

The Nephroprotective Effect of *Andrographis Paniculata* Ethanol Extract Poly-Lactic-Co-Glycolic Acid (PLGA) Nanoparticles on Vero Cell

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ABSTRACT

Sambiloto (*Andrographis paniculata*) comprises andrographolide, a compound characterized by inherently low bioavailability. Consequently, it is formulated into nanoparticles to enhance its pharmacokinetic profile. Andrographolide is able to fight free radicals that cause cell death. This study was conducted to determine nephroprotective activity of poly-lactic-co-glycolic acid (PLGA)-based *Andrographis paniculata* ethanol extract nanoparticles in Vero cell line. *Andrographis paniculata* ethanol extract nanoparticles (50, 100, 250, 500, and 1000 ppm) were tested for cytotoxicity and nephroprotective activity. Nephroprotective tests consisted of acute nephroprotection, chronic nephroprotection, and recovery tests using concentrations of 100, 250 and 500 ppm. The protective effect was tested by administering nanoparticles, followed by H₂O₂. The recovery effect was tested by administering H₂O₂, followed by nanoparticles. All tests were carried out using the 3-(4, 5-dimethyl tiazol-2-yl)-5-diphenyl tetrazolium bromide (MTT) assay method and data analysis was performed using *one-way* ANOVA. *Andrographis paniculata* ethanol extract nanoparticles (50 – 1000 ppm) did not have any toxic effect on Vero cell line. Nanoparticles provide nephroprotective activity based on concentration-dependent trend. High concentration nanoparticles (500 ppm) were able to provide better acute protective effect than vitamin E. Nanoparticles 500 ppm provided a better recovery effect ($p < 0,05$) than vitamin E, but the chronic protection effect was lower than vitamin E. It can be concluded that *Andrographis paniculata* ethanol extract PLGA nanoparticles with concentration of 500 ppm provide effective nephroprotective activity against H₂O₂-induced toxicity in Vero cells line.

Keywords: *Andrographis paniculata*, PLGA nanoparticles, cytotoxicity, nephroprotective, Vero cell line

INTRODUCTION

Acute renal failure (ARF) is characterized by an abrupt decline in the glomerular filtration rate. This condition represents a significant global health challenge, affecting approximately 13.3 million individuals annually. The pathogenesis of ARF is multifactorial, with oxidative stress being a principal contributor. Among the various

etiological factors, aminoglycoside antibiotics are notably implicated. Nephrotoxicity induced by pharmacological agents is observed in 19-26% of hospitalized patients, with aminoglycosides such as gentamicin being prominent culprits (Kwiatkowska et al., 2021). Gentamicin has the potential to precipitate acute renal failure by augmenting the generation of reactive oxygen

species (ROS), including superoxide anions, hydroxyl radicals, hydrogen peroxide, and reactive nitrogen species within the renal cortex. This escalation in ROS production results in substantial structural and functional impairment of renal tissues (Kandemir et al., 2015).

The administration of medicinal plants exhibiting nephroprotective properties offers the dual benefits of cost-effectiveness and minimal adverse effects. Among such botanicals, *Andrographis paniculata*, commonly known as sambiloto, has garnered attention for its nephroprotective efficacy. *Andrographis paniculata* is renowned for its multifaceted biological activities, including anti-inflammatory and antioxidant effects and hepatoprotective and renoprotective capacities (Adeoye et al., 2019). *Andrographis paniculata* encompasses bioactive constituents belonging to the diterpene lactone class, among which andrographolide has been documented to exhibit significant antioxidant properties. Andrographolide exerts its effects by directly neutralizing free radicals through electron donation. Moreover, andrographolide confers indirect protective benefits by preserving mitochondrial integrity to avert apoptosis and by activating endogenous antioxidant enzymes, including glutathione dismutase and superoxide dismutase (Mussard et al., 2019). Andrographolide exhibits significant antioxidant properties with an IC₅₀ value of 3.2 µg/mL and demonstrates cytoprotective effects against carbon tetrachloride (CCl₄)-induced toxicity in HepG2 cells. This protective effect is achieved through the inhibition of CCl₄-mediated lipid peroxidation. Furthermore, the administration of andrographolide enhances cell viability and augments MTT metabolism (Krithika et al., 2013). In the investigation conducted by Adeoye et al. (2019), the administration of an ethanol extract derived from *Andrographis paniculata* leaves to mice subjected to cisplatin induction demonstrated a capacity to attenuate the inflammatory response, suppress oxidative stress, and enhance glomerular function.

