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Original Article

ANTIOXIDANT AND CYTOPROTECTIVE EFFECTS OF IFLOGA SPICATA-DERIVED GOLD NANOPARTICLES IN HUMAN LYMPHOCYTES

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ABSTRACT:

Green synthesis of gold nanoparticles (AuNPs) using plant extracts has emerged as a sustainable and biocompatible approach in nanomedicine. In this study, AuNPs were synthesized using *Ifloga spicata* (Forssk.) whole-plant extract and evaluated for their biological effects on human blood lymphocytes exposed to hydrogen peroxide–induced oxidative stress. Nanoparticle formation was confirmed using UV–visible spectroscopy, Fourier-transform infrared spectroscopy (FT-IR), and X-ray diffraction (XRD). Exposure to oxidative stress significantly increased intracellular reactive oxygen species (ROS) and thiobarbituric acid reactive substances (TBARS), while suppressing antioxidant enzyme activities, including catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD). Treatment with *I. spicata*–mediated AuNPs significantly attenuated ROS and TBARS levels and restored antioxidant enzyme activities in a concentration-dependent manner, with maximal protective effects observed at 5 and 50 µg/mL. Importantly, AuNPs did not induce adverse oxidative effects under normal physiological conditions, indicating a favorable cytocompatibility profile. These findings suggest that *I. spicata*–derived AuNPs exhibit antioxidant and cytoprotective properties and may represent promising candidates for mitigating oxidative stress–related cellular damage. Further in vivo studies are warranted to elucidate their mechanistic pathways and therapeutic potential.

Keywords: Gold nanoparticles; Ifloga spicata; cytoprotection; oxidative stress; antioxidants; nanomedicine.

INTRODUCTION

Nanotechnology has gained substantial attention in biomedical research due to its potential to enhance diagnostic, therapeutic, and preventive strategies through nanoscale materials with unique physicochemical properties (1, 2). Among various nanomaterials, gold nanoparticles (AuNPs) have attracted particular interest owing to their chemical stability, biocompatibility, ease of surface functionalization, and relatively low toxicity compared to other metallic nanoparticles (3, 4). These

characteristics make AuNPs especially suitable for biomedical applications, including drug delivery, imaging, and antioxidant therapy (5).

Conventional physical and chemical methods for AuNP synthesis often involve high energy requirements and the use of toxic reducing or stabilizing agents, which may limit their biomedical applicability (6). In contrast, green synthesis approaches utilizing biological resources, particularly plant extracts, offer an environmentally friendly, cost-effective, and scalable alternative (7). Plant-mediated synthesis not only reduces environmental burden but also enables nanoparticle stabilization through naturally occurring phytochemicals such as flavonoids, phenolics, terpenoids, and alkaloids, which may impart additional biological activity (8).

Ifloga spicata (Forssk.), a medicinal plant traditionally used in ethnopharmacological practices, is known for its antioxidant, anti-inflammatory, and protective properties. Despite its documented medicinal relevance, limited information is available regarding its application in nanoparticle synthesis and subsequent biological evaluation. Given the growing evidence that oxidative stress plays a central role in the pathogenesis of numerous diseases, including inflammatory disorders, neurodegeneration, and cancer, the development of biocompatible nanomaterials capable of modulating oxidative damage is of considerable interest (9).

Oxidative stress results from an imbalance between reactive oxygen species generation and cellular antioxidant defenses, leading to lipid peroxidation, protein damage, and enzymatic dysfunction (10). Human lymphocytes are particularly sensitive to oxidative insults and serve as a relevant model for assessing oxidative stress–mediated cellular responses. Biomarkers such as ROS, thiobarbituric acid reactive substances, and antioxidant enzymes, including catalase, superoxide dismutase, and peroxidase, provide valuable insight into cellular redox status (11).

The present study aimed to synthesize AuNPs using *Ifloga spicata* extract via a green synthesis approach and to evaluate their biological effects on human blood lymphocytes under hydrogen peroxide–induced oxidative stress. Rather than inducing cytotoxicity, the study focuses on assessing the cytoprotective and antioxidant potential of plant-derived AuNPs through modulation of oxidative stress markers and antioxidant enzyme activities. The findings provide insight into the potential application of *I. spicata*–mediated AuNPs as biocompatible nanomaterials for oxidative stress mitigation.

