Ind. J. Pharm. Edu. Res., 2026; 60(4):1777-1786.
https://www.ijper.org

Original Article

Flax-Based Green-Synthesized Gold Nanoparticles Alleviate the Severity of Multiple Sclerosis Symptoms in Mice

Yuanhong Li, Yan Jiang*

Department of Neurology, The Second People's Hospital of Hunan Province, Brain Hospital of Hunan Province, No. 427, Section 3, Furong Middle Road, Changsha, CHINA.

ABSTRACT

Aim: This study aimed to explore the green synthesis and characterization of Gold Nanoparticles (GNPs) using an aqueous extract of *Linum usitatissimum* L. (Flax) and assess their potential role in neuroinflammatory disease management. **Background:** The therapeutic applications of green-synthesized GNPs are gaining prominence in addressing neurodegenerative and autoimmune disorders such as Multiple Sclerosis (MS). **Objectives:** To evaluate the antioxidant properties of flax-mediated GNPs and their therapeutic efficacy in Experimental Autoimmune Encephalomyelitis (EAE), a widely accepted animal model of MS. **Materials and Methods:** GNPs were synthesized by reducing Chloroauric Acid ($HAuCl_4$) with Flax extract. Characterization was performed using Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS), and Zetasizer analysis. Antioxidant activity was assessed using the DPPH assay. EAE was induced in female C57BL/6 mice via myelin oligodendrocyte glycoprotein peptide and Freund's adjuvant. Disease severity was evaluated by monitoring clinical scores and body weight changes. **Results:** The synthesized GNPs were less than 100 nm in size. Antioxidant activity, as determined by the DPPH assay, varied with GNP concentration. Notably, GNP treatment significantly reduced the severity of MS symptoms in EAE mice. **Conclusion:** These findings suggest that GNPs derived from Flax extract hold promise as therapeutic agents for alleviating clinical symptoms associated with multiple sclerosis. This study lays the groundwork for further investigation into the application of GNPs in the treatment of autoimmune diseases.

Keywords: Autoimmune Encephalomyelitis, Gold Nanoparticles, Flax (*Linum usitatissimum* L.) Extract.

Correspondence:
Dr. Yan Jiang
Department of Neurology, Brain Hospital of Hunan Province (The Second People's Hospital of Hunan Province), No. 427, Section 3, Furong Middle Road, Changsha-410000, CHINA.
Email: yanjiang6210@outlook.com

Received: 06-03-2026;
Revised: 14-05-2026;
Accepted: 24-07-2026.

INTRODUCTION

Autoimmune Encephalitis (AE) is a rare neuroinflammatory disorder in which the immune system mistakenly attacks normal brain and spinal cord tissues, leading to abrupt physical and psychological changes. Some patients exhibit disease-associated Antibodies (Ab) in blood or Cerebrospinal Fluid (CSF), while others remain seronegative yet display similar clinical features.[1-3] Experimental Autoimmune Encephalomyelitis (EAE), an induced inflammatory demyelinating model in animals, closely mimics AE pathology by targeting the myelin sheath of Central Nervous System (CNS) nerve fibers.[4-6]

Brain inflammation can manifest through a broad spectrum of neurological and psychiatric symptoms, including rapid cognitive decline, speech impairment, abnormal movements or seizures, vision loss, limb weakness, and sleep disturbances. Psychiatric symptoms such as anxiety, mood instability, psychosis, hallucinations, delusions, or catatonia may also occur, typically developing over weeks to months. However, long-standing mental health conditions persisting for years are not necessarily indicative of autoimmune encephalitis.[7-10] These clinical features, diagnostic challenges, and management approaches have been reviewed in recent literature and consensus statements.[11-14]

In chronic neuroinflammatory disorders such as Multiple Sclerosis (MS), excessive generation of Reactive Oxygen Species (ROS) contributes to extensive cellular and tissue damage.[15,16] ROS also modulate immune mechanisms by promoting Dendritic Cell (DC) maturation and enhancing MHC II and co-stimulatory molecule expression, ultimately activating $CD4^+$ T cells.[17-20] Although small-molecule antioxidants have demonstrated efficacy in reducing EAE severity,[21-23] their therapeutic use remains limited due to poor bioavailability, rapid renal clearance, and diminished *in vivo* stability.[24,25] These limitations highlight the need for novel antioxidant strategies with improved systemic retention and therapeutic potential.

Nanotechnology offers a promising platform to overcome these challenges. Among various nanomaterials, metallic

DOI: 10.5530/ijper.20262115

nanoparticles—particularly Gold Nanoparticles (GNPs)—have gained significant attention due to their unique physicochemical characteristics, including high surface-area-to-volume ratio, ease of functionalization, and excellent biocompatibility.[26-30] Such features make GNPs valuable for diverse biomedical applications, including drug delivery, imaging, and the treatment of inflammatory disorders.[31,32]

Recent advances in green nanotechnology have further enhanced the appeal of GNPs synthesized using plant extracts. The biosynthesis of nanoparticles using medicinal plants is considered eco-friendly, cost-effective, and safe for biomedical use. Phytochemicals serve as natural reducing and stabilizing agents in nanoparticle formation, imparting additional bioactivities, including antioxidant and anti-inflammatory effects.[33-35]

Linum usitatissimum (Flax) is a rich source of essential fatty acids, lignans, and polyphenolic compounds known for their neuroprotective and antioxidant properties. Flax has shown potential in managing various neurological and inflammatory disorders, including anxiety, bipolar disorder, and neurodegenerative diseases such as Parkinson's and Alzheimer's.[36-39] Its phytoconstituents are believed to enhance nerve conduction, reduce oxidative stress, and modulate inflammatory responses, making it a promising candidate for neuroprotective interventions.

Based on this background, the present study aimed to synthesize and characterize Flax-mediated GNPs, evaluate their antioxidant activity, and assess their therapeutic potential in EAE, a validated animal model of MS. The objective was to develop a green-synthesized, plant-based nanotherapeutic system with enhanced antioxidant capacity and potential utility in autoimmune neuroinflammation.

MATERIALS AND METHODS

Chemical and reagents

$HAuCl_4$ (Sigma Chemical Co. Philadelphia, PA), myelin oligodendrocyte glycoprotein peptide (MOG35-55, SICBD), ketamine/xylazine (Merck, Germany), pertussis toxin (Sigma-Aldrich, P7208), Complete Freund's Adjuvant (CFA, Sigma-Aldrich, USA) and polysorbate 80 (Merck, Germany) were used in this study. The plants (winter flax species) were purchased from the Linea industry (Wavignies, France).