Andrographolide exhibits limited bioavailability and poor aqueous solubility, necessitating strategies to enhance its solubility and permeability. One method to achieve this is through the manipulation of particle size, particularly via the formulation of nanoparticles (Casamonti et al., 2019). Nanoparticles exhibit diminutive particle sizes ranging from 10 to 1000 nm, coupled with an extensive surface area, thereby enhancing drug solubility and dissolution

rates. The preparation of nanoparticles can be accomplished through various techniques, among which the emulsion solvent evaporation method stands out. This particular method involves the integration of volatile organic solvents with aqueous solutions, followed by emulsification at high agitation speeds, ultimately leading to the evaporation of the volatile solvents. This technique is preferred due to its non-toxic nature and its efficacy in producing small particles (Hernández-Giottonini et al., 2020). Nanoparticles are synthesized utilizing synthetic polymers, specifically poly-lactic-co-glycolic acid (PLGA), due to its exemplary biodegradability, superior biocompatibility, and minimal systemic toxicity. Furthermore, PLGA has received approval from the Food and Drug Administration (FDA) (Makadia & Siegel, 2011).

Poly(lactic-co-glycolic acid) (PLGA)-based nanoparticles encapsulating *Andrographis paniculata* ethanol extract were evaluated for their nephroprotective efficacy using Vero cell lines. These Vero cell lines, which originate from the renal tissues of African green monkeys, represent a widely-utilized mammalian model in molecular and cellular biology investigations. The nephroprotective potential of the nanoparticles was assessed in vitro by monitoring the cellular responses to external stimuli via the MTT colorimetric assay (Okamoto et al., 2020). The MTT colorimetric assay was conducted to evaluate cell viability, predicated on the enzymatic activity of mitochondrial succinate dehydrogenase, which facilitates the reduction of yellow MTT to insoluble purple formazan crystals (Movaliya, 2020).

According to the aforementioned description, it is imperative to conduct an investigation into the nephroprotective efficacy of PLGA-based ethanol extract nanoparticles derived from Sambiloto (*Andrographis paniculata*) on the Vero cell line.

MATERIALS AND METHODS

The materials utilized in this study include *Andrographis paniculata* [purchased from Toko Ting Bao, Purwokerto, Indonesia], Poly(lactic-co-glycolic acid) (PLGA)[Sigma], deionized water, distilled water, polyvinyl alcohol (PVA) [Sigma], and ethyl acetate [Bratachem]. Additionally, the study employs 3-(4, 5-dimethylthiazol-2-yl)-5-diphenyltetrazolium bromide (MTT)[Sigma], Vero cell lines [BRIN], 10% Fetal Bovine Serum (FBS)[Gibco], Phosphate Buffered Saline (PBS)[Gibco], Dulbecco's Modified Eagle Medium

(DMEM)[Gibco], trypsin-EDTA solution (0.25% trypsin)[Sciencebio], streptomycin, penicillin [Elabscience], dimethyl sulfoxide (DMSO)[Sigma], Sodium Dodecyl Sulfate (SDS)[Sigma], hydrogen peroxide (H₂O₂)[Sigma], vitamin E [Sigma], and hydrochloric acid (HCl)[Bratachem].

Preparation of *Andrographis paniculata* ethanol extract

The *Andrographis paniculata* herbs were finely pulverized using a blender, resulting in a powder form. This powder was subsequently mixed with 70% ethanol at a ratio of 1:10. Specifically, 0.5 kg of the powdered *Andrographis paniculata* herbs were weighed and combined with 5 liters of 70% ethanol solvent. The powdered simplicia underwent an extraction process via maceration for a duration of 72 hours, with periodic stirring at ambient temperature. The resulting filtrate was then filtered and concentrated using a rotary evaporator until a thick, solvent-free extract was obtained (Verma et al., 2020).

Preparation of *Andrographis paniculata* ethanol extract-PLGA nanoparticles

Two grams of *Andrographis paniculata* ethanol extract were dissolved in 100 mL of deionized water within a 100 mL volumetric flask (Gaur et al., 2018). A polyvinyl alcohol (PVA) solution was prepared with a concentration of 2% (aqueous phase) and 0.3% (hardener) utilizing deionized water as the solvent. The solution was homogenized using a magnetic stirrer operating at 600 rpm and maintained at a temperature of 70°C (Kurniawan et al., 2024). This solution will be known as the aqueous phase. Poly-(lactic-co-glycolic-acid) (PLGA) was precisely weighed to 2.5 mg and subsequently dissolved in 100 µL of ethyl acetate in an Eppendorf tube. To this PLGA-ethyl acetate solution, 100 µL of *Andrographis paniculata* extract was added dropwise. The resulting mixture was then subjected to vortexing for 2 minutes (Kurniawan et al., 2018). This mixture is subsequently referred to as the organic phase.

