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IN VIVO EVALUATION OF GOLD NANOPARTICLES FUNCTIONALIZED WITH *ARTHROSPIRA PLATENSIS* PROTEIN EXTRACT

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ABSTRACT

Introduction Gold nanoparticles (AuNPs) have gained increasing attention in nanomedicine due to their biocompatibility and unique physicochemical properties, which make them suitable for targeted therapies against cancer and other chronic diseases. Functionalizing AuNPs with bioactive compounds further enhances their biocompatibility and therapeutic potential. This study aimed to evaluate the effects of 10 nm citrate-stabilized AuNPs functionalized with a protein extract from *Arthrospira platensis* (AuNPs-APE) on biodistribution, as well as hematological and biochemical parameters, in Wistar rats.

Material and methods FTIR spectroscopy confirmed AuNPs' functionalization. Wistar rats received oral doses of AuNPs or AuNPs-APE for 28 days, followed by a 28-day clearance period. Gold biodistribution was analyzed in organs collected at the end of the experiment. Hematological and biochemical profiles were assessed using standard methods.

Results FTIR analysis confirmed stable AuNPs-protein interactions. Both AuNPs and AuNPs-APE exhibited notable renal accumulation, suggesting extraglomerular retention. The protein extract showed mild immunomodulatory effects, reducing oxidative stress and inflammation associated with nanoparticle exposure.

Conclusions The AuNPs–APE complex shows promise as a renal-targeted nanotherapeutic system, demonstrating improved biocompatibility and reduced systemic stress compared with unmodified AuNPs. Further studies are required to elucidate the underlying mechanisms and to optimize the formulation and safety profile.

Keywords Gold nanoparticles, *Arthrospira platensis*, protein extract, Wistar rats, biodistribution, hematological assessment, biochemical profile.

EVALUAREA *IN VIVO* A NANOPARTICULELOR DE AUR FUNCȚIONALIZATE CU EXTRACT PROTEIC DIN *ARTHROSPIRA PLATENSIS*

Introducere Nanoparticulele de aur (AuNP) sunt utilizate tot mai frecvent în nanomedicină datorită biocompatibilității și proprietăților fizico-chimice unice, care le susțin aplicabilitatea în terapiile țintite pentru cancer și boli cronice. Funcționalizarea cu ajutorul compușilor bioactivi le optimizează potențialul terapeutic și biocompatibilitatea. Acest studiu a evaluat efectele funcționalizării AuNP stabilizate cu citrat de 10 nm, cu un extract proteic din *Arthrospira platensis* (AuNP-APE) asupra biodistribuției, profilurilor hematologic și biochimic la șobolani Wistar.

Material și metode Funcționalizarea AuNP a fost caracterizată prin spectroscopie FTIR. Șobolanii au primit oral AuNP sau AuNP-APE timp de 28 de zile, urmate de o perioadă de repaus de 28 de zile. Biodistribuția aurului a fost analizată în organele colectate la finalul experimentului. Profilurile biochimic și hematologic au fost evaluate prin metode standard.

Rezultate Spectroscopia FTIR a confirmat interacțiuni stabile între AuNP și proteinele din extract. Atât AuNP, cât și AuNP-APE s-au acumulat în rinichi, sugerând mecanisme extraglomerulare de retenție. Extractul proteic a avut efecte imunomodulatoare favorabile, reducând stresul oxidativ și inflamația sistemică, cauzată de nanoparticule.

Concluzii Complexul AuNP–APE prezintă potențial ca sistem nanoterapeutic, demonstrând biocompatibilitate superioară și un impact sistemic redus, față de AuNP nefuncționalizate. Sunt necesare cercetări suplimentare pentru clarificarea mecanismelor implicate și optimizarea profilului de siguranță și al formulării.

Cuvinte-cheie Nanoparticule de aur, *Arthrospira platensis*, extract proteic, șobolani Wistar, biodistribuție, evaluare hematologică, profil biochimic.

INTRODUCTION

Gold nanoparticles have emerged as essential tools in nanomedicine due to their significant potential in cancer diagnosis and treatment. In recent years, their applications have expanded considerably to include therapies for degenerative and infectious diseases and innovative oncological treatment strategies (1-3). Their unique physicochemical properties and nanoscale dimensions allow AuNPs to accumulate preferentially in tumor tissues and interact selectively with target cells while maintaining low systemic toxicity (4).