Extraction and Purification

The aerial parts of *Linum usitatissimum* were first broken down using a blade crusher equipped with 1 mm mesh screens. The coarse powder was then further processed in an electric mill to reduce the particle size, thereby improving extraction efficiency. A total of 500 g of the fine powder was extracted in 10 L of a hydroalcoholic solution composed of distilled water and ethanol (50:50, v/v). The extraction was carried out at room temperature for 1 hr under continuous stirring using a magnetic stirrer. The resulting extract was filtered through a Buchner funnel to remove solid residues. To remove the ethanol from the filtrate, the extract was subjected to rotary evaporation under reduced pressure at 40°C. The aqueous concentrate was then subjected to adsorption on Amberlite® XAD-16N resin (Sigma-Aldrich), which was pre-treated by sequential washing with methanol and water to activate the resin and remove any potential contaminants. A total of 4 L of the extract was loaded onto the column packed with pre-activated resin. After adsorption, the column was washed with 2 L of distilled water to eliminate non-retained polar impurities. Subsequently, elution was performed with 2 L of 80% ethanol in water to recover the polyphenol-rich fraction. This eluted fraction was again concentrated under reduced pressure and lyophilized to yield the dry extract of *Linum usitatissimum* (LOH), which was used for nanoparticle synthesis.

Determination of the Total Phenolic Content

The Total Phenolic Content (TPC) of the flax extract and synthesized GNPs was determined using the Folin-Ciocalteu reagent method. Briefly, 600 µL of the appropriately diluted sample was mixed with 600 µL of 10% Folin-Ciocalteu reagent and incubated for 5 min at room temperature. Then, 600 µL of 7.5% Sodium Carbonate (Na_2CO_3) solution was added. The mixture was incubated in the dark at room temperature for 30 min, and the absorbance was measured at 765 nm using a UV-visible spectrophotometer. Gallic acid was used as the standard, and results were expressed as mg Gallic Acid Equivalents (GAE)/g of dry extract. All experiments were performed in triplicate (n=3), and the results are presented as mean±Standard Deviation (SD). Proper dilutions were made for all samples to ensure absorbance values fell within the linear range of the calibration curves.

Determining the Total Flavonoids Content of the Sample

For the Total Flavonoid Content (TFC), 500 µL of the diluted sample was mixed with 150 µL of 5% Sodium Nitrite ($NaNO_2$). After 5 min, 150 µL of 10% Aluminum Chloride ($AlCl_3$) was added, followed by 1 mL of 1 M Sodium Hydroxide (NaOH). The final volume was adjusted to 3 mL with distilled water, mixed well, and absorbance was recorded at 510 nm. Quercetin was used as the reference standard, and the results were expressed as mg Quercetin Equivalents (QE)/g of dry extract.

All experiments were performed in triplicate (n=3), and the results are presented as mean±Standard Deviation (SD). Proper dilutions were made for all samples to ensure absorbance values fell within the linear range of the calibration curves.

Synthesis of gold nanoparticles

The green chemistry was applied to synthesis the GNPs. Briefly, the obtained extract (10 mg/mL) was blended with the gold salt ($HAuCl_4 \cdot 3H_2O$, 1 mM) and stirred at 80°C. During the synthesis

the color of the solution change into cherry red in color indicating the reduction of Au^{+} ions to Au^{0} nanoparticles.

Characterizations

The UV-vis spectral analysis of the biogenic GNPs was performed using double beam UV-Vis spectrophotometer with quartz cell (Varian's Cary 100, USA) at a wavelength of 200 nm to 800 nm (1 nm resolution). The experiment was conducted on colloidal dispersion of GNPs. The morphology of the extract-mediated GNPs was observed using a TEM (Philips EM 208 S system, Netherlands). The imaging was conducted on a diluted dispersion of GNPs after placing on carbon-coated copper grid and drying at ambient conditions. The same experiment was conducted to visualize the biogenic GNPs using the SEM imaging technique. The hydrodynamic diameter and zeta potential of the biogenic GNPs was measured via the DLS technique using Zetasizer Nano ZS (Malvern Panalytical, UK). The experiment was conducted on dispersion of GNPs in PBS buffer (pH 7.4).

Antioxidant activity

The antioxidant properties of the biogenic GNPs was measured using the DPPH assay protocol, according to the previous studies with a slight modification.[40,41] The DPPH solution (ETOH, 3 mL, 0.1M) was incubated with 2 mL of different concentrations of the biogenic GNPs at 37°C in the dark. Then, the absorbance of the samples and controls was read at 517 nm using a UV-vis spectrophotometer.

Cell toxicity evaluation

The MTT assay was employed to measure the toxicity of the biogenic GNPs. In this experiment, the cells were seeded and cultured in 96-well plates 24 hr before the experiment at a cell density of 2.5×10^3 per well. The cell culture medium was DMEM plus streptomycin (100 mg mL^{-p}), penicillin (100 U mL^{-}), and heat-inactivated FBS (10%). The seeded cells were washed with sterilized PBS and incubated with different concentrations of the biogenic GNPs for 24 hr at 37°C, in a 5% CO_2 atmosphere. After the incubation time, the MTT salt was added to the wells and the cells incubated for another 4 hr at 37°C. Then, DMSO was added to the wells to solubilize the crystals and the absorbance of the samples was read using a plate reading spectrophotometer at 570 nm.

Animal studies

The animal studies were conducted on Female C57BL/6 mice with the weight of 18±3 g and 8 to 10 weeks old. The mice were housed and kept at standard conditions (12-hr light/dark cycle, constant temperature of 22±0.5°C, and free access to food and water under pathogen-free conditions. Animal Care and Use Committees approved the experimental procedures and animal care. The animals were divided into three groups (6 mice in each): PBS group (healthy mice without EAE injected by PBS), EAE group (EAE model without treatment) and EA+GNPs (EAE model treated by the synthesized GNPs).

Induction and assessment of EAE

The EAE was induced according to the previous studies[21,42] with slight alteration. Briefly, 300 µg of MOG35-55 peptide emulsified in CFA was subcutaneously injected (in four parts of the body of the mice) to immunized the animals. Moreover, the mice received i.p injections of 400 ng pertussis toxin in PBS at the time of immunization and 2 days after. Polysorbate 80 (1%) was mixed with PBS and GNPs to facilitate the transport of components across the BBB. The clinical symptoms of the animals were evaluated according to the following scale: 0, normal; 1, loss of tail tonicity; 2, limp tail and partial paralysis of hind legs; 3, hind limb paralysis; 4, paralysis of hind limb and forelimb; and 5, moribund state. A blind observer recorded the body weight and the clinical symptoms of animals.

Statistical analysis

One- or two-way Analysis of Variance (ANOVA) was conducted to conduct Statistical comparison of the data using SPSS statistical analysis software version 16. The obtained results were presented mean SEM (standard error of mean) and p values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Biogenic GNPs characteristics

The physicochemical characterization of nanoparticles plays a pivotal role in understanding their biological interactions, stability, and efficacy in therapeutic applications. In the present study, the hydrodynamic diameter and surface charge (zeta potential) of the synthesized biogenic Gold Nanoparticles (GNPs) were evaluated using the Dynamic Light Scattering (DLS) technique.