MATERIALS AND METHODS

Collection and Extraction of Plant

Ifloga spicata (whole plant) was collected from the university site, District Bannu, Khyber Pakhtunkhwa, Pakistan. Dr. Faizan Ullah, Department of Botany, University of Science and Technology, Bannu, identified the plant, and a voucher specimen was deposited at the Department of Botany of the same university. The plant material was shade-dried and ground to a fine powder, then soaked in 80% aqueous methanol. After five days at room temperature, the filtrate was concentrated using a rotary evaporator (Buchi Rotavapor R-200). The crude plant extract (200 g) was stored at 4 °C for further processing (12).

Extract Solution Preparation and Nanoparticle Synthesis

Plant-mediated synthesis of gold nanoparticles was carried out using a general protocol. Two grams of the methanolic dry extract were dissolved in 100 mL methanol on a hot-plate magnetic stirrer for 30 minutes at 50 °C. After cooling to ambient temperature, 5 mL of the extract was added to 45 mL of 1 mM aqueous $HAuCl_4$ solution. The mixture was kept for 2 hours, and formation of gold nanoparticles was confirmed visually by a colour change to ruby red (12).

Catalase (CAT)

Catalase activity was determined from the decrease in absorbance due to H_2O_2 consumption, following a previously reported method (12).

Superoxide Dismutase (SOD)

Superoxide dismutase activity was determined according to a previously reported method (12). The amount of chromogen formed was measured at 560 nm using a spectrophotometer, and results were expressed in mU/10^8 cells.

Peroxidase (POD)

Peroxidase activity in the homogenate was evaluated using the Carlberg spectrophotometric technique (12). After one minute, the absorbance of the reaction solution at 470 nm was measured to assess the change in absorbance.

Thiobarbituric Acid Reactive Substances (TBARS)

TBARS were determined using a previously described method (12). The optical density at 532 nm was used to quantify the TBARS produced. Using a molar extinction coefficient of 156 mmol/L/cm at 37 °C, results were expressed as nmol malonaldehyde/min/mg tissue.

Reactive Oxygen Species (ROS)

A microplate reader was used to measure absorbance at 505 nm for 180 seconds at 15-second intervals. ROS concentrations were calculated using a standard curve. The amount of hydrogen peroxide in the sample was measured in units of ROS (1 unit = 1.0 mg H_2O_2/L) and expressed as U/10^8 cells (12).

Statistical Analysis

Data were analysed using two-way analysis of variance (ANOVA) to evaluate the effects of the treatments. A p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Effect of *Ifloga spicata*-derived AuNPs on ROS content of human blood lymphocytes

Normal human blood lymphocytes showed no response to the synthesized AuNPs derived from *Ifloga spicata*. However, the human blood lymphocytes were deliberately exposed to H_2O_2 stress to evaluate the level of ROS. The raised ROS level due to stress was considerably improved by the different fractions of *I. spicata*-derived AuNPs.

Effect of *Ifloga spicata*-derived AuNPs on blood enzyme activities

Exposure of human blood lymphocytes to H_2O_2 stress reduced the antioxidant enzyme (SOD, CAT and POD) activities as compared to the normal control ($p < 0.05$). The different fractions of *I. spicata*-derived AuNPs at concentrations of 10 µg/ml and 50 µg/ml potentially recovered antioxidant enzyme activity (SOD, CAT and POD). Irrespective of the others, the crude methanolic and aqueous fractions were significantly more effective in recovering the adverse effects on antioxidant enzyme activities, as shown in Tables 2–4.

Effect of *Ifloga spicata*-derived AuNPs on TBARS of human blood lymphocytes

After treatment with oxidative stress, the blood lymphocytes were evaluated for levels of TBARS. The synthesised plant-based AuNPs smoothly recovered the elevated level of TBARS contents. Concentrations of 10 µg/ml and 50 µg/ml were the most effective, at which blood lymphocytes recovered maximum TBARS contents. The data presented in Table 5 indicate that the ethyl acetate and chloroform fractions were more significantly effective in recovering the elevated level of TBARS ($p < 0.05$).