To enhance therapeutic effectiveness and improve the risk-benefit balance, recent research has focused on functionalizing AuNPs with bioactive molecules or complex delivery systems (4-6). Standard methods included functionalization with polyethylene glycol (PEG) or citrate, which boost colloidal stability and influence biodistribution (7-9). Additionally, attaching peptides, amino acids, or other specific ligands has shown potential for increasing the selectivity of AuNPs for tumor cells (4, 10).

Bionanosynthesis has emerged as a novel approach to nanoparticle functionalization, using natural reducing agents derived from plant or microbial sources to generate nanoparticles with enhanced biological properties (5, 6, 11). One such innovative strategy involves the incorporation of gold nanoparticles into the biomass of the cyanobacterium *Spirulina (Arthrospira) platensis*, followed by functionalization with bioactive protein fractions extracted from this biomass. These strategies improve the biocompatibility of nanoparticles and provide new opportunities for the development of advanced targeted therapies (12, 13).

The biomass of *Arthrospira (Spirulina) platensis* is recognized as a natural source of bioactive compounds with well-documented immunomodulatory and antioxidant properties, making it an ideal biological matrix for nanoparticle biofunctionalization (12–14).

This study aimed to evaluate the effects of 10 nm citrate-stabilized AuNPs functionalized with a protein extract from *Arthrospira platensis* biomass on biodistribution and *in vivo* hematological and biochemical profiles in a Wistar rat model.

MATERIAL AND METHODS

Preparation of *Arthrospira platensis* Protein Extract

The protein extract was prepared from the biomass of the cyanobacterial strain *Arthrospira (Spirulina) platensis* CNMN-CB-02, maintained in the National Collection of Non-Pathogenic Microorganisms at the Institute of Microbiology and Biotechnology, Technical University of Moldova. The strain was cultivated under photoautotrophic conditions in a mineral growth medium with the following composition (g/L): $NaNO_3$, 2.25; $NaHCO_3$, 8.0; NaCl, 1.0; K_2SO_4, 0.3; Na_2HPO_4, 0.2; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2$, 0.024; $FeSO_4$, 0.01; EDTA, 0.08; H_3BO_3, 0.00286; $MnCl_2 \cdot 4H_2O$, 0.00181; $ZnSO_4 \cdot 7H_2O$, 0.00022; $CuSO_4 \cdot 5H_2O$, 0.00008; MoO_3, 0.000015. The protein content of the dry biomass ranged from 68% to 72%, as determined by the Lowry method (15). For protein extraction, the bio-

mass was treated with a 0.45% (w/v) sodium hydroxide solution (SIGMA-ALDRICH CHEMIE GmbH, Germany) at a ratio of 1 g of biomass to 0.5 L of extraction solution. The extraction was carried out at 25 ± 1 °C with constant stirring for 60 min. The mixture was subsequently centrifuged, and the supernatant was collected. A second extraction was performed using an additional 200 mL of the same alkaline solution for 30 min under identical conditions. The two supernatants were combined and dialyzed until a neutral pH (7.0–7.2) was reached. The final *Arthrospira platensis* protein extract (APE) was standardized to a 1% (w/v) dry matter concentration, containing a total protein content of 52%.

Functionalization of AuNPs and Preparation of the AuNPs-APE Functional Complex (12)

Citrate-stabilized gold nanoparticles (AuNPs) with an average diameter of 10 ± 0.2 nm (0.02 mg/mL; SIGMA-ALDRICH CHEMIE GmbH, Germany) were used to prepare the functional complex. A 20 mL volume of the AuNP suspension was slowly added to 200 mL of a 1% (w/v) *Arthrospira platensis* protein extract (APE) solution. The mixture was stirred at 2000 rpm for 120 minutes at 25 ± 1 °C to facilitate interaction between the protein extract and the nanoparticles. The resulting bio-nanoconjugate (AuNPs–APE) had a final concentration of 1.0 mg/mL APE and 1 µg/mL AuNPs.

Characterization of AuNPs-APE

Fourier Transform Infrared (FTIR) Spectroscopy was performed on both APE and AuNPs-APE to assess functional group interactions. After being dehydrated at 40°C, the samples were analyzed at room temperature. Spectra were recorded in the range of 4000–400 cm^{-1} using a PerkinElmer spectrometer equipped with a DTGS detector.

