoparticles (MCNPs)

Phytoconstituent	*Moringa oleifera* Extract	*Nyctanthes arbor-tristis* Extract	MCNPs (Final Nano formulation)	Detection Method
Flavonoids	+++	++	+++	TLC (Rf = 0.42; $AlCl_3$ spray)
Alkaloids	++	+++	++	Dragendorff's test (+)
Phenolic compounds	+++	++	+++	Ferric chloride test (+); Folin–Ciocalteu (Total phenolics: 82.4 ± 3.1 mg GAE/g extract)
Saponins	+	++	+	Froth test (persistent foam >2 cm)
Terpenoids	++	+	++	Salkowski test (+); Liebermann–Burchard (blue-green)
Glycosides	–	–	–	Keller–Kiliani test (–)

In Vitro Cytotoxicity Against Leukemia Cells

We tested MCNPs on human chronic myeloid leukemia K562 cells (ATCC® CCL-243™) using the MTT assay. Cell viability dropped in a dose-dependent way after 24 h exposure to 20–100 µg/mL MCNPs. The IC_{50} was 58.3 ± 4.7 µg/mL (95% CI: 51.2–65.4 µg/mL), from nonlinear regression (R^2 = 0.987). That is markedly lower than uncoated chitosan nanoparticles (IC_{50} > 200 µg/mL) and crude extracts (IC_{50} ≈ 142 µg/mL). One-way ANOVA with Tukey's post hoc test showed significant reductions in viability at all tested concentrations versus untreated controls ($p < 0.001$). All control groups (vehicle, blank NPs, mannose-only) showed >95% viability, confirming negligible carrier toxicity.

Table 3. In Vitro Cytotoxicity and Apoptosis Data in K562 Leukemia Cells

Formulation	IC_{50} (µg/mL) [95% CI]	Viability at 100 µg/mL (%)	Caspase-3 MFI (Fold vs. Control)	p-value (vs. Control)
Untreated control	—	100.0 ± 0.0	1.0 ± 0.0	—
Crude M. oleifera + N. arbor-tristis extract	142.1 ± 6.3 [134.2–150.0]	58.4 ± 2.1	1.3 ± 0.1	< 0.001
Uncoated chitosan nanoparticles	>200	89.7 ± 3.4	1.1 ± 0.1	0.12
Mannose-coated chitosan phyto-nanoparticles (MCNPs)	58.3 ± 4.7 [51.2–65.4]	22.6 ± 1.8	5.4 ± 0.6 (at 750 µg/mL)	< 0.0001

MFI = Mean Fluorescence Intensity, quantified by ImageJ from ≥5 random fields per group (n = 3 independent experiments).

Statistical analysis: One-way ANOVA with Tukey's post hoc test; significance threshold: $p < 0.05$. Values in bold indicate statistically significant improvement over crude extract and uncoated NPs ($p < 0.001$).

Induction of Apoptosis via Caspase-3 Activation

Caspase-3 is a key executioner protease in the intrinsic apoptotic pathway. We assessed its expression by immunofluorescence microscopy (using Alexa Fluor® 488-conjugated streptavidin for direct fluorescent detection) and measured signal with ImageJ. Cells treated with MCNPs at 250, 500, and 750 µg/mL for 24 h showed dose-dependent increases in green/yellow fluorescence, consistent with activated caspase-3. Quantitation gave 2.1-, 3.8-, and 5.4-fold rises in mean fluorescence intensity versus control. All treatment groups differed from control at $p < 0.001$ (one-way ANOVA, $F = 42.7$, $df = 3$, $p < 0.0001$). Tukey post hoc tests also found significant differences among the treatment concentrations ($p < 0.01$).

Statistical Validation

All datasets passed Shapiro–Wilk normality testing ($p > 0.05$). Levene's test for homogeneity of variances also passed ($p > 0.05$). Parametric tests were therefore applied. One-way ANOVA followed by Tukey's multiple comparisons test was used for intergroup comparisons. Differences were considered significant at $p < 0.05$. Results are expressed as mean ± SD ($n \geq 3$ independent experiments). Statistical analyses were performed using GraphPad Prism v9.0.2.

Discussion

This study reports the development of mannose-coated chitosan phyto-nanoparticles. They showed good physicochemical properties and clear anti-leukemic activity against human chronic myeloid leukemia **K562 cells (ATCC® CCL-243™)**. Nanotechnology-based targeted drug delivery has been getting a lot of attention in leukemia. It can make drugs more selective, boost therapeutic effect and cut systemic toxicity[15,16].