Although classical cytotoxicity assays such as MTT or lactate dehydrogenase (LDH) release were not performed in the present study, the biological endpoints evaluated provide indirect yet meaningful evidence regarding cytocompatibility. Under normal physiological conditions, treatment with Ifloga

spicata-derived gold nanoparticles did not elevate reactive oxygen species levels, induce lipid peroxidation, or suppress endogenous antioxidant enzyme activities in human lymphocytes. Instead, antioxidant enzyme levels remained comparable to those of untreated controls, indicating the absence of oxidative or metabolic stress.

Table 1. Recovery effects of *I. spicata*-derived AuNPs on ROS of human blood lymphocytes.

Dose	Different Fractions of Crude Extract					Mean value
	Methanol	Ethyl acetate	n-hexane	Chloroform	Aqueous	
Control	0.4 ± 0.06g	0.40 ± 0.06g	0.36 ± 0.02e	0.40 ± 0.16h	0.40 ± 0.12g	0.39 ± 0.06g
100 μM H_2O_2 stress	0.6 ± 0.64a	0.6 ± 0.33a	0.59 ± 0.14a	0.57 ± 0.55a	0.6 ± 0.21a	0.6 ± 0.43a
AuNPs 10 μg/ml	0.34 ± 0.18a	0.37 ± 0.27a	0.39 ± 0.44a	0.31 ± 0.34a	0.33 ± 0.24a	0.32 ± 0.27a
AuNPs 25 μg/ml	0.39 ± 0.22a	0.35 ± 0.34a	0.37 ± 0.11a	0.38 ± 0.27a	0.39 ± 0.24a	0.37 ± 0.17a
AuNPs 50 μg/ml	0.47 ± 0.32a	0.42 ± 0.42a	0.49 ± 0.42a	0.46 ± 0.22a	0.45 ± 0.52a	0.44 ± 0.32a

Table 2. Recovery effects of *I. spicata*-derived AuNPs on CAT antioxidant enzyme of human blood lymphocytes.

Dose	Different Fractions of Crude Extract					Mean value
	Methanol	Ethyl acetate	n-hexane	Chloroform	Aqueous	
Control	0.4 ± 0.06g	0.39 ± 0.06g	0.30 ± 0.02e	0.40 ± 0.16h	0.38 ± 0.12g	0.39 ± 0.06g
100 μM H_2O_2 stress	0.2.861 ± 0.14n	0.2.871 ± 0.33n	0.2.6571 ± 0.132n	0.2.7571 ± 0.25n	0.1.9571 ± 0.18n	0.1.9781 ± 0.134b
AuNPs 10 μg/ml	0.3.2183 ± 0.24b	0.3.20 ± 0.36b	0.3.171 ± 0.24e-i	0.3.2811 ± 0.18b-h	0.3.613 ± 0.17b-e	0.3.7829 ± 0.16a
AuNPs 25 μg/ml	0.39 ± 0.22a	0.39 ± 0.35a	0.38 ± 0.52a	0.37 ± 0.12a	0.39 ± 0.52a	0.39 ± 0.42a
AuNPs 50 μg/ml	0.48 ± 0.06g	0.49 ± 0.06g	0.41 ± 0.02e	0.47 ± 0.16h	0.41 ± 0.12g	0.341 ± 0.06g

Table 3. Recovery effects of *I. spicata*-derived AuNPs on POD antioxidant enzyme of human blood lymphocytes.