The DLS analysis revealed that the synthesized biogenic GNPs exhibited an average hydrodynamic diameter of approximately 192±XX nm with a Polydispersity Index (PDI) of XX, indicating a moderately uniform size distribution (Figure 1A). The hydrodynamic diameter, which reflects the size of nanoparticles in a colloidal suspension along with their surface-bound solvent layer, is generally larger than the core particle size observed under electron microscopy. A PDI value below 0.3 is typically considered acceptable for nanomedicine applications, implying colloidal stability and reproducibility of synthesis.

The surface charge of the nanoparticles, as determined by zeta potential measurements, was found to be -9.9 mV (Figure 1B). Zeta potential is an essential parameter that indicates the electrostatic repulsion between adjacent, similarly charged particles in suspension. It is widely used to predict the stability of colloidal dispersions.[43,44] In general, nanoparticles with zeta potential values greater than ±30 mV are considered highly stable due to strong electrostatic repulsion. Although the GNPs

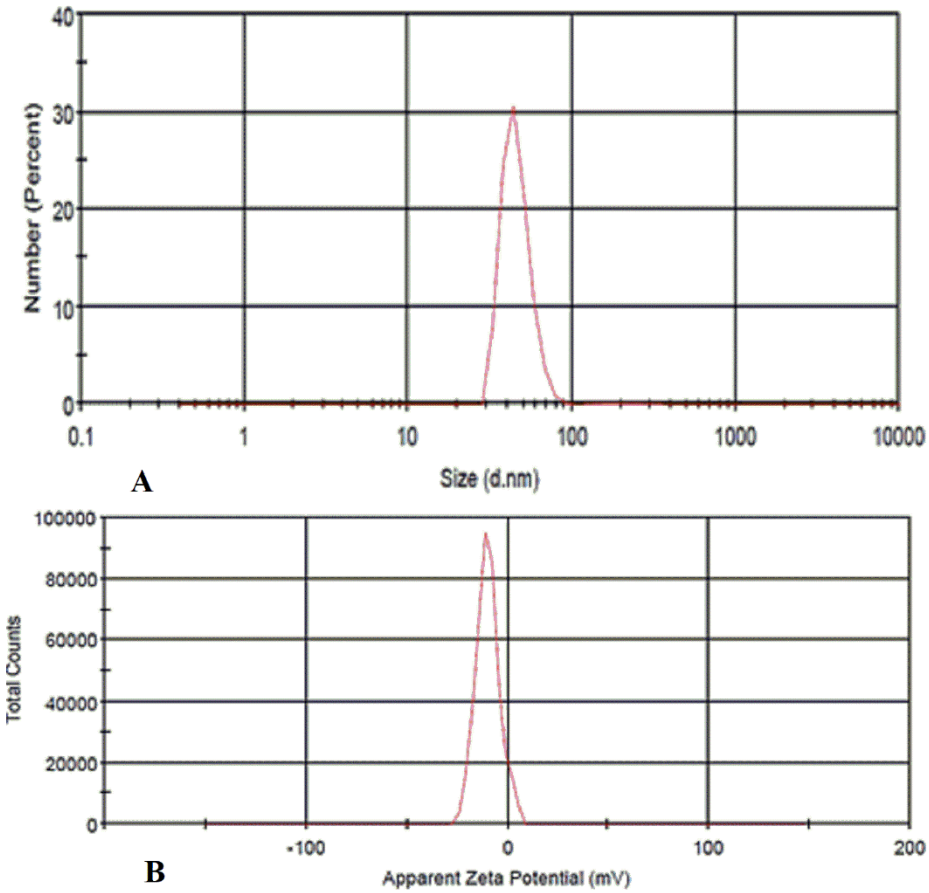

Figure 1: Hydrodynamic diameter of the biogenic GNPs measured by DLS technique (A). Zeta potential of the biogenic GNPs measured by DLS technique (B).

synthesized in this study exhibit a moderately negative surface charge, the colloidal system remained physically stable without signs of aggregation or sedimentation, suggesting that other stabilizing factors, such as phytoconstituent capping from *Linum usitatissimum*, may contribute to their stability.

Furthermore, the DLS and zeta potential data are in agreement with the observed biological activity and compatibility of these nanoparticles in subsequent *in vitro* and *in vivo* experiments. The slightly negative zeta potential may also influence the interaction of GNPs with cellular membranes, affecting uptake and biodistribution. It is also important to note that the DLS measurements were performed in distilled water at ambient pH (approximately 6.8-7.0), which reflects the native colloidal state of these green-synthesized nanoparticles.

Although DLS provides quick and valuable insights into the nanoparticle size distribution and colloidal behavior, it does not offer morphological details. Therefore, the GNPs were further characterized using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM), which confirmed that the particles were predominantly spherical with uniform morphology and a smaller core diameter compared to DLS measurements. These findings support the successful synthesis of stable, well-dispersed gold nanoparticles using *Linum usitatissimum* extract as a reducing and capping agent (Figure 1).

Although the DLS is very helpful and informative technique to measure the NPs diameter, direct observation using electronic microscopies are more appreciated for NPs characterization. Accordingly, we applied Scanning (SEM) and Transmittance (TEM) electron microscopy to directly visualize and evaluate the synthesized biogenic GNPs. SEM image (Figure 2) showed that the synthesized biogenic GNPs have a spherical shape with uniform morphology.

The primary distinction between SEM and TEM is that SEM generates an image by the detection of reflected or knocked-off electrons, whereas TEM generates an image through the use of transmitted electrons (electrons that are moving through the material). Furthermore, one of the most notable distinctions between the two approaches is the highest possible spatial resolution that each of them is capable of achieving. SEM resolution is restricted to 0.5 nm, although with the recent advancement in aberration-corrected TEMs, pictures with spatial resolution of even less than 50 pm have been observed. This contrasts with the fact that the resolution of the SEM is limited. Accordingly, it is proposed that TEM is more appropriate to visualize nanoobjects with a size less than 100 nm. The results

of TEM imaging (Figure 2) are consistent with the results of SEM indicating the spherical shape and monodispersed morphology of the synthesized biogenic GNPs.

To investigate the elemental composition and confirm the successful synthesis of Gold Nanoparticles (GNPs) using *Linum usitatissimum* extract, Energy-Dispersive X-ray (EDAX) spectroscopy was performed along with elemental mapping.

The EDAX spectrum (Figure 3, top panel) revealed prominent peaks corresponding to Gold (Au) at ~2.1 keV and ~9.7 keV, which are characteristic for elemental gold, confirming the successful formation of GNPs. The presence of Carbon (C) and Oxygen (O) was also detected, which can be attributed to the capping and stabilizing phytochemicals derived from the flax extract, such as polyphenols, flavonoids, and other plant-based organics (Figure 3).

In addition to these major peaks, the spectrum also showed minor signals for Chlorine (Cl) and Potassium (K). The presence of Cl might result from residual Chloroauric Acid ($HAuCl_4$) used as a gold precursor or from natural plant constituents. The detection of potassium is consistent with its abundance in biological tissues and plant extracts, suggesting its co-existence or possible involvement in reduction/stabilization mechanisms.