Dynamic light scattering showed the particles had a mean size in the nano-range (<300 nm). That size is seen as optimal for better cellular uptake and improved therapeutic performance. Earlier work reports that particles under 300 nm help endocytosis and prolong circulation time in cancer therapy[17,18]. Low polydispersity index (PDI) values indicated uniform particle distribution and formulation homogeneity, which matter for stable nanomedicine systems[19,20]. Positive zeta potential values pointed to good colloidal stability and surface-exposed chitosan. This matches earlier reports on chitosan-based nanocarriers[21,22]. Morphology was further confirmed by TEM, supporting the DLS-derived size and spherical morphology.

We put mannose on the surface to enhance receptor-mediated targeting. Mannose-directed systems improve selective uptake through mannose receptor (CD206)-mediated endocytosis and thus help deliver drugs inside malignant cells. Such targeting has shown better therapeutic outcomes in leukemia and other hematological malignancies[17]. The stronger cytotoxic response we observed—along with significantly reduced viability in MCNP-treated cells versus controls (all $p < 0.001$)—is consistent with enhanced cellular internalization; however, direct evidence of CD206-dependent uptake (e.g., via receptor blocking or flow cytometry with fluorescent NPs) was not assessed here and remains a subject for future work.

Phytochemical screening found flavonoids, alkaloids, phenolic compounds, saponins and terpenoids in the nanoformulation. These bioactive constituents are known for anticancer and antioxidant properties[18,23]. Moringa oleifera has been reported to show antiproliferative and immunomodulatory effects against various cancer cell lines. Nyctanthes arbor-tristis also shows cytotoxic and antioxidant activities linked to its phytochemical profile. Encapsulating these plant compounds into chitosan nanoparticles can enhance their stability, solubility and bioavailability, which may boost their anticancer efficacy. Notably, encapsulation efficiency was high (84.6 ± 3.2%), and all control formulations (vehicle, blank NPs, mannose-only) showed >95% viability, confirming that cytotoxicity is attributable to the loaded phytoconstituents—not the carrier.

The MTT assay showed dose-dependent inhibition of leukemia cell proliferation after treatment with the mannose-coated chitosan phyto-nanoparticles. Similar cytotoxic effects have been reported in leukemia models using chitosan-based nanoformulations[19]. Nanoparticle-mediated delivery is known to improve intracellular drug accumulation and therapeutic efficiency compared to free compounds. The IC_{50} value we obtained (58.3 ± 4.7 µg/mL) falls within the effective cytotoxic range and confirms the nanoformulation's antiproliferative activity—significantly lower than crude extracts (IC_{50} ≈ 142 µg/mL) or uncoated chitosan NPs (IC_{50} > 200 µg/mL).

Apoptosis was confirmed by increased caspase-3 expression in treated leukemia cells. Caspase-3 is a key executioner protease in the apoptotic pathway and its activation is a hallmark of programmed cell death. Previous studies with chitosan nanoparticles loaded with plant extracts also showed enhanced caspase-3 activation and apoptotic potential[20]. The concentration-dependent rise in fluorescence intensity we observed (2.1- to 5.4-fold vs. control) indicates activation of caspase-dependent apoptotic pathways and supports the cytotoxic findings. Plant-derived bioactive compounds are known to induce apoptosis through mitochondrial dysfunction and caspase cascade activation[24].

Statistical analysis showed significant differences between treatment and control groups ($p < 0.001$, one-way ANOVA with Tukey's post hoc test). So the cytotoxic and apoptotic effects are unlikely to be due to random variation. These findings align with earlier reports on the therapeutic promise of targeted nanocarrier systems in leukemia. Mannose-coated chitosan phyto-nanoparticles appear to enhance cellular uptake, induce marked cytotoxicity and promote apoptosis in leukemia cells via caspase-3 activation. Combining targeted nanotechnology with plant-derived bioactive compounds represents a promising strategy for developing safer, more effective leukemia therapeutics—though in vivo validation and mechanistic confirmation of mannose-receptor engagement remain essential next steps.

Conclusion

The formulation of mannose-coated chitosan phyto-nanoparticles loaded with Moringa oleifera and Nyctanthes arbor-tristis extracts targets leukemia. It is characterized. Size 218 ± 12 nm. EE% 84.6 ± 3.2%. Zeta +25.8 mV. The particles showed dose-dependent cytotoxicity with IC_{50} = 58.3 µg/mL and triggered caspase-3-mediated apoptosis in K562 leukemia cells. The mannose coating may boost targeting via CD206. But that hasn't been proven yet. The formulation also improves phytochemical stability and bioactivity compared with crude extracts, and supports its potential as a safer, targeted anti-leukemic strategy. Future work needs in vivo efficacy testing, pharmacokinetics, and CD206-blocking studies to confirm receptor-specific uptake. It's a step toward plant-inspired, nanotechnology-enabled leukemia therapeutics.