A 2% PVA solution was prepared using 100 µL in an Eppendorf tube, followed by the gradual addition of the organic phase into the aqueous phase, with vortex mixing for 2 minutes (Kurniawan et al., 2024). A 40 mL solution of 0.3% PVA was prepared in a beaker. The aqueous phase and organic phase mixture were gradually introduced into the 0.3% PVA solution and homogenized using a magnetic stirrer at a speed of

600 rpm. Subsequently, the mixture was allowed to stand for 24 hours to facilitate the evaporation of ethyl acetate (Kurniawan et al., 2018). Nanoparticles were subjected to centrifugation for a duration of 10 minutes at a rotational speed of 10,000 rpm. The resultant precipitate was subsequently dissolved in 1 mL of deionized water within an Eppendorf tube, yielding a nanosuspension (Kurniawan et al., 2024).

Characterization of *Andrographis paniculata* ethanol extract PLGA nanoparticles

PLGA nanoparticles encapsulating ethanol extract of *Andrographis paniculata* were characterized with respect to particle size, size distribution, zeta potential, and morphological attributes. Particle size and size distribution were assessed by diluting a 50 µL sample of the nanoparticle suspension with 1000 µL of distilled water, followed by measurement using a particle size analyzer (PSA) (Kurniawan et al., 2018). The zeta potential was determined by initially obtaining 50 µL of nanoparticle suspension, which was subsequently diluted with 1000 µL of 1N KCl solution. The measurement was conducted using a particle size analyzer/zeta sizer (Tarwadi et al., 2023). The morphological observation of nanoparticles was conducted by first subjecting the synthesized nanoparticles to lyophilization. Subsequently, the lyophilized nanoparticles were positioned onto a scanning electron microscope (SEM) holder for detailed examination (Kurniawan et al., 2018)

Preparation of media and cell culture

Stock cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), penicillin (100 IU/mL), and streptomycin (10 mg/mL) in a CO₂ incubator at 5% concentration and a temperature of 37°C until they reached 70-80% confluence. Cells were subsequently dissociated using a 0.25% Trypsin-EDTA solution (Silva et al., 2019).

Cytotoxicity test using MTT assay

The monolayer cell culture was harvested utilizing 0.25% Trypsin-EDTA and subsequently seeded at a density of 1.0 x 10⁴ cells per well in culture medium. A 100 µL cell suspension was added to each well of a 96-well microtiter plate, followed by incubation for 24 hours. Post-incubation, the medium was aspirated and replaced with 100 µL of culture medium containing *Andrographis paniculata* nanoparticle solutions at

concentrations of 1000, 500, 250, 100, and 50 ppm. The cells were then incubated for an additional 24 hours at 37°C in a 5% CO₂ atmosphere. After the second incubation period, the medium was removed and 100 µL of MTT solution was added to each well, followed by a 4-hour incubation. Subsequently, 100 µL of SDS in HCl solution was added to the wells and incubated for 24 hours at 37°C in a 5% CO₂ environment. Cellular morphology was examined microscopically using a light microscope. The absorbance of the 96-well microtiter plate was measured using a microplate reader at a wavelength of 595 nm (Kurniawan et al., 2024).

The determination of cell viability percentage was conducted based on the absorbance readings obtained from the microplate reader. The percentage of cell viability (% viability) was calculated using the formula:

$$\% \text{cell viability} = \frac{Abs_{\text{sample}} - Abs_{\text{media control}}}{Abs_{\text{control}} - Abs_{\text{media control}}} \times 100\%$$

Acute protective test

The monolayer cell culture was harvested using 0.25% Trypsin-EDTA and seeded at a density of 1.0×10^4 cells/well in culture medium. A 100 µL aliquot of the cell suspension was added to each well of a 96-well microtiter plate, followed by incubation for 24 hours. Post-incubation, the medium was aspirated. Each well was then treated with 100 µL of *Andrographis* nanoparticle solution at serial concentrations of 500, 250, and 100 ppm; 20 µM vitamin E; 0.5% DMSO; and culture medium. Subsequent incubation was carried out for 2 hours at 37°C with 5% CO₂. The medium was then removed, and 100 µL of 1.6 mM H₂O₂ was added to each well, followed by another 2-hour incubation. After this period, the medium was aspirated, and 100 µL of fresh culture medium was added to the wells. The plate was incubated for an additional 24 hours, after which 100 µL of MTT reagent was introduced, followed by a 4-hour incubation. A 100 µL aliquot of SDS solution was then added to the wells, and the plate was incubated for 24 hours at 37°C in a 5% CO₂ environment. Cellular morphology was examined using a microscope. Absorbance readings of the 96-well microtiter plate were obtained using a microplate reader at a wavelength of 595 nm (Nurulita et al., 2018).