Animals and Experimental Design

The *in vivo* study was conducted at the Laboratory of Stress Physiology, Adaptation, General Sanocreatology and Vivarium of the Institute of Physiology and Sanocreatology, Republic of Moldova. All procedures were approved by the Institute's Ethics Committee (Approval No. IREC/12/03.11.2020). A total of 48 adult Wistar albino rats (28 males and 20 females) were used. The animals were randomly divided into four experimental groups (n=12 per group, with 7 males and 5 females in each) and housed under standard laboratory conditions (22 ± 2 °C, 55 ± 5% relative humidity, 12-hour light/dark cycle) with *ad libitum* access to food and water. The experimental groups received the following dietary regimens for 28 consecutive days: C (–): negative control – standard diet only; C (+): positive control – standard diet supplemented with APE; Group 1 (AuNPs): standard diet with non-functionalized gold nanoparticles; and group 2 (AuNPs-APE): standard diet with the functionalized AuNPs-APE complex. Each animal received a daily dose of 1 µg of gold, administered in breadcrumbs made from whole rye flour. After the 28-day treatment period, seven animals from each group (4 males and 3 females) were euthanized for the collection of blood and organs (brain, liver, spleen, kidneys, testes, and ovaries). The remaining animals were maintained on a standard diet for a further 28 days to assess nanoparticle clearance.

Analysis of Gold Content in Animal Tissues by ICP-MS

Tissue samples were dried at 70 °C until a constant weight was achieved. For gold analysis, a 0.1 g aliquot of each sample was digested with 3 mL of HNO_3 and 1 mL of H_2O_2 (both from Sigma-Aldrich, Germany) in an ETAS-6 autoclave at 180 °C, 20 bar, and 400 W for 90 minutes. After cooling, the digests were diluted to a final volume of 20 mL with deionized water. Gold concentrations were determined using inductively coupled plasma mass spectrometry (ICP-MS; XSeries II, Thermo Scientific, USA), with a measurement uncertainty of 5–10%.

Hematological and Biochemical Analysis

Hematological parameters were analyzed using an automated hematology analyzer (Sysmex XT-2000i, GMI Inc., USA). Biochemical parameters were measured with a semi-automatic photometer (StarDust MC15, DiaSys Diagnostic Systems, Germany).

Statistical Analysis

All experiments were conducted in triplicate, and data are expressed as the mean ± standard deviation. Statistical comparisons between groups were made using Student's t-test, with a $p < 0.05$ considered statistically significant.

RESULTS

Characteristics of AuNPs Biofunctionalized with *Arthrospira platensis* Protein Extract (AuNPs-APE)

The FTIR spectra in Figure 1 illustrate the structural characteristics of the *Arthrospira platensis* protein extract (APE) and the changes following its interaction with gold nanoparticles (AuNPs-APE).

The APE spectrum (blue curve) shows characteristic absorption bands for protein structures. The peaks at 3138 and 3050 cm^{-1} are assigned to O–H and N–H stretching vibrations, respectively, while the band at 2853 cm^{-1} is attributed to C–H stretching in alkyl side chains. The amide I and II bands appear at ~1657 and 1618 cm^{-1}, respectively. A peak at 1403 cm^{-1} corresponds to symmetric COO^- stretching, and the band at 1284 cm^{-1} is assigned to C–N stretching vibrations from amines or amide III. Additional vibrations between 1218–1125 cm^{-1} and 1200–1000 cm^{-1} are attributed to C–O and C–N bonds in ether, alcohol, and amide groups (fig. 1).

The AuNPs–APE spectrum (black curve) retains several native features but exhibits noticeable shifts and variations in band intensity, indicating interactions between the biomolecule and the nanoparticle. A weak, broadened band observed around 3260–3270 cm^{-1} (O–H/N–H stretching) shows decreased intensity, suggesting its involvement in nanoparticle binding. The C–H stretching bands (2920–2850 cm^{-1}) remain detectable but appear less intense. The amide I (~1640–1650 cm^{-1}) and amide II (~1530–1540 cm^{-1}) bands exhibit reduced intensity, implying interactions likely mediated by peptide carbonyl and amine groups (fig. 1).