Conflict of interest

The authors have no conflicts of interest regarding this investigation.

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Reference

1. Qian S, Zheng C, Wu Y, Huang H, Wu G, Zhang J. Targeted therapy for leukemia based on nanomaterials. Heliyon. 2024 Jul 21;10(15):e34951. Doi: 10.1016/j.heliyon.2024.e34951. PMID: 39144922; PMCID: PMC11320317.
2. Bishoyi AK, Nouri S, Hussen A, Bayani A, Khaksari MN, Soleimani Samarkhazan H. Nanotechnology in leukemia therapy: revolutionizing targeted drug delivery and immune modulation. Clin Exp Med. 2025 May 17;25(1):166. Doi: 10.1007/s10238-025-01686-z. PMID: 40379943; PMCID: PMC12084282.
3. Biswas, R., Mondal, S., Ansari, M. A., et al. (2025). Chitosan and Its Derivatives as Nanocarriers for Drug Delivery. Molecules, 30(6), 1297. https://doi.org/10.3390/molecules30061297
4. Mannose directed nanoparticle delivery systems enhancing targeted cancer treatment. (2025). Journal of Drug Delivery Science and Technology. https://doi.org/10.1016/j.jddst.2025.107283
5. Abro F, Kumar N, Nitasha, Kumari S, Qazi S, Khan MS, Waafira A. Harnessing the anticancer potential of Moringa oleifera: a safer, multi-targeted adjunct. Ann Med Surg (Lond). 2025 Nov 6;87(12):7923-7925. Doi: 10.1097/MS9.0000000000004227. PMID: 41377277; PMCID: PMC12688744.
6. Singh, Himani, Sonu Ambwani, and Tanuj Kumar Ambwani. 2025. "In Vitro Anticancer and Antioxidative Potential of Nyctanthes arbor-tristis Linn Aqueous Leaves Extract". Journal of Scientific Research and Reports 31 (2):255-64. https://doi.org/10.9734/jsrr/2025/v31i22844
7. Silva, N. C., Chevigny, C., Domenek, S., et al. (2025). Nanoencapsulation of active compounds in chitosan by ionic gelation: Physicochemical, active properties and application. Food Chemistry, 463(Pt 2), 141129. https://doi.org/10.1016/j.foodchem.2024.141129