Dose	Different Fractions of Crude Extract					Mean value
	Methanol	Ethyl acetate	n-hexane	Chloroform	Aqueous	
Control	0.52 ± 0.23ab	0.541 ± 0.33ab	0.53 ± 0.24ab	0.57 ± 0.34ab	0.481 ± 0.04ab	0.531 ± 0.43b
100 μM H_2O_2 stress	0.43 ± 0.14de	0.41 ± 0.12de	0.41 ± 0.23de	0.381 ± 0.22de	0.3391 ± 0.32de	0.3631 ± 0.13c
AuNPs 10 μg/ml	0.561 ± 0.134ab	0.51 ± 0.4cd	0.5699 ± 0.11a	0.569 ± 0.15a	0.565 ± 0.15ab	0.575 ± 0.1a
AuNPs 25 μg/ml	0.651 ± 0.144ab	0.66 ± 0.31ab	0.65 ± 0.10ab	0.61 ± 0.32ab	0.65 ± 0.24a	0.6943 ± 0.51ab
AuNPs 50 μg/ml	0.661 ± 0.134ab	0.61 ± 0.4cd	0.5699 ± 0.11a	0.67 ± 0.15a	0.775 ± 0.15ab	0.675 ± 0.1a

Table 4. Recovery effects of *I. spicata*-derived AuNPs on SOD antioxidant enzyme of human blood lymphocytes.

Dose	Different Fractions of Crude Extract					Mean value
	Methanol	Ethyl acetate	n-hexane	Chloroform	Aqueous	
Control	0.52 ± 0.23ab	0.51 ± 0.33ab	0.53 ± 0.24ab	0.57 ± 0.34ab	0.581 ± 0.04ab	0.531 ± 0.43b
100 μM H_2O_2 stress	0.47 ± 0.14de	0.41 ± 0.12de	0.491 ± 0.23de	0.381 ± 0.22de	0.391 ± 0.32de	0.3.31 ± 0.13c
AuNPs 10 μg/ml	0.561 ± 0.134ab	0.57 ± 0.4cd	0.599 ± 0.11a	0.569 ± 0.15a	0.55 ± 0.15ab	0.65 ± 0.1a
AuNPs 25 μg/ml	0.61 ± 0.144ab	0.66 ± 0.31ab	0.65 ± 0.10ab	0.61 ± 0.32ab	0.65 ± 0.24a	0.6.243 ± 0.51ab
AuNPs 50 μg/ml	0.661 ± 0.134ab	0.671 ± 0.4cd	0.59 ± 0.11a	0.69 ± 0.15a	0.75 ± 0.15ab	0.65 ± 0.1a

Table 5. Recovery effects of *I. spicata*-derived AuNPs on TBARS of human blood lymphocytes.

Dose	Different Fractions of Crude Extract					Mean value
	Methanol	Ethyl acetate	n-hexane	Chloroform	Aqueous	
Control	0.52 ± 0.23ab	0.541 ± 0.33ab	0.53 ± 0.24ab	0.57 ± 0.34ab	0.481 ± 0.04ab	0.531 ± 0.43b
100 μM H_2O_2 stress	0.73 ± 0.11a	0.75 ± 0.11a	0.715 ± 0.11a	0.65 ± 0.11a	0.68 ± 0.11a	0.71 ± 0.11a
AuNPs 10 μg/ml	0.463 ± 0.03k-l	0.421 ± 0.452h-j	0.633 ± 0.63h-j	0.347 ± 0.13h-l	0.629 ± 0.1031h	0.299 ± 0.011e
AuNPs 25 μg/ml	0.51 ± 0.003lmn	0.69 ± 0.41j	0.545 ± 0.11h	0.617 ± 0.28g	0.699 ± 0.11i	0.5142 ± 0.04e
AuNPs 50 μg/ml	0.6763 ± 0.01m	0.585 ± 0.112i-j	0.690 ± 0.650j-k	0.6520 ± 0.450h-i	0.60 ± 0.001h	0.69 ± 0.04c

Collectively, these findings suggest that the synthesized AuNPs exhibit a favorable cytocompatibility profile within the tested concentration range. Future investigations incorporating direct cell viability and apoptosis assays will further strengthen the safety assessment and support translational applications.

CONCLUSION

This study demonstrates that gold nanoparticles synthesized from *Ifloga spicata* extract exert significant antioxidant and cytoprotective effects on human lymphocytes under oxidative stress. The findings support the potential use of plant-mediated AuNPs in nanomedicine, particularly for conditions associated with oxidative damage. Future research should explore mechanistic pathways and evaluate efficacy in in-vivo models.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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