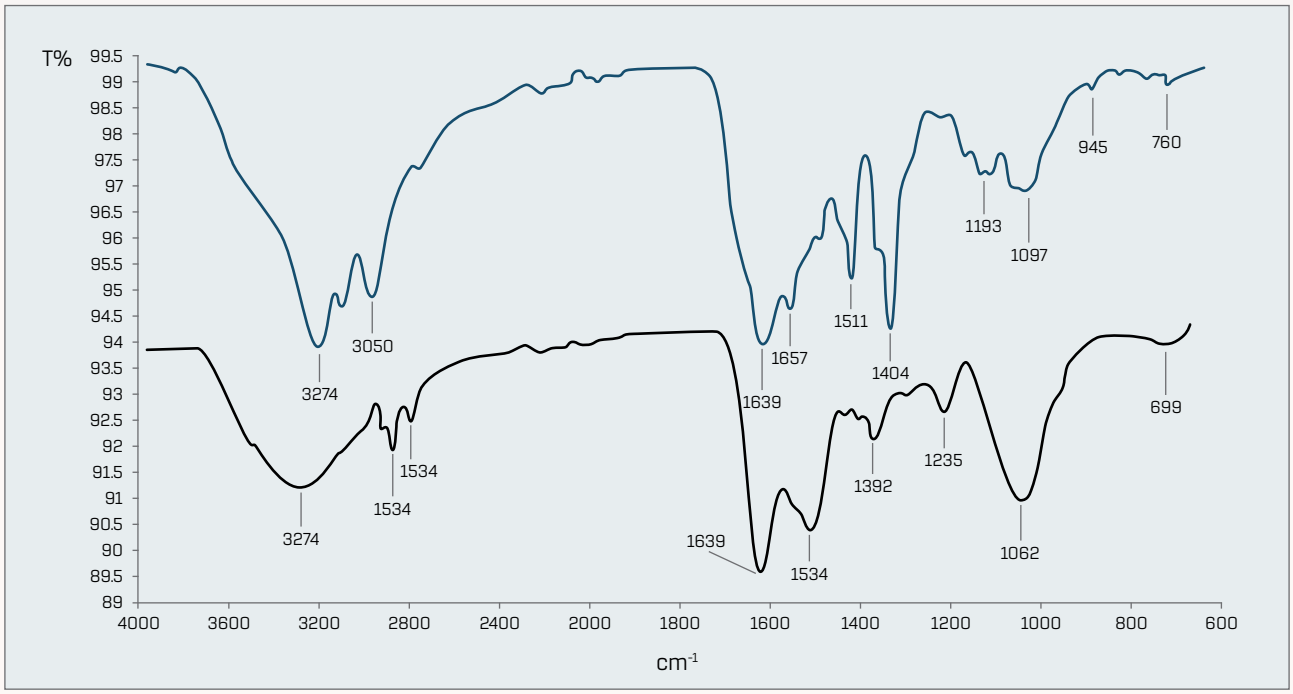

Figure 1. FTIR spectra of the *Arthrospira platensis* protein extract (APE) and the functionalized AuNPs–APE complex. Blue curve – the APE spectrum, and black curve – the AuNPs–APE spectrum.

Changes in the 1400–1200 cm^{-1} region, including band shape and position, suggest a structural reorganization of functional groups upon nanoparticle binding. In the 1100–1000 cm^{-1} region, the C–O stretching bands become less defined, likely due to reduced mobility of the involved groups. Further evidence of interaction with the metallic surface comes from the 1000–800 cm^{-1} region, where bands between 900–800 cm^{-1} are more attenuated. Finally, a weak band at 650–600 cm^{-1} may indicate coordination of the gold nanoparticles with donor atoms like nitrogen or sulfur from amino acids or peptide residues (fig. 1).

Gold Accumulation in Rat Organs

Table 1 shows the gold concentration in the kidneys of rats following 28 days of oral administration of AuNPs and the AuNPs-APE complex. Gold was detected only in the kidneys and was measured immediately after the administration and clearance period (CET). It was not detected in any other organs examined, including the brain, liver, ovaries, and testes.

Table 1. Gold accumulation in rat organs following AuNPs and AuNPs-APE administration, and a subsequent 28-day clearance period.

Experimental Groups	Kidney (ng/g)	Kidney, CET (ng/g)	Spleen (ng/g)	Spleen, CET (ng/g)
AuNPs	6.4±0.42	2.8±0.38	ND	ND
AuNPs-APE	10.4±0.66*	4.3±0.41	1.0±0.40	ND

Note: Concentrations were measured immediately after 28 days of treatment or following a 28-day clearance period (CET). *p < 0.01 indicates statistically significant differences between adjacent groups. ND – non-detected.

Quantitative analysis of kidney tissue revealed significantly different gold concentrations between non-functionalized gold nanoparticles (AuNPs) and those functionalized with *Arthrospira platensis* protein extract (AuNPs-APE). Following administration, the gold concentration in the kidneys was 6.4 ng/g in the AuNPs group, compared to 10.4 ng/g in the AuNPs-APE group – a 62.59% increase (tab. 1).