Recovery test

The monolayer cell culture was harvested with the aid of 0.25% Trypsin-EDTA and seeded at

a density of 1.0×10^4 cells per well using culture medium. A 100 µL cell suspension was added to each well in a 96-well microtiter plate, followed by incubation for 24 hours. Post-incubation, the medium was aspirated. Subsequently, 100 µL of 1.6 mM H₂O₂ was added to each well and incubated for 2 hours. The medium was then discarded, and 100 µL of *Andrographis paniculata* nanoparticles at concentrations of 500, 250, and 100 ppm; 20 µM vitamin E; 0.5% DMSO; and culture medium were added. The plate was incubated for an additional 2 hours at 37°C in a 5% CO₂ atmosphere. After this incubation period, the medium was removed and replaced with 100 µL of fresh culture medium. The plate was further incubated for 24 hours, after which 100 µL of MTT was added and incubated for 4 hours. Subsequently, 100 µL of SDS solution was added to each well and incubated for 24 hours at 37°C in a 5% CO₂ environment. Cellular morphology was observed using a microscope. Absorbance of the 96-well microtiter plate was measured using a microplate reader at a wavelength of 595 nm (Nurulita et al., 2018).

The chronic protection test

The monolayer cell culture was harvested using 0.25% Trypsin-EDTA and seeded at a density of 0.5×10^4 cells per well in culture medium. A cell suspension of 100 µL was added to each well of a 96-well microtiter plate and incubated for 24 hours. Post incubation, the medium was aspirated. Subsequently, 100 µL of *Andrographis paniculata* nanoparticles, at concentrations of 500, 250, and 100 ppm; 20 µM vitamin E; 0.5% DMSO; and culture medium were added to each well. The plate was then incubated for 2 hours at 37°C with 5% CO₂. Following this, the medium was removed, and 100 µL of 1.6 mM H₂O₂ was added to the wells and incubated for another 2 hours. Afterward, the medium was aspirated and replaced with 100 µL of culture medium, followed by a 24-hour incubation. This treatment was repeated at 24, 48, and 72 hour intervals. According to Macko et al. (2021), an incubation period of 72 hours can be characterized as a chronic condition in in vitro experimentation. Subsequently, 100 µL of MTT was added to the plate and incubated for 4 hours. After the incubation, 100 µL of SDS solution was added to each well, and the plate was incubated for 24 hours at 37°C with 5% CO₂. Cellular morphology was observed under a microscope. Absorbance was measured using a microplate reader at a wavelength of 595 nm (Nurulita et al., 2018).

Data analyses

The morphology of the untreated Vero cell line is observed to be in optimal condition when compared to the Vero cell line that has undergone treatment. The statistical analysis of cell viability percentages was performed using GraphPad 8.0 software, with results expressed as mean \pm standard deviation (SD). One-way ANOVA was employed to determine statistical significance, with a significance threshold set at $p < 0.05$.

RESULTS AND DISCUSSION

Based on the results of the test, nanoparticles with a PLGA weight of 2 mg produced a particle size of 466.4 ± 31.6 nm. The polydispersity index value of these nanoparticles was < 0.5 , specifically 0.365 ± 0.032 , indicating that the particles produced were relatively homogeneous. The zeta potential value of the PLGA-*Andrographis paniculata* ethanol extract nanoparticles obtained from this study was -1.48 mV, characterizing them as anionic (negative).

The morphological observations of PLGA nanoparticles encapsulating ethanolic extract of *Andrographis paniculata*, as examined through Scanning Electron Microscopy (SEM) (Figure 1).

The PLGA nanoparticles derived from *Andrographis paniculata* ethanol extract exhibit an irregular morphology intermediate between spherical and cubic shapes, consistent with the findings of Kurniawan et al. (2018) (Figure 1). The obtained PLGA nanoparticle morphology is anticipated to deliver the desired efficacy.

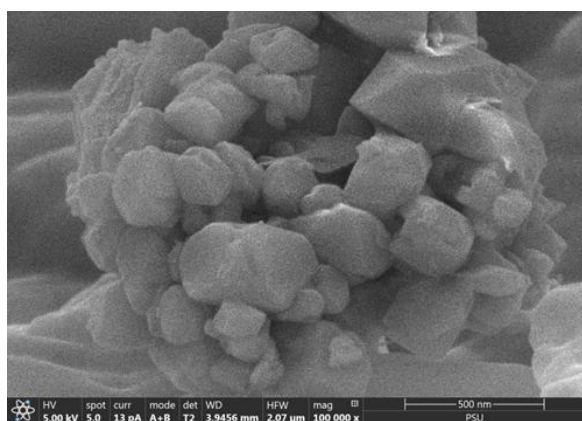


Figure 1. The morphological observations of PLGA nanoparticles encapsulating *Andrographis paniculata* ethanol extract at a magnification of 100,000x.