The elemental mapping images further support the EDAX findings by displaying the spatial distribution of individual elements across the sample surface. Uniform and abundant distribution of Gold (Au) (yellow dots) confirms homogeneous deposition of GNPs. The widespread distribution of C (red), O (green), Cl (blue), and K (cyan) suggests their co-localization with gold nanoparticles, likely as capping agents or residuals from the extract and precursor solution.These data collectively confirm the biogenic origin of the GNPs and validate the presence of plant-derived moieties on their surface, which are crucial for their stability, bioactivity, and dispersion in aqueous media.

The role that ROS play in the operation of living organisms is critical. Initially, ROS production was believed to be random and uncontrolled, with its effects mainly contributing to disease progression and aging. However, increasing evidence has established that ROS generation is a normal physiological process. This process is vital for proper immune function and the regulation of several signal transduction pathways. When produced under tightly regulated conditions-in appropriate amounts, time, and locations-ROS help maintain cellular homeostasis.[45,46]

Studies have shown that nasal mucosal cells in individuals with allergic rhinitis produce elevated ROS levels, disrupting the oxidant-antioxidant balance and compromising the antioxidant defense system, thereby contributing to the pathogenesis of asthma and allergic rhinitis.[47-51] ROS such as hydroxyl radicals, superoxides, and peroxides can induce lipid peroxidation, enhance nasal mucosal sensitivity and secretions, increase vascular permeability, and exacerbate inflammatory responses-factors commonly observed in chronic airway inflammation.

Given the close link between oxidative stress and inflammation, we evaluated the antioxidant potential of the synthesized biogenic GNPs using the DPPH assay. The results (Figure 4) demonstrated a concentration-dependent increase in radical scavenging activity, with percent inhibition ranging from 3.7%

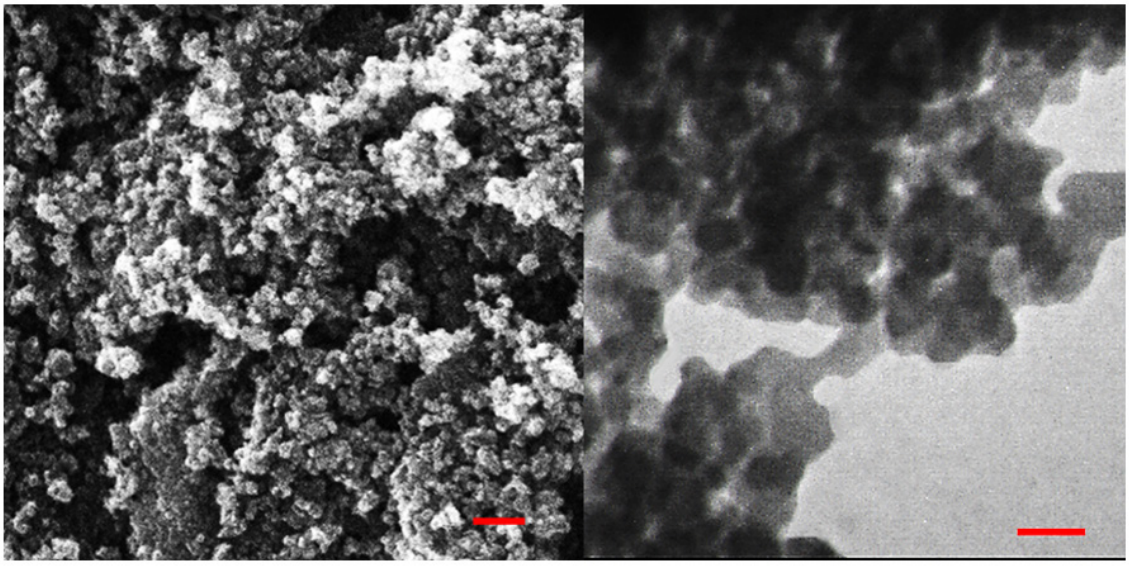

Figure 2: (A) SEM image of the synthesized biogenic GNPs showing spherical morphology and moderate uniformity. (B) TEM image confirming nanoscale size and well-dispersed, spherical particles with minimal agglomeration, supporting successful green synthesis via flax extract.

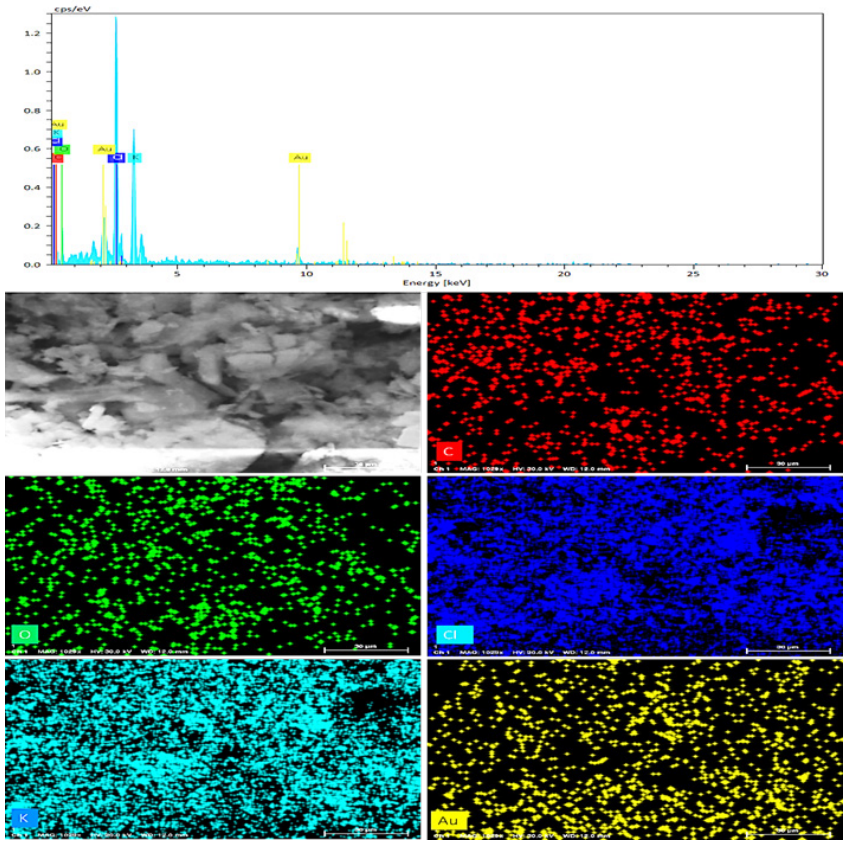

Figure 3: EDAX spectrum and elemental mapping images of the synthesized biogenic GNPs confirming the presence and spatial distribution of Au, C, O, Cl, and K.