After the clearance period, gold levels in renal tissue decreased to 2.8 ng/g in the AuNPs group and 4.3 ng/g in the AuNPs-APE group. This corresponds to the elimination of 56.25% and 58.65% of the initially accumulated gold from the AuNPs and AuNPs-APE groups, respectively (tab. 1).

In the spleen, gold accumulation was detected only in the AuNPs-APE group (1.0 ng/g) and was undetectable after the clearance period (tab. 1).

Hematological and Biochemical Profiles in Experimental Animals

Table 2 presents the hematological and biochemical parameters measured in the experimental groups at two time points: following nanoparticle administration and at the end of the clearance period (CET).

Table 2. Hematological and biochemical indices in rats after 28 days of AuNPs and AuNPs-APE administration, followed by a clearance period of 28 days.

Indices	C (-)	AuNPs	AuNPs, CET	C (+)	AuNPs-APE	AuNPs-APE, CET
HB g/L	156.43±5.9	155.2±3.3	152.0±8.5	145.57±7.1	151.40±3.0	158.5±0.7
RBC, 10^{12}/L	8.77±0.30	8.56±0.39	8.14±0.08	8.15±0.38	8.21±0.46	8.53±0.15
WBC, 10^{9}/L	17.08±2.55	11.96±3.49*	9.70±1.65*	12.73±3.52	15.18±2.52*	12.85±0.05
PMN, %	28.59±6.69	22.38±2.77*	22.3±1.13*	22.37±7.51	26.42±2.38*	23.06±1.20
LY, %	57.40±6.21	67.36±5.78*	62.30±3.11	62.00±9.19	59.22±3.74	61.17±1.65
MON, %	7.56±2.33	4.98±4.59*	8.0±1.13	7.36±2.55	8.06±2.34	9.43±1.24*
EOS, %	5.43±1.61	4.78±0.96*	6.6±1.56*	6.33±1.67	5.70±1.98	5.85±0.49
BAS, %	0.53±0.33	0.56± 0.25	0.7±0.71	0.5 ±0.22	0.60 0.42	0.5±0.28
RET, %	3.35±1.08	3.45±1.15	5.08±0.16*	3.66±0.81	4.20±0.38	3.66±0.46
Prot, g/L	58.97±5.68	64.71±7.13	62.46±4.04	64.17±6.47	61.53±8.81	57.57±6.25
Glu, mmol/L	5.49± 0.77	4.64±1.10	5.65±0.47	5.87±0.86	5.42±0.75	4.91±1.05
Crea, μM/L	86.43±14.9	69.64±28.7	68.04±3.92*	100.93±7.77	71.70±10.6*	58.53±2.13*
Urea, mg/dL	29.03±5.69	25.65±5.51	33.72±0.36	25.53±5.97	24.27±1.73	23.71±1.59
ALT, U/L	162.4±39.6	131.0±27.9	188.2±13.5	184.9±50.2	218.6±23.5*	208.6±10.6
AST, U/L	3.57±2.31	5.61±2.57	5.43±0.80	2.84±1.90	2.83±1.08	7.03±0.68

Note: C (-) – Negative control; C (+) – Positive control (*Arthrospira platensis* protein extract); CET – clearance period; AuNPs – Experimental group administered with gold nanoparticles; AuNPs-APE – Experimental group administered with AuNPs functionalized with *Arthrospira platensis* protein extract; HB – hemoglobin; RBC – erythrocytes; WBC – leukocytes; PMN – polymorphomultinuclear neutrophil granulocytes; LY – lymphocytes; EOS – eosinophils; BAS – basophils; MON – monocytes; RET – reticulocyte; Prot – Total protein; Glu – Glucose; Crea – Creatinine; ALT – Alanine aminotransferase; AST – Aspartate aminotransferase; *p<0.05.

At the end of the 28-day daily administration period of non-functionalized gold nanoparticles, the treated rats exhibited a significant 30% decrease ($p < 0.05$) in total white blood cell count. Among the leukocyte subpopulations, neutrophil counts decreased by 21.72% ($p < 0.05$) and monocyte counts by 34.13% ($p < 0.05$) (tab. 2).

After the clearance period, monocytes showed signs of recovery. However, the total leukocyte count continued to decline, reaching a level 43.21% lower ($p<0.05$) than that of the negative control group, while neutrophil levels remained low and unchanged. Notably, significant increases were recorded in eosinophils (21.55%, $p<0.05$) and reticulocytes (51.64%, $p<0.05$). Most biochemical parameters did not remain within normal range and showed no significant changes (tab. 2).