Cytotoxicity assay of *Andrographis paniculata* ethanol extract-loaded PLGA nanoparticles on Vero cells

The cytotoxicity assay was conducted by introducing nanoparticles at concentrations of 1000, 500, 250, 100, and 50 ppm into the wells, followed by an incubation period of 24 hours.

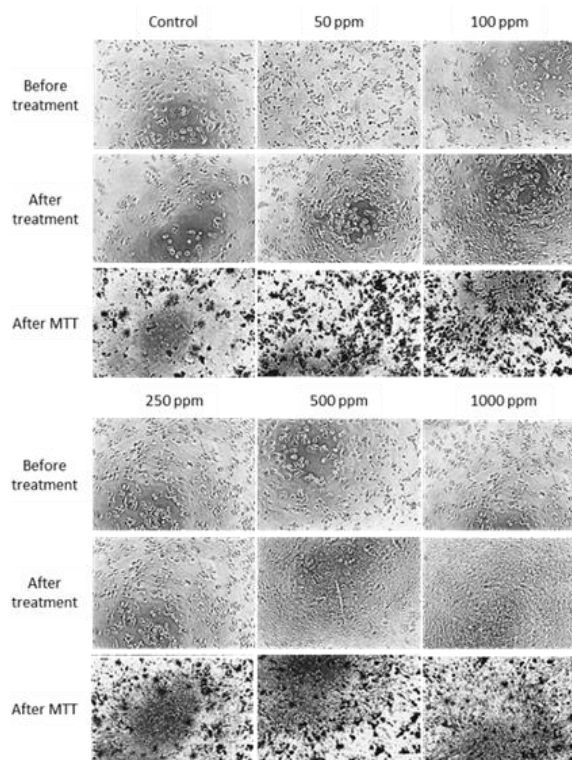


Figure 2. The effect of PLGA nanoparticle *Andrographis paniculata* on Vero cell morphology at 100x magnification. The cytotoxicity assay was conducted in triplicate

The morphology of Vero cells was analyzed using microscopy to observe the differences before and after treatment (Figure 2). Treatment with *Andrographis paniculata* nanoparticles resulted in altered cell morphology, characterized by a more rounded and smaller appearance (Figure 2). Such morphological alterations in Vero cells may include cell shrinkage with distorted shapes and a reduction in cell number (Hussein et al., 2019).

The cytotoxicity assay of nanoparticles demonstrated a dose-dependent trend in cell viability, where an increase in nanoparticle concentration corresponded with enhanced cell viability. Specifically, at concentrations of 50, 100,

250, 500, and 1000 ppm, the cell viability was observed to be 90.54%, 86.824%, 97.297%, 116.892%, and 153.378%, respectively. These results indicate that the nanoparticles do not exert a toxic effect on the cells, as evidenced by cell viability exceeding 50%. According to Desai et al. (2023), nanoparticles are considered toxic to normal cells if the cell viability is equal to or less than 50%.

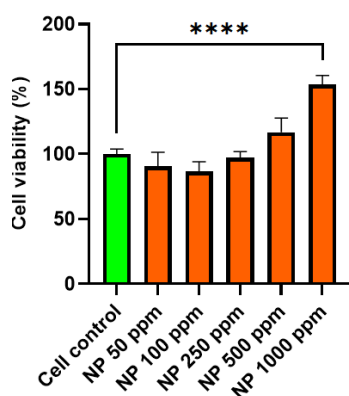


Figure 2. The viability assay results of PLGA nanoparticles containing ethanol extract of *Andrographis paniculata* on Vero cells (n=3).

Based on the results of the cytotoxicity assay, *Andrographis paniculata* nanoparticles at concentrations of 100, 250, and 500 ppm were employed for nephroprotective evaluation. The nephroprotective assessment encompassed acute protection tests, recovery tests, and chronic protection tests. The nephroprotective assays utilized H₂O₂ as an inducer of oxidative stress and vitamin E as a positive control.

