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RESEARCH PAPER

Exploring the Protective role of Olive Oil Against Gold Nanoparticle-Induced Hepatotoxicity in Sprague Dawley Rats

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ABSTRACT

The potential hepatotoxic effects of gold nanoparticles (AuNPs) have become a concern due to their widespread use. Olive oil, with its rich antioxidant properties, may offer protective benefits against such toxicity. This study assesses the protective role of olive oil against AuNP-induced hepatotoxicity in male Sprague Dawley rats. The experiment was conducted with 20 adult male Sprague Dawley rats, divided into four groups: control, olive oil only, AuNPs only, and a combined treatment of AuNPs and olive oil. Parameters such as liver weight and volume, hepatocyte count, central venous volume, and liver enzymes (LDH, ALP, GGT) were measured after a 42-day treatment period. Rats treated with AuNPs exhibited a significant decrease in relative liver weight (6.85 ± 0.45g compared to 7.81 ± 0.46g in controls) and elevated liver enzymes (LDH, ALP, and GGT levels increased to 6.99 ± 0.49 U/L, 120.96 ± 34.28 U/L, and 175.59 ± 20.77 U/L, respectively). The combined treatment group showed a notable improvement in these parameters, with liver weight and enzyme levels approaching those of the control group. Additionally, hepatocyte cell volume significantly increased in the AuNPs group (7547.80 ± 923.19 μm^3) compared to the combined treatment group (6007.35 ± 579.85 μm^3). Olive oil significantly mitigates AuNP-induced hepatotoxicity in male Sprague Dawley rats. This study underscores the potential of natural antioxidants in reducing liver damage caused by nanoparticles and suggests further research into dietary interventions for toxin exposure.

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INTRODUCTION

Gold nanoparticles (AuNPs) stand at the forefront of nanotechnology's revolutionary impact across various industries, playing a pivotal role due to their unique properties. These nano-sized materials, particularly AuNPs, have become integral in multiple fields, accounting for an estimated 20% of nanotechnology products. Their unique physical and chemical properties enable their versatile applications in medicine, environmental science, and chemical engineering [1,2].

In the medical field, AuNPs have revolutionized targeted drug delivery systems, particularly in cancer therapy. AuNPs enhance the precision of chemotherapeutic drug delivery to tumor cells, significantly minimizing the adverse effects on healthy tissues [3]. Moreover, their exceptional optical properties make them invaluable in diagnostic procedures, including bioimaging and biosensors [4].

Beyond their medical applications, AuNPs play a significant role in environmental protection. They contribute to water purification and treatment processes, breaking down contaminants at a molecular level, thereby providing cleaner and safer water supplies [5–7].

In the chemical industry, the catalytic properties of AuNPs are exploited for more efficient and environmentally friendly chemical reactions. These nanoparticles facilitate various chemical processes, leading to greener and more sustainable production methods [8,9].

Despite these advantages, the extensive application of AuNPs raises concerns about potential health risks [10]. Their small size, often ranging from 1 to 100 nm, allows them unprecedented access to various tissues, cells, and biological molecules within living organisms. However, this can lead to undesirable accumulation in vital organs, especially when used in concentrations exceeding 50 mg/mL [11]. The liver, being the primary detoxification organ, is particularly vulnerable to such exposure. Overexposure to AuNPs can disrupt liver functions, potentially leading to hepatic stress or damage [12].

Moreover, there is evidence suggesting that nanoparticles below 10 nm can cross the blood-brain barrier, posing potential risks to the central nervous system [13]. The interaction of AuNPs with cellular components like mitochondria and DNA, could induce oxidative stress, leading to cellular damage and apoptosis, particularly concerning in the context of long-term exposure or high concentrations [14–16].

Liver damage resulting from exposure to AuNPs can be a serious health concern. Though reversible in some cases, it has the potential to lead to acute liver failure and even death. Studies indicate that AuNPs, especially those smaller than 50 nm, can accumulate in the liver and induce oxidative stress, disrupting its normal functioning [17,18]. Oxidative stress occurs due to a disparity between the generation of free radicals and the liver's capacity to neutralize their damaging impacts. The liver's antioxidant defense system, comprising both enzymatic and non-enzymatic mechanisms, plays a critical role in neutralizing these free radicals [19]. However, when the burden of nanoparticles exceeds the liver's capacity to cope, it can lead to cellular damage and impaired liver function.

Given these risks, it becomes crucial to explore potential safeguards. Olive oil, widely acclaimed for its high levels of oleic acid, monounsaturated fats, and potent antioxidants such as polyphenols and vitamin E, is recognized for its extensive health advantages [20–22]. Its anti-inflammatory effects, ability to enhance heart health, and role in safeguarding crucial organs like the liver are well-documented in the literature [23].