In contrast, rats treated with protein extract-functionalized gold nanoparticles showed a significant increase in total leukocyte count (19.25%, $p<0.05$) and neutrophil levels (18.10%) after the 28-day administration period. A significant elevation in serum ALT levels (18.20%, $p<0.05$) was also observed (tab. 2).

Following the clearance period, most hematological parameters returned to baseline. Monocyte levels, however, continued to rise, reaching values 17% higher than those recorded during the administration period and 28.12% above those in the positive control group ($p < 0.05$). ALT levels remained elevated, and a significant increase in AST was recorded (41.90%, $p < 0.05$; tab. 2).

A decrease in serum creatinine levels was observed across all experimental groups. In the AuNPs and AuNPs (CET) groups, creatinine levels decreased by 19.43% and 21.28% ($p < 0.05$), respectively. The reductions were more pronounced in the AuNPs-APE and AuNPs-APE (CET) groups, with decreases of 28.93% and 42% ($p < 0.05$), respectively (tab. 2).

DISCUSSIONS

This study provides valuable insights into the functionalization of gold nanoparticles with protein extract components from derived the cyanobacterium *Arthrospira platensis*. FTIR spectral analysis revealed significant structural and functional changes upon the formation of the AuNPs–protein complex. Specifically, a reduction in the intensity of O–H and N–H bands, together with shifts in the carbonyl and amine regions, indicates coordinative or electrostatic interactions between the AuNPs and the protein matrix.

The emergence of characteristic signals for direct coordination bonds between the nanoparticles and donor atoms—such as nitrogen or sulfur from amino acids and peptides—further confirms the stable anchoring of biomolecules onto the gold surface. When compared to the original protein extract, the spectral changes in the fingerprint region of the AuNPs-APE complex confirm the formation of a new bio-nano system where the extract components interact directly with the AuNP surface.

The biodistribution data in rat organs are particularly noteworthy. Both non-functionalized and functionalized AuNPs were detected in the kidney, with a notable distinction: AuNPs-APE also accumulated in the spleen, albeit in very low amounts. For 10 nm citrate-stabilized AuNPs, intestinal transit and biliary excretion are the primary elimination routes, resulting in significant systemic bioavailability, particularly in the liver and spleen. In fact, 10 nm AuNPs are typically known to preferentially accumulate in the liver. As AuNPs are among the most inert nanoparticles and do not release gold ions, their absence in brain tissue is consistent with previous findings. The most prominent difference between AuNPs-APE and unmodified AuNPs is the significantly higher accumulation of the functionalized nanoparticles in renal tissue (13, 16, 17).

The stabilization medium of nanoparticles influences their aggregation behavior, absorption, and circulation properties (16). For instance, PEG-stabilized AuNPs have been detected in the brain, liver, kidneys, and testes. Likewise, functionalization with compounds derived from *Spirulina platensis* biomass has resulted in accumulation in the ovaries (13). AuNPs of 20 nm diameter, functionalized with peptides and administered intravenously to rats, showed predominant hepatic accumulation with minimal renal retention (10). However, other studies have experimentally demonstrated AuNP accumulation in the kidneys.

While 10 nm nanoparticles exceed the glomerular filtration threshold (~6–8 nm), they can still cross the fenestrated endothelium. This alternative route, independent of classical glomerular filtration, has been documented for PEGylated AuNPs ranging from 26 to 100 nm in size (9).

The exclusive detection in kidney tissue of AuNPs functionalized with *Arthrospira platensis* protein extract supports the hypothesis that the mesangium is the primary retention site for 10 nm particles. The proteins may facilitate receptor-mediated recognition, which could prolong circulation time and enhance renal retention (18).

The accumulation of citrate-stabilized 10 and 30 nm gold nanoparticles (AuNPs) in the kidneys was confirmed after intraperitoneal administration. The low urinary gold content indicates that these particles were not eliminated by glomerular filtration. Instead, they likely accumulated via extraglomerular pathways or were retained in the tubules through cellular interactions. These findings support the hypothesis that AuNPs smaller than 30 nm can be retained in renal tissue and are not efficiently excreted. Their accumulation appears to depend more on structural properties and specific tissue interactions than on classical glomerular filtration (7). This result highlights a promising research direction, particularly given the growing interest in kidney-targeted nanoparticles for drug delivery. This approach aims to deliver therapeutics directly to diseased tissue, minimize off-target effects, and improve treatment tolerability in patients requiring long-term pharmacotherapy for chronic kidney disease (4).