Acute protection assay

An acute protection assay was conducted to ascertain the potential of PLGA nanoparticles encapsulating *Andrographis paniculata* ethanol extract in mitigating oxidative stress induced by H₂O₂ administration (Figure 4). The administration of *Andrographis paniculata* nanoparticles exhibited a trend indicating that an increase in cell viability is directly proportional to the increase in concentration. Specifically, *Andrographis paniculata* nanoparticles at concentrations of 100, 250, and 500 ppm resulted in vero cell viabilities of 75%; 80.978%; 91.304%, respectively. *Andrographis paniculata* nanoparticles at a concentration of 500 ppm exhibited the most substantial protective effects in comparison to nanoparticles at other

concentrations. Vitamin E demonstrated a protective effect by enhancing the viability of Vero cells to 88.587%; however, this increase was not statistically significant (p>0.05).

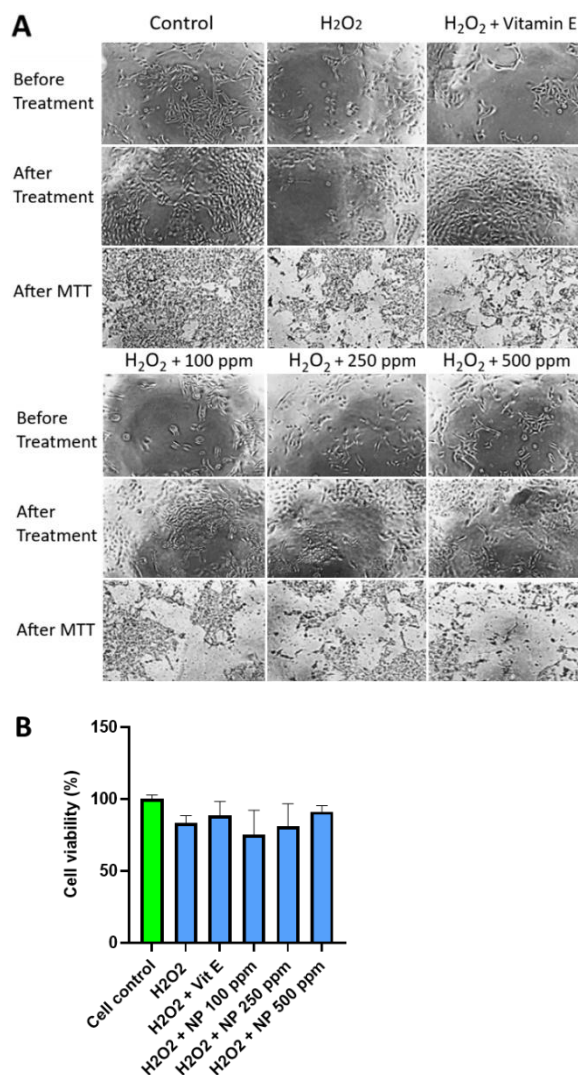


Figure 3. Acute protection assay results of PLGA nanoparticles containing ethanol extract of *Andrographis paniculata* on Vero cells. The morphology of Vero cells at 100x magnification (A). The quantification of Vero cells viability post H₂O₂ induction compared to the positive control (vitamin E) (B). The acute protection test was conducted with three replicates. Data are presented as mean ± SD.

The protective effects of high-concentration nanoparticles surpass those of vitamin E, attributable to the superior antioxidant activity of andrographolide compared to vitamin C, with an IC₅₀ value of 0.514 mg/mL (Adam et al., 2023).