This study, therefore, investigates the protective and antioxidant effects of olive oil on male Sprague Dawley rats treated with AuNPs. Drawing from the research of Mihailovic et al. [24], which explored the impact of dietary antioxidants on nanoparticle-induced toxicity, we aim to understand how olive oil can mitigate the adverse health impacts of AuNPs. This exploration is critical, not only for its potential in reducing the risks associated with AuNPs in medical and industrial applications but also for its implications in broader fields of toxicology and environmental health.

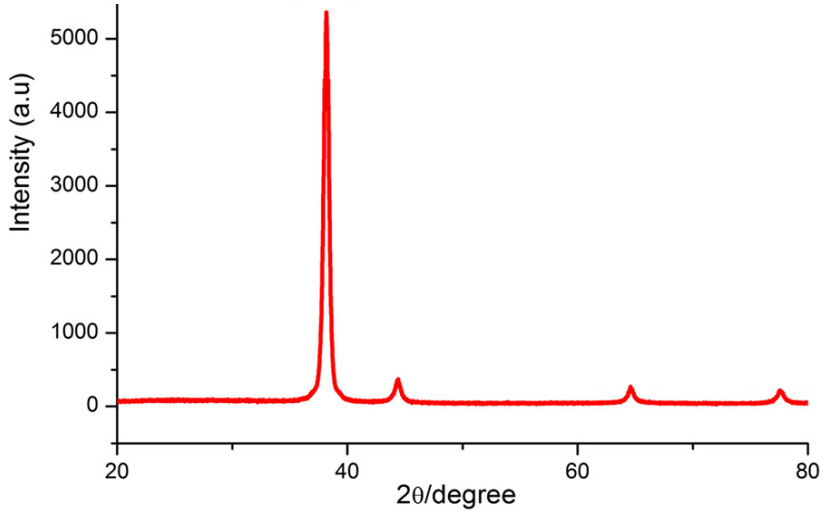
MATERIALS AND METHODS

This research was conducted in strict compliance with the ethical standards set forth by the Animal Care Committee, ensuring adherence to the highest ethical guidelines for animal experimentation. In this experiment, 20 adult male Sprague Dawley (SD) rats were housed in the specialized Animal House Unit at the University of Baghdad. These rats were provided with an environment optimized for their well-being,

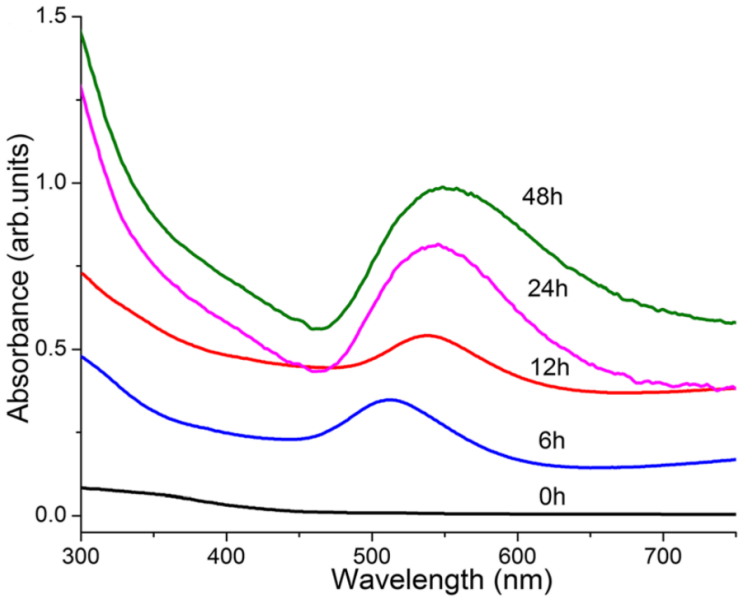
maintaining a stable temperature of 23 ± 1°C and a balanced light-dark cycle, comprising 12 hours of light followed by 12 hours of darkness. Throughout the study, the SD rats had unlimited access to both food and water, ensuring their nutritional needs were consistently met.

Prior to the commencement of the experimental procedures, a two-week acclimation period was observed, allowing the rats to comfortably adjust to their new surroundings and minimize stress. For the purpose of this study, the SD rats were meticulously divided into four distinct groups, each comprising five rats (n=5). The allocation of the groups was as follows [25]:

1. The control group, which received no treatment.
2. A group treated with AuNPs, receiving a dose of 450 mg/kg/day administered via gavage.



(a)



(b)

Fig. 1. Analysis results of AuNPs: (a) XRD measurement, (b) UV-Vis.

3. A group given olive oil, at a dosage of 10 ml/kg/day, also administered via gavage.

4. A final group that received a combined treatment of both AuNPs and olive oil, in the same dosages and method as the individual treatment groups.

The X-ray diffraction (XRD) pattern and Ultraviolet-Visible (UV-Vis) spectrophotometry of the AuNPs employed in