Correlations between the hematological and biochemical data present a coherent picture of the differential systemic impact of functionalized

versus non-functionalized nanoparticles. In the AuNP-treated groups, the decrease in total leukocytes, neutrophils, and monocytes suggests either immune suppression from oxidative stress or a subacute inflammatory response consuming these cells. This systemic reaction could also explain the moderate rise in AST without a corresponding ALT increase, pointing to extrahepatic mitochondrial damage, most likely in the kidneys, as further suggested by alterations in renal function markers.

Key Improvements and Rationale:

In contrast, the groups treated with AuNPs-APE exhibited a more stable immunological profile, with preserved or even elevated levels of monocytes and total leukocytes. This pattern suggests that the bioactive components in the protein extract may have an immunomodulatory or protective function. However, the functionalization process appeared to enhance hepatic toxicity, as indicated by high ALT levels and a further increase in AST in the post-clearance group.

This divergence between hematological stability and biochemical liver impairment suggests a potential compensatory mechanism against hepatotoxicity induced by the complexation of AuNPs with the protein matrix. It is also plausible that the hepatic toxicity might be an indirect effect of bioactive metabolites generated from the AuNPs–APE complex during enteric transit or systemic circulation.

Notably, the positive control group, which received only the *Arthrospira platensis* protein extract, exhibited hepatic and renal biochemical parameters comparable to, or even better than, those of the negative control group. No signs of enzymatic, hematological, or renal impairment were observed, indicating that the protein extract itself is non-toxic. Moreover, functionalizing the AuNPs the *Arthrospira platensis* protein extract appears to reduce their overall toxicity.

A common observation across all experimental groups was decreased serum creatinine levels. This reduction was particularly pronounced in female mice treated with PEGylated AuNPs, which showed a significant decline in creatinine without a corresponding changes in urea levels. This phenomenon may be linked to a subtle impairment of glomerular filtration or to transient metabolic alterations (8).

These findings highlight the need to further explore the biological mechanisms by which protein-extract-functionalized gold nanoparticles influence immune and biochemical parameters. Such research is essential for determining the optimal balance between the therapeutic benefits and toxicological risks of this nanotherapeutic approach.

CONCLUSIONS

1. FTIR analysis confirms the successful biofunctionalization of the AuNPs with *Arthrospira platensis* protein extract by forming direct interactions between the functional groups of the protein extract and the nanoparticle surface, indicating stabilization of the complex through coordinative or electrostatic bonds.
2. Unlike non-functionalized nanoparticles, AuNPs-APE accumulated in the kidneys at a three-fold higher level, thus, supporting the hypothesis that 10 nm particles penetrate renal tissue through extraglomerular mechanisms. This characteristic, combined with a noticeable reduction in systemic toxicity, highlights the potential of these bio-nano complexes in therapeutic for targeted renal therapy.
3. Combined hematological and biochemical data indicate that the APE has a beneficial immunomodulatory effect, attenuating the oxidative stress and systemic inflammation triggered by the nanoparticles. When administered alone, the *Arthrospira platensis* protein extract was non-toxic and showed a potentially protective effect. Functionalization with APE thus improves the stability and biocompatibility of the AuNPs, alters their tissue distribution, and reduces their overall toxicity.
4. The AuNP–APE complex emerges as a highly promising candidate for developing new targeted renal nanotherapeutics. Further research is needed on elucidating the underlying molecular mechanisms and establishing an optimal therapeutic window to ensure both efficacy and safety.

CONFLICT OF INTEREST The authors of the article deny the existence of any conflict of interest in the publication of this material.