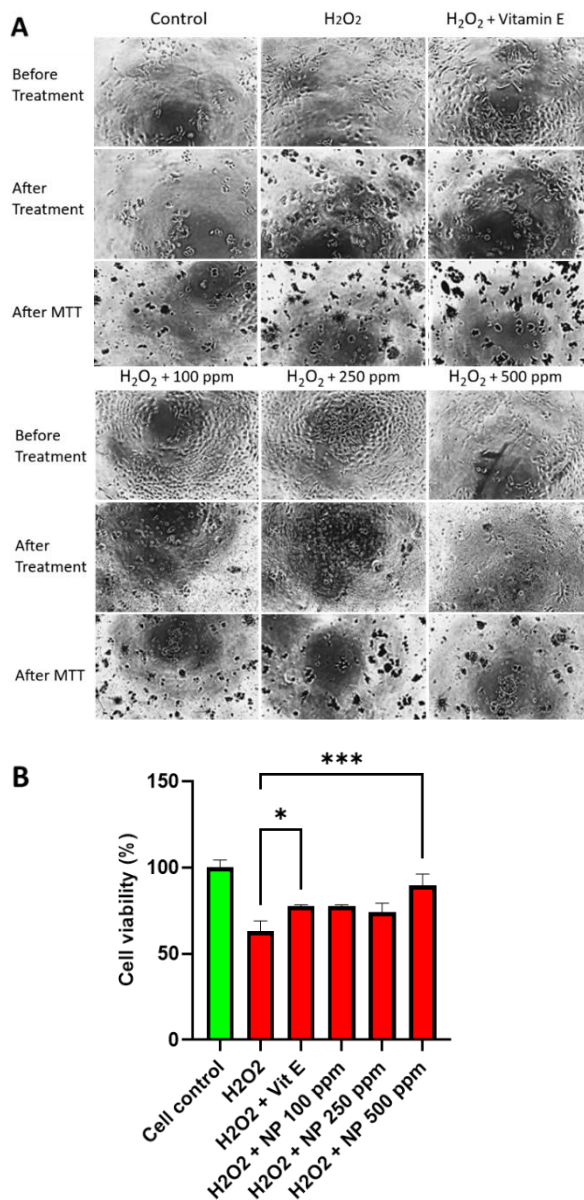


Figure 4. The results of the recovery assay of PLGA nanoparticles containing ethanolic extract of *Andrographis paniculata* on Vero cells. Morphology of Vero cells at 100x magnification (A). The calculation of Vero cells viability post-induction with H₂O₂ compared to the positive control, vitamin E (B). Testing was conducted in three replicates (n=3). Data are presented as mean ± SD.

Andrographolide mitigates oxidative stress by inhibiting the release of reactive oxygen species (ROS). Specifically, it reduces NADPH oxidase activity, which catalyzes the oxidation of NADPH by oxygen (O₂) to produce NADP⁺, H⁺, and O₂^{•-}, thereby diminishing ROS production. Additionally,

andrographolide effectively impedes the synthesis of pro-inflammatory cytokines by downregulating the expression of NLRP3, caspase-1, and IL-1β (Mussard et al., 2019).

Recovery assay

The recovery assay was conducted to evaluate the efficacy of nanoparticles in restoring Vero cells that had sustained damage due to H₂O₂ induction (Figure 5).

Induction with H₂O₂ for a duration of 2 hours significantly reduces cell viability ($p < 0.05$) to 63.006%. Vitamin E at a concentration of 20 mM, employed as a positive control, demonstrates a marked recovery effect ($p < 0.05$) by enhancing cell viability to 97.26%. Nanoparticles at concentrations of 100, 250, and 500 ppm respectively elevate cell viability to 77.457%, 73.988%, and 89.595%. The 100 ppm nanoparticles exhibit a significant recovery effect ($p < 0.05$) that closely approximates that of Vitamin E. This phenomenon may be attributed to the presence of phenolic compounds in the ethanol extract of *Andrographis paniculata*, which are capable of neutralizing H₂O₂ to H₂O (Sinha, 2020).

Chronic protection assay

The chronic protection assay was conducted over a period of 3 days to observe the continuous protective effects of PLGA nanoparticles encapsulating *Andrographis paniculata* ethanol extract against chronic H₂O₂ induction (Figure 6).

The induction of H₂O₂ resulted in a reduction of cell viability to 88.082%. The administration of H₂O₂ did not cause significant cellular damage ($p > 0.05$) as H₂O₂ can facilitate cell cycle progression by oxidizing thiol proteins, thus forming disulfide bonds that stabilize the protein structure (Heo et al., 2020). Increasing the concentration of H₂O₂ could lead to a more pronounced reduction in cell viability. The administration of vitamin E increased the viability of Vero cells to 97.26%, although this increase was not statistically significant ($p > 0.05$). *Andrographis paniculata* nanoparticle treatment exhibited a trend where higher concentrations corresponded to enhanced protective effects. Treatment with *Andrographis paniculata* nanoparticles at concentrations of 100, 250, and 500 ppm resulted in cell viabilities of 67.808%, 80.822%, and 90.411%, respectively. A concentration of 500 ppm provided superior protective effects against H₂O₂ induction compared to other concentrations, although this was not statistically significant ($p > 0.05$).