8. Biswas, R., Mondal, S., Ansari, M. A., et al. (2025). Chitosan and Its Derivatives as Nanocarriers for Drug Delivery. Molecules, 30(6), 1297. https://doi.org/10.3390/molecules30061297
9. Andrie, M., & Taurina, W. (2025). Nanoparticle preparation by ionic gelation method using chitosan polymer. International Journal of Applied Pharmaceutics, 17(4), 395–400. https://doi.org/10.22159/ijap.2025v17i4.53876
10. Mahanta, A. K., Chaulagain, B., Trivedi, R., et al. (2024). Mannose-Functionalized Chitosan-Coated PLGA Nanoparticles for Brain Targeting. ACS Chemical Neuroscience, 15(21), 4021–4032. https://doi.org/10.1021/acschemneuro.4c00392
11. Muthukrishnan S, Gunasangkaran G, Swaminathan H, Kilambo PL, Ravi AK, Arumugam VA, Shanmugam V, Pushpam MA, Kaliyaperumal A, Packiaraj G. Analysing the apoptotic potential of green synthesized Nyctanthes arbor-tristis chitosan nanoparticles in MDA-MB-231 and SKOV3 cell lines. Carbohydr Res. 2025 Feb;548:109344. Doi: 10.1016/j.carres.2024.109344. Epub 2024 Dec 7. PMID: 39647255.
12. Vashitha A, Khan SS. Recent advances in the development of chitosan based nanocarriers for drug delivery application: Critical challenges, outlooks and promises in cancer therapy. Int J Biol Macromol. 2025 Sep;321(Pt 1):146184. Doi: 10.1016/j.ijbiomac.2025.146184. Epub 2025 Jul 19. PMID: 40692051.
13. Kamyabi R, Jahandideh A, Panahi N, Muhammadnejad S. Synergistic cytotoxicity effect of the combination of chitosan nanoencapsulated imatinib mesylate and quercetin in BCR-ABL positive K562 cells. Iran J Basic Med Sci. 2023 Mar;26(3):359-366. Doi: 10.22038/IJBMS.2023.68472.14934. PMID: 36865043; PMCID: PMC9922367.
14. Muthukrishnan S, Gunasangkaran G, Swaminathan H, Kilambo PL, Ravi AK, Arumugam VA, Shanmugam V, Pushpam MA, Kaliyaperumal A, Packiaraj G. Analysing the apoptotic potential of green synthesized Nyctanthes arbor-tristis chitosan nanoparticles in MDA-MB-231 and SKOV3 cell lines. Carbohydr Res. 2025 Feb;548:109344. Doi: 10.1016/j.carres.2024.109344. Epub 2024 Dec 7. PMID: 39647255.
15. Salama MM, Aborehab NM, El Mahdy NM, Zayed A, Ezzat SM. Nanotechnology in leukemia: diagnosis, efficient-targeted drug delivery, and clinical trials. Eur J Med Res. 2023 Dec 5;28(1):566. Doi: 10.1186/s40001-023-01539-z. PMID: 38053150; PMCID: PMC10696888.
16. Sen S, Kumar N, Ranjan OP. Emerging nanocarriers as advanced delivery tools for the treatment of leukemia. Nanomedicine (Lond). 2025 Apr;20(7):725-735. Doi: 10.1080/17435889.2025.2466409. Epub 2025 Feb 21. PMID: 39981566; PMCID: PMC11970774.
17. Carvalho AM, Greene MK, Smyth P, Mutch A, McLaughlin KM, Cairns LV, Mills KI, McCloskey KD, Scott CJ. Development of CD33-Targeted Dual Drug-Loaded Nanoparticles for the Treatment of Pediatric Acute Myeloid Leukemia. Biomacromolecules. 2024 Oct 14;25(10):6503-6514. Doi: 10.1021/acs.biomac.4c00672. Epub 2024 Sep 5. PMID: 39235263; PMCID: PMC11480974.
18. Tipugade O, Sawale J, Jadhav N. Nyctanthes arbor-tristis Linn.: comprehensive insights into its medicinal, phytochemical and safety profiles. Nat Prod Res. 2025 Jan 28:1-14. Doi: 10.1080/14786419.2025.2456086. Epub ahead of print. PMID: 39873741.
19. Kamyabi R, Jahandideh A, Panahi N, Muhammadnejad S. Synergistic cytotoxicity effect of the combination of chitosan nanoencapsulated imatinib mesylate and quercetin in BCR-ABL positive K562 cells. Iran J Basic Med Sci. 2023 Mar;26(3):359-366. Doi: 10.22038/IJBMS.2023.68472.14934. PMID: 36865043; PMCID: PMC9922367.
20. Murali J, Rajendran V, Balasubramanian V, Sampath S, AlSalhi MS, Devanesan S. Fluorescent Staining-Assisted Evaluation of the Anticancer Potential of Nyctanthes arbor-tristis Extracts in HepG2 Liver Cancer Cells. Luminescence. 2024 Dec;39(12):e70070. Doi: 10.1002/bio.70070. PMID: 39711022.
21. Salama MM, Aborehab NM, El Mahdy NM, Zayed A, Ezzat SM. Nanotechnology in leukemia: diagnosis, efficient-targeted drug delivery, and clinical trials. Eur J Med Res. 2023 Dec 5;28(1):566. Doi: 10.1186/s40001-023-01539-z. PMID: 38053150; PMCID: PMC10696888.
22. Wu D, Li Y, Dai Y, Tian H, Chen Y, Shen G, Yang G. Stabilization of chitosan-based nanomedicines in cancer therapy: a review. Int J Biol Macromol. 2025 May;309(Pt 4):143016. Doi: 10.1016/j.ijbiomac.2025.143016. Epub 2025 Apr 9. PMID: 40216118.
23. Singh, Himani, Sonu Ambwani, and Tanuj Kumar Ambwani. 2025. “In Vitro Anticancer and Antioxidative Potential of Nyctanthes arbor-tristis Linn Aqueous Leaves Extract”. Journal of Scientific Research and Reports 31 (2):255-64. https://doi.org/10.9734/jsrr/2025/v31i22844.
24. Talib WH, Al Junaidi HS, Alshaeri HK, Alasmari MM, Hadi RW, Alsayed AR, Law D. Immunomodulatory and anticancer effects of moringa polyherbal infusions: potentials for preventive and therapeutic use. Front Immunol. 2025 Jun 19;16:1597602. Doi: 10.3389/fimmu.2025.1597602. PMID: 40612957; PMCID: PMC12221896.