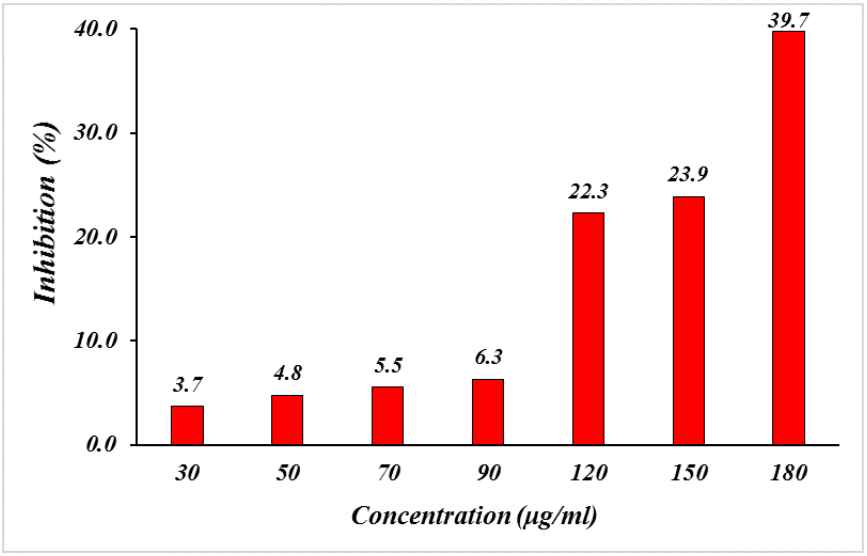

Figure 4: Dose-dependent DPPH radical scavenging activity of the synthesized biogenic GNPs. % inhibition increased from 3.7±0.4% at 30 µg/mL to 39.7±1.2% at 180 µg/mL (p<0.001 vs. control). IC_{50} for GNPs was 164.2 µg/mL; for ascorbic acid (positive control), it was 41.3 µg/mL.

at 30 µg/mL to 39.7% at 180 µg/mL. The IC_{50} value for biogenic GNPs was calculated to be 164.2 µg/mL, indicating moderate antioxidant potency. For comparison, ascorbic acid-the standard reference antioxidant-exhibited a much lower IC_{50} value of 41.3 µg/mL, confirming its higher efficacy. These findings underscore the free radical scavenging ability of the GNPs, though less potent than the standard, and support their potential utility in oxidative stress-related conditions.

The cytocompatibility of the synthesized biogenic GNPs was evaluated using the MTT assay and the results are presented in Figure 5. Although GNPs have emerged as potential instruments for a wide variety of medicinal applications, the evaluation of

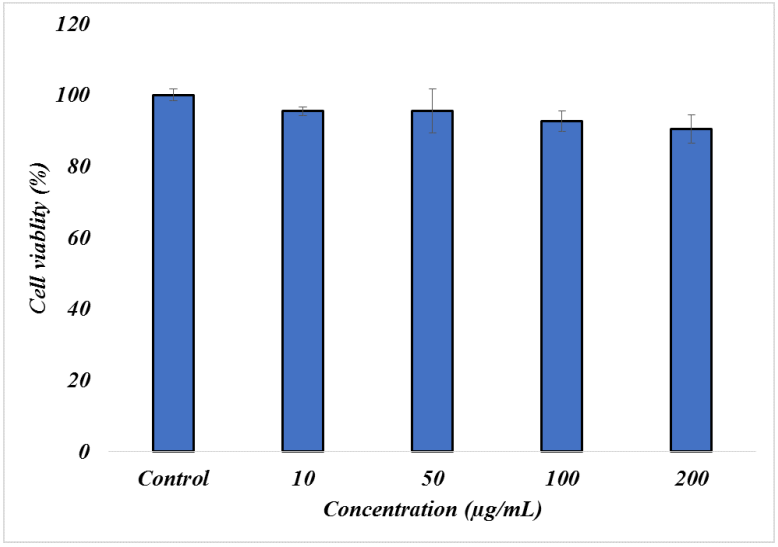

Figure 5: Cytocompatibility of the synthesized biogenic Gold Nanoparticles (GNPs), assessed via MTT assay. Cells were exposed to varying concentrations of GNPs (10-200 µg/mL) for 24 hr. Cell viability remained above 85% across all tested concentrations, indicating low cytotoxicity and high biocompatibility. Data are presented as Mean±SD (*n*=3). Statistical analysis was performed using one-way ANOVA followed by Tukey's *post hoc* test; no significant differences were observed compared to the control group ($p>0.05$).

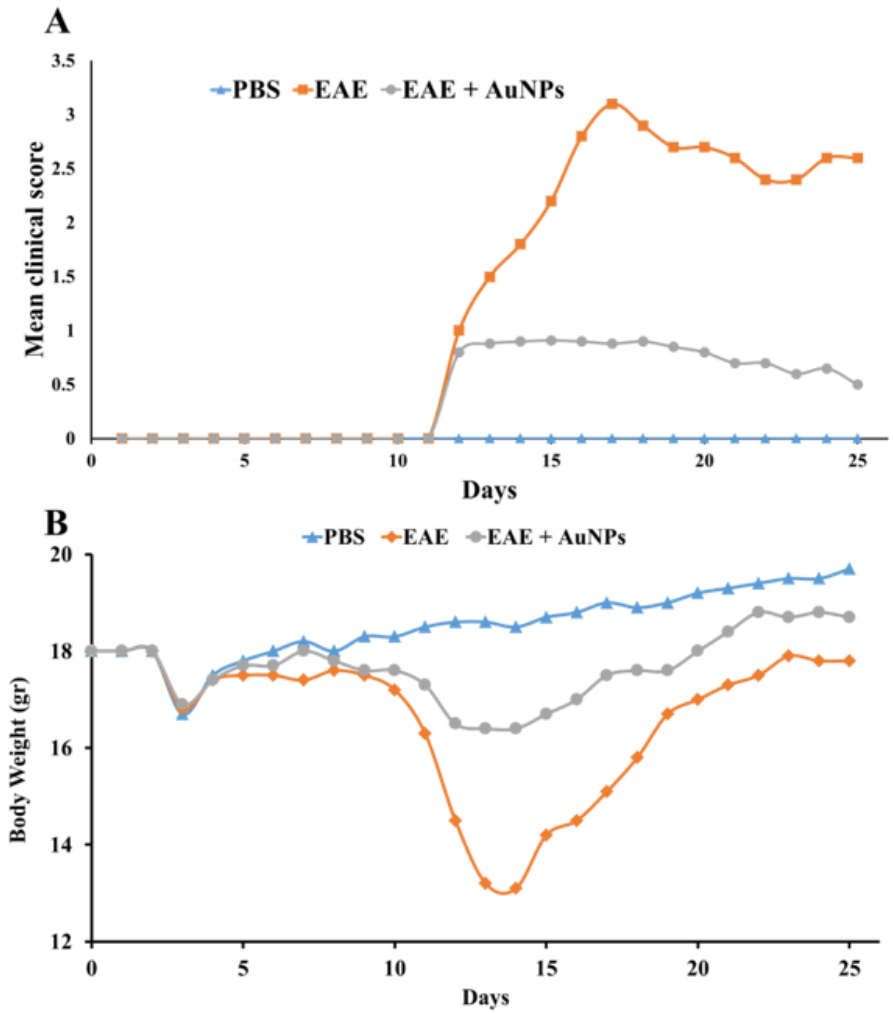

Figure 6: Effect of synthesized biogenic GNPs on EAE clinical signs and body weight, (A) Clinical scores of EAE mice over time show that GNP-treated animals exhibited reduced neurological symptoms compared to untreated EAE controls. (B) Body weight trends indicate that GNP treatment mitigated EAE-associated weight loss. Values are Mean±SEM (*n*=6); $p<0.05$ vs. EAE control.