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REFERENCES

1. Eker F, Akdaşçi E, Duman H, Bechelany M, Karav S. Gold Nanoparticles in Nanomedicine: Unique Properties and Therapeutic Potential. *Nanomaterials*. 2024;14(22):1854. https://doi.org/10.3390/nano14221854.
2. Haume K, Rosa S, Grellet S, Śmiałek MA, Butterworth KT, Solov'yov AV, Prise KM, Golding J, Mason NJ. Gold nanoparticles for cancer radiotherapy: a review. *Cancer Nanotechnol.* 2016;7(1):8. https://doi.org/10.1186/s12645-016-0021-x.
3. Bansal SA, Kumar V, Karimi J, Singh AP, Kumar, S. Role of Gold Nanoparticles in Advanced Biomedical Applications. *Nanoscale Adv.* 2020;2:3764–3787. https://doi.org/10.1039/D0NA00472c.
4. Gerosa C, Crisponi G, Nurchi VM, Saba L, Cappai R, Cau F, Faa G, Van Eyken P, Scartozzi M, Floris G, Fanni D. Gold Nanoparticles: A New Golden Era in Oncology? *Pharmaceuticals.* 2020;13(8):192. https://doi.org/10.3390/ph13080192.
5. Niżnik Ł, Noga M, Kobylarz D, Frydrych A, Krośniak A, Kapka-Skrzypczak L, Jurowski K. Gold Nanoparticles (AuNPs)—Toxicity, Safety and Green Synthesis: A Critical Review. *Int J Mol Sci*. 2024;25(7):4057. https://doi.org/10.3390/ijms25074057.
6. Yadav P, Bandyopadhyay A, Sarkar K. Enhancement of gold-curcumin nanoparticle mediated radiation response for improved therapy in cervical cancer: a computational approach and predictive pathway analysis. *Discover Nano.* 2024;19(1):153. https://doi.org/10.1186/s11671-024-04104-7.
7. Lopez-Chaves C, Soto-Alvaredo J, Montes-Bayon M, Bettmer J, Llopis J, Sanchez-Gonzalez C. Gold nanoparticles: Distribution, bioaccumulation and toxicity. In vitro and in vivo studies. *Nanomedicine.* 2018;14(1):1-12. https://doi.org/10.1016/j.nano.2017.08.011.
8. Chen J, Wang H, Long W, Shen X, Wu D, Song SS, Sun YM, Liu PX, Fan S, Fan F, Zhang XD. Sex differences in the toxicity of polyethylene glycol-coated gold nanoparticles in mice. *Int J Nanomedicine.* 2013;8:2409-2419. https://doi.org/10.2147/IJN.S46376.
9. Choi HS, Liu W, Misra P, Tanaka E, Zimmer JP, Itty Ipe B, Bawendi MG, Frangioni JV. Renal clearance of quantum dots. *Nat Biotechnol.* 2007;25(10):1165-70. https://doi.org/10.1038/nbt1340.
10. Morais T, Soares ME, Duarte JA, Soares L, Maia S, Gomes P, Pereira E, Fraga S, Carmo H, Bastos Mde L. Effect of surface coating on the biodistribution profile of gold nanoparticles in the rat. *Eur J Pharm Biopharm.* 2012;80(1):185-193. https://doi.org/10.1016/j.ejpb.2011.09.005.
11. Hanisha R, Balaganapathy M, Eswar B, Kathirvelan P, Rajabathar JR, Dinakarkumar Y. Biogenic synthesis of silver nanoparticles using *Spirulina maxima* extract and their bactericidal activity. J *Umm Al-Qura Univ Appl Sci.* 2024. https://doi.org/10.1007/s43994-024-00203-4.
12. Rudi L, Zinicovscaia I, Cepoi L, Chiriac T, Grozdov D, Kravtsova A. The Impact of Silver Nanoparticles Functionalized with *Spirulina* Protein Extract on Rats. *Pharmaceuticals*. 2024; 17(9):1247. https://doi.org/10.3390/ph17091247.
13. Cepoi L, Rudi L, Chiriac T, Zinicovscaia I, Grozdov D, Rudic V. Effect of Gold Nanoparticles Functionalized by *Arthrospira Platensis* on Rats. In: Sontea, V., Tiginyanu, I., Railean, S. eds. *6th International Conference on Nanotechnologies and Biomedical Engineering. ICNBME 2023.* Vol 91. Springer; 2023. https://doi.org/10.1007/978-3-031-42775-6_40.
14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265-275.
15. Huang H, Lang Y, Zhou M. A comprehensive review on medical applications of microalgae. *Algal Res.* 2024;80:103504. https://doi.org/10.1016/j.algal.2024.103504.
16. Kozics K, Sramkova M, Kopecka K, Begerova P, Manova A, Krivosikova Z, Sevcikova Z, Liskova A, Rollerova E, Dubaj T, et al. Pharmacokinetics, Biodistribution, and Biosafety of PEGylated Gold Nanoparticles In Vivo. *Nanomaterials.* 2021;11(7):1702. https://doi.org/10.3390/nano11071702.
17. Abdelhalim MAK, Moussa SAA. The gold nanoparticle size and exposure duration effect on the liver and kidney function of rats: In vivo. *Saudi J Biol Sci.* 2013;20(2):177-181. https://doi.org/10.1016/j.sjbs.2013.01.007.
18. Huang Y, Wang J, Jiang K, Chung EJ. Improving kidney targeting: The influence of nanoparticle physicochemical properties on kidney interactions. J Control Release. 2021;334:127-137. https://doi.org/10.1016/j.jconrel.2021.04.016.

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