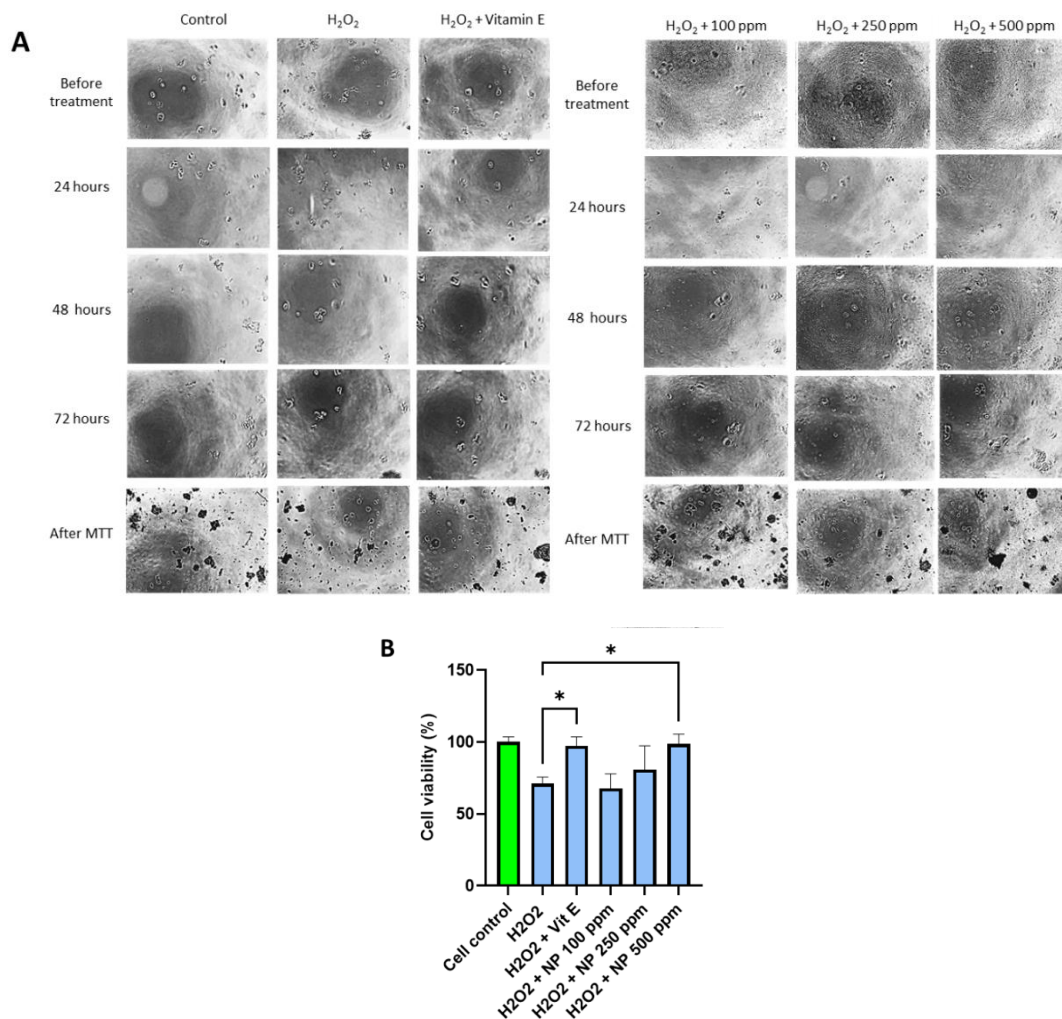


Figure 5. Chronic protection assay results of PLGA nanoparticles encapsulating *Andrographis paniculata* ethanolic extract on Vero Cells. The morphology of Vero cells at 100x magnification (A). The quantification of Vero cells viability post H₂O₂ induction compared to the positive control, vitamin E (B). The experiments were conducted in triplicate (n=3). Data are presented as mean ± standard deviation (SD).

These protective effects are consistent with the findings of Liu et al. (2021), which demonstrated that treatment with andrographolide (5 and 10 μM) for 36 hours could protect human renal tubular epithelial HK-2 cells by reducing apoptosis induced by high glucose concentrations.

Vitamin E offers superior protection compared to *Andrographis paniculata* nanoparticles, albeit the difference is not statistically significant (p > 0.05). In the study conducted by Afandi et al. (2020), Vitamin E demonstrated nephroprotective effects against acute kidney injury induced by cisplatin. Vitamin E was shown to reduce Blood Urea Nitrogen (BUN) and serum creatinine levels in male Wistar rats

(*Rattus norvegicus*). As a potent antioxidant, Vitamin E has long been recognized for its ability to neutralize reactive oxygen species (ROS), thereby maintaining the balance between free radical production and antioxidant capacity in the body (Yilmaz et al., 2012). Vitamin E protects cell membranes and lipids from oxidation and peroxidation through the donation of phenolic hydrogen atoms (Lee et al., 2023).

CONCLUSION

Andrographis paniculata nanoparticles at concentrations ranging from 50 to 1000 ppm exhibited no cytotoxic effects on Vero cells. Moreover, concentrations between 100 and 500

ppm demonstrated nephroprotective effects against H₂O₂-induced oxidative stress. Notably, higher concentrations of these nanoparticles correlated with enhanced nephroprotective efficacy.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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