their biosafety is necessary before their widespread adoption. There is an increasing need to assess the effect that these materials have on people's health and to broaden our understanding of the toxicity and biocompatibility of these substances. When a new nanomaterial is discovered, its cytotoxic effect-that is, the possibility that it could change fundamental biological functions-is typically the first thing that is investigated. Nevertheless, the absence of cytotoxicity does not automatically imply that these materials are biocompatible. This ought to be evaluated as a separate criterion in its own right. The idea of biocompatibility is predicated on the material's ability to interact appropriately with its surrounding biological environment; more specifically, it refers to the lack of a toxic or immunological reaction coming from the treated biomaterial (cell, tissue, or organism). The results of the cytocompatibility showed that the synthesized biogenic GNPs did not induce significant toxicity and are cytocompatible.

The protective/therapeutic efficacy of the fabricated GNPs was evaluated in EAE mice model and the clinical symptoms (Figure 6A) and body weight change (Figure 6B) were recorded as the indication of the healing. The results showed that treatments with Flax-mediated GNPS alleviated the neurological and behavioral symptoms of mice suffered from EAE. We observed that the onset of EAE-related clinical symptoms was 13th day postimmunization. Compared with the control (EAE model without treatment) treatment with GNPs significantly improved clinical scores. The observed findings were in agreement with previous reports. Aghaie *et al.*,[42] synthesized polyethylene glycol-stabilized GNPs and evaluated their ameliorative effects on EAE animal model. They reported that during the experiment (27 days) and under 13 injections in total of PEG-GNPs the severity of EAE symptoms was significantly decreased. They proposed that the healing potential of the PEG-GNPs is due to their antioxidant properties.

CONCLUSION

In this study, we utilized a conjugate of Flax-Gold Nanoparticles (F-GNPs) to investigate their therapeutic potential in an experimental model of Multiple Sclerosis (MS). Gold Nanoparticles (GNPs) have garnered considerable attention in drug delivery due to their excellent biocompatibility, low toxicity and immunogenicity, chemical stability, and ease of functionalization. Their modifiable surface allows conjugation with various bioactive compounds, making them highly suitable for biomedical applications. Plant-derived phytochemicals have previously been shown to possess intrinsic reducing and stabilizing properties for the green synthesis of biocompatible GNPs. Consistent with these findings, our study successfully synthesized GNPs using flax (*Linum usitatissimum*) extract, yielding nanoparticles with favorable physicochemical properties, high biocompatibility, and significant antioxidant activity. Importantly, when tested in the Experimental Autoimmune Encephalomyelitis (EAE) mouse model-a well-established model for MS-the synthesized GNPs significantly alleviated clinical symptoms compared to the control group. The reduction in neurological deficits was attributed to the anti-inflammatory and antioxidative properties of the biogenic GNPs, suggesting a neuroprotective role in modulating disease progression.

These findings highlight the therapeutic promise of flax-mediated GNPs as a novel strategy to manage neuroinflammatory conditions such as MS. Nevertheless, further in-depth studies are warranted to elucidate their precise mechanisms of action, optimize dosage and delivery, and evaluate long-term safety and efficacy in preclinical and clinical settings.

ACKNOWLEDGEMENT

The authors gratefully acknowledge financial support from Brain Hospital of Hunan Province (The Second People's Hospital of Hunan Province).

ABBREVIATIONS

GNPs: Gold Nanoparticles; **F-GNPs:** Flax-mediated Gold Nanoparticles; **EAE:** Experimental Autoimmune Encephalomyelitis; **AE:** Autoimmune Encephalitis; **MOG:** Myelin Oligodendrocyte Glycoprotein; **CFA:** Complete Freund's Adjuvant; **ROS:** Reactive Oxygen Species; **MHC:** Major Histocompatibility Complex; **PBS:** Phosphate Buffered Saline; **TEM:** Transmission Electron Microscopy; **SEM:** Scanning Electron Microscopy; **UV-vis:** Ultraviolet-Visible Spectroscopy; **FCR:** Folin-Ciocalteu Reagent; **GAE:** Gallic Acid Equivalents; **RE:** Rutin Equivalents; **DMSO:** Dimethyl Sulfoxide; **DMEM:** Dulbecco's Modified Eagle Medium; **FBS:** Fetal Bovine Serum; **ANOVA:** Analysis of Variance; **DLS:** Dynamic Light Scattering; **EDAX/EDS:** Energy-Dispersive X-ray Analysis/ Spectroscopy; **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl; **MTT:** 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; **PEG:** Polyethylene Glycol; **EGCG:** Epigallocatechin Gallate; **MS:** Multiple Sclerosis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL COMPLIANCE

This study adheres to internationally accepted standards for animal research, following the 3Rs principle. The ARRIVE guidelines were employed for reporting experiments involving live animals, promoting ethical research practices. The animal research conducted in this article was approved by the approved by the Ethics Committee of Brain Hospital of Hunan Province, China (no. 20230216).

AUTHOR CONTRIBUTIONS

All authors participated in the conception and design of the study. Additionally, all authors have reviewed and endorsed the final manuscript.

DATA AVAILABILITY

The datasets produced and/or analyzed during the present study can be obtained from the corresponding author upon reasonable request.

SUMMARY

This study reports the green synthesis of Gold Nanoparticles (GNPs) using aqueous extract of *Linum usitatissimum* (Flax) and evaluates their therapeutic efficacy in Experimental Autoimmune Encephalomyelitis (EAE), a widely accepted murine model of Multiple Sclerosis (MS). The GNPs were synthesized by the reduction of chloroauric acid with Flax extract and were thoroughly characterized using SEM, TEM, DLS, and Zetasizer analysis. The nanoparticles exhibited a spherical shape with a size below 100 nm and a zeta potential indicative of colloidal stability. Antioxidant potential was confirmed through the DPPH radical scavenging assay. *In vivo* evaluation involved induction of EAE in C57BL/6 mice using myelin oligodendrocyte glycoprotein and Freund's adjuvant. Treatment with Flax-mediated GNPs resulted in a significant reduction in clinical symptoms and weight loss associated with EAE. These findings suggest that biogenically synthesized GNPs possess potent antioxidant and immunomodulatory properties, and may serve as promising therapeutic agents in the management of neuroinflammatory conditions such as MS.

REFERENCES

1. Xu L, Zhang C, He D, Jiang N, Bai Y, Xin Y. Rapamycin and MCC950 modified gut microbiota in experimental autoimmune encephalomyelitis mouse by brain gut axis. Life Sci. 2020;253:117747. doi: 10.1016/j.lfs.2020.117747, PMID 32376270.
2. Mapunda JA, Tibar H, Regragui W, Engelhardt B. How does the immune system enter the brain? Front Immunol. 2022;13:805657. doi: 10.3389/fimmu.2022.805657, PMID 35273596.
3. Malter MP, Elger CE, Surges R. Diagnostic value of CSF findings in antibody-associated limbic and anti-NMDAR-encephalitis. Seizure. 2013;22(2):136-40. doi: 10.1016/j.seizure.2012.12.013, PMID 23318046.
4. Constantinescu CS, Farooqi N, O'Brien K, Gran B. Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). Br J Pharmacol. 2011;164(4):1079-106. doi: 10.1111/j.1476-5381.2011.01302.x, PMID 21371012.
5. Braitch M, Constantinescu CS. The role of osteopontin in experimental autoimmune encephalomyelitis (EAE) and multiple sclerosis (MS), Inflammation and Allergy-Drug Targets (Formerly Current Drug Targets-Inflammation and Allergy)(Discontinued). 2010;9:249-56.
6. Barclay W, Shinohara ML. Inflammasome activation in multiple sclerosis and experimental autoimmune encephalomyelitis (EAE). Brain Pathol. 2017;27(2):213-9. doi: 10.1111/bpa.12477, PMID 27997058.
7. Morris G, Berk M, Walder K, Maes M. Central pathways causing fatigue in neuro-inflammatory and autoimmune illnesses. BMC Med. 2015;13:28. doi: 10.1186/s12916-014-0259-2, PMID 25856766.
8. Broadley J, Seneviratne U, Beech P, Buzzard K, Butzkueven H, O'Brien T, *et al.* Prognosticating autoimmune encephalitis: a systematic review. J Autoimmun. 2019;96:24-34. doi: 10.1016/j.jaut.2018.10.014, PMID 30595145.
9. Lancaster E. The diagnosis and treatment of autoimmune encephalitis. J Clin Neurol. 2016;12(1):1-13. doi: 10.3988/jcn.2016.12.1.1, PMID 26754777.
10. Deng S, Qiu K, Liu H, Wu X, Lei Q, Lu W. Clinical characteristics and short-term prognosis of autoimmune encephalitis: a single-center cohort study in Changsha, China. Front Neurol. 2019;10:539. doi: 10.3389/fneur.2019.00539, PMID 31178819.
11. Graus F, Titulaer MJ, Balu R, Benseler S, Bien CG, Cellucci T, *et al.* A clinical approach to diagnosis of autoimmune encephalitis. Lancet Neurol. 2016;15(4):391-404. doi: 10.1016/S1474-4422(15)00401-9, PMID 26906964.
12. Hermetter C, Fazekas F, Hochmeister S. Systematic review: syndromes, early diagnosis, and treatment in autoimmune encephalitis. Front Neurol. 2018;9:706. doi: 10.3389/fneur.2018.00706, PMID 30233481.
13. Ganesh A, Wesley SF. Practice current: when do you suspect autoimmune encephalitis and what is the role of antibody testing? Neurol Clin Pract. 2018;8(1):67-73. doi: 10.1212/CPJ.0000000000000423, PMID 29517071.
14. Ellul MA, Wood G, Tooren HV, Easton A, Babu A, Michael BD. Update on the diagnosis and management of autoimmune encephalitis. Clin Med (Lond). 2020;20(4):389-92. doi: 10.7861/clinmed.2020-0241, PMID 32675144.
15. Cruz de Carvalho MHC. Drought stress and reactive oxygen species: production, scavenging and signaling. Plant Signal Behav. 2008;3(3):156-65. doi: 10.4161/psb.3.3.5536, PMID 19513210.
16. Barja G. Mitochondrial oxygen consumption and reactive oxygen species production are independently modulated: implications for aging studies. Rejuvenation Res. 2007;10(2):215-24. doi: 10.1089/rej.2006.0516, PMID 17523876.
17. Kumari A, Singh RK. Synthesis, molecular Docking and biological evaluation of N-substituted indole derivatives as potential anti-inflammatory and antioxidant agents. Chem Biodivers. 2022;19(9):e202200290. doi: 10.1002/cbdv.202200290, PMID 35818885.
18. Sheng KC, Pietersz GA, Tang CK, Ramsland PA, Apostolopoulos V. Reactive oxygen species level defines two functionally distinctive stages of inflammatory dendritic cell development from mouse bone marrow. J Immunol. 2010;184(6):2863-72. doi: 10.4049/jimmunol.0903458, PMID 20176741.
19. Previte DM, Piganelli JD. Reactive oxygen species and their implications on CD4+ T cells in type 1 diabetes. Antioxid Redox Signal. 2018;29(14):1399-414. doi: 10.1089/ars.2017.7357, PMID 28990401.
20. Previte DM, O'Connor EC, Novak EA, Martins CP, Mollen KP, Piganelli JD. Reactive oxygen species are required for driving efficient and sustained aerobic glycolysis during CD4+ T cell activation. PLOS One. 2017;12(4):e0175549. doi: 10.1371/journal.pone.0175549, PMID 28426686.
21. Kim TW, Kim Y, Jung W, Kim DE, Keum H, Son Y, *et al.* Bilirubin nanomedicine ameliorates the progression of experimental autoimmune encephalomyelitis by modulating dendritic cells. J Control Release. 2021;331:74-84. doi: 10.1016/j.jconrel.2021.01.019, PMID 33450316.
22. Hendriks JJ, Alblas J, van der Pol SM, van Tol EA, Dijkstra CD, de Vries HE. Flavonoids influence monocytic GTPase activity and are protective in experimental allergic encephalitis. J Exp Med. 2004;200(12):1667-72. doi: 10.1084/jem.20040819, PMID 15611292.
23. Stanislaus R, Gilg AG, Singh AK, Singh I. N-acetyl-L-cysteine ameliorates the inflammatory disease process in experimental autoimmune encephalomyelitis in Lewis rats. J Autoimmune Dis. 2005;2(1):4. doi: 10.1186/1740-2557-2-4, PMID 15869713.
24. Moriya M, Nakatsuji Y, Miyamoto K, Okuno T, Kinoshita M, Kumanogoh A, *et al.* Edaravone, a free radical scavenger, ameliorates experimental autoimmune encephalomyelitis. Neurosci Lett. 2008;440(3):323-6. doi: 10.1016/j.neulet.2008.05.110, PMID 18579300.
25. Lee Y, Kim H, Kang S, Lee J, Park J, Jon S. Bilirubin nanoparticles as a nanomedicine for anti-inflammation therapy. Angew Chem Int Ed Engl. 2016;55(26):7460-3. doi: 10.1002/anie.201602525, PMID 27144463.
26. Smith DM, Simon JK, Baker JR Jr. Applications of nanotechnology for immunology. Nat Rev Immunol. 2013;13(8):592-605. doi: 10.1038/nri3488, PMID 23883969.
27. West JL, Halas NJ. Applications of nanotechnology to biotechnology: commentary. Curr Opin Biotechnol. 2000;11(2):215-7. doi: 10.1016/S0958-1669(00)00082-3, PMID 10753774.
28. Mehta S, Sharma AK, Singh RK. Therapeutic journey of *Andrographis paniculata* (Burm.f.) Nees from natural to synthetic and nanoformulations. Mini Rev Med Chem. 2021;21(12):1556-77. doi: 10.2174/1389557521666210315162354, PMID 33719961.
29. Murty B, Shankar P, Raj B, Rath B, Murday J, Murty B, *et al.* Unique properties of nanomaterials, Textbook of nanoscience and nanotechnology; 2013. p. 29-65.
30. Andrievski RA, Glezer AM. Size effects in properties of nanomaterials. Scr Mater. 2001;44(8-9):1621-4. doi: 10.1016/S1359-6462(01)00786-2.
31. Yeh YC, Creran B, Rotello VM. Gold nanoparticles: preparation, properties, and applications in bionanotechnology. Nanoscale. 2012;4(6):1871-80. doi: 10.1039/C1NR11188D, PMID 22076024.
32. Kumar S, Singh RK, Murthy RS, Bhardwaj TR. Synthesis and evaluation of substituted poly(organophosphazenes) as a novel nanocarrier system for combined antimalarial therapy of primaquine and dihydroartemisinin. Pharm Res. 2015;32(8):2736-52. doi: 10.1007/s11095-015-1659-5, PMID 25777611.
33. Si A, Pal K, Kralj S, El-Sayyad GS, de Souza FG, Narayanan T. Sustainable preparation of gold nanoparticles via green chemistry approach for biogenic applications. Mater Today Chem. 2020;17:100327. doi: 10.1016/j.mtchem.2020.100327.
34. Gurunathan S, Han J, Park JH, Kim JH. A green chemistry approach for synthesizing biocompatible gold nanoparticles. Nanoscale Res Lett. 2014;9:1-11.

35. Rai A, Bhatia R, Singh RK. Nature-inspired anticancer agents: the synergy of phytochemicals and synthetic analogs (2019-2024). Chem Biodivers. Published online April11. 2025; 22(9):e202500162. doi: 10.1002/cbdv.202500162, PMID 40213994.
36. Goyal A, Sharma V, Upadhyay N, Gill S, Sihag M. Flax and flaxseed oil: an ancient medicine and modern functional food. J Food Sci Technol. 2014;51(9):1633-53. doi: 10.1007/s13197-013-1247-9, PMID 25190822.
37. Jhala AJ, Hall LM, Flax (*Linum usitatissimum* L.): current uses and future applications. Aust J Basic Appl Sci. 2010;4:4304-12.
38. Palla AH, Gilani AU, Bashir S, Ur Rehman NUr. Multiple mechanisms of flaxseed: effectiveness in inflammatory bowel disease. Evid Based Complement Alternat Med. 2020; 2020:7974835. doi: 10.1155/2020/7974835, PMID 32765633.
39. Basch E, Bent S, Collins J, Dacey C, Hammerness P, Harrison M, *et al.* Flax and flaxseed oil (*Linum usitatissimum*): a review by the Natural Standard Research Collaboration. J Soc Integr Oncol. 2007;5(3):92-105. doi: 10.2310/7200.2007.005, PMID 17761128.
40. Begum I, Shamim S, Ameen F, Hussain Z, Bhat SA, Qadri T, *et al.* A combinatorial approach towards antibacterial and antioxidant activity using tartaric acid capped silver nanoparticles. Processes. 2022;10(4):716. doi: 10.3390/pr10040716.
41. Xiao F, Xu T, Lu B, Liu R. Guidelines for antioxidant assays for food components, Food Frontiers. 2020;1:60-9.
42. Aghaie T, Jazayeri MH, Avan A, Anissian A, Salari AA. Gold nanoparticles and polyethylene glycol alleviate clinical symptoms and alter cytokine secretion in a mouse model of experimental autoimmune encephalomyelitis. IUBMB Life. 2019;71(9):1313-21. doi: 10.1002/iub.2045, PMID 30957389.
43. Honary S, Zahir F. Effect of zeta potential on the properties of nano-drug delivery systems-a review (Part 1). Trop J Pharm Res. 2013;12:255-64.
44. Kamble S, Agrawal S, Cherumukkil S, Sharma V, Jasra RV, Munshi P. Revisiting zeta potential, the key feature of interfacial phenomena, with applications and recent advancements. ChemistrySelect. 2022;7(1):e202103084. doi: 10.1002/slct.202103084.
45. Kumar S, Singh RK, Bhardwaj TR. Therapeutic role of nitric oxide as emerging molecule. Biomed Pharmacother. 2017;85:182-201. doi: 10.1016/j.biopha.2016.11.125, PMID 27940398.
46. Saed-Moucheshi A, Pakniyat H, Pirasteh-Anosheh H, Azooz M. Role of ROS as signaling molecules in plants. In: Oxidative damage to plants. Amsterdam: Elsevier; 2014. p. 585-620. doi: 10.1016/B978-0-12-799963-0.00020-4.
47. Chauhan B, Gupta M, Chauhan K. Role of antioxidants on the clinical outcome of patients with perennial allergic rhinitis. Allergy Rhinol (Providence). 2016;7(2):74-81:ar. 2016.2017. doi: 10.2500/ar.2016.7.0163, PMID 27658183.
48. Han M, Lee D, Lee SH, Kim TH. Oxidative stress and antioxidant pathway in allergic rhinitis. Antioxidants (Basel). 2021;10(8):1266. doi: 10.3390/antiox10081266, PMID 34439514.
49. Barnes PJ. Reactive oxygen species and airway inflammation. Free Radic Biol Med. 1990;9(3):235-43. doi: 10.1016/0891-5849(90)90034-G, PMID 2272532.
50. Kumari A, Singh RK. Synthesis, Molecular Docking and ADME Prediction of 1H-indole/5- substituted indole Derivatives as Potential Antioxidant and anti-inflammatory Agents. Med Chem. 2023;19(2):163-73. doi: 10.2174/1573406418666220812152950, PMID 35959908.
51. Aksoy F, Yıldırım YS, Veyseller B, Demirhan H, Özturan O. Serum levels of advanced oxidation protein products in response to allergen exposure in allergic rhinitis. Ear Nose Throat J. 2012;91(8):E32-5. PMID 22930093.

Cite this article: Li Y, Jiang Y. Flax-Based Green-Synthesized Gold Nanoparticles Alleviate the Severity of Multiple Sclerosis Symptoms in Mice. Indian J of Pharmaceutical Education and Research. 2026;60(4):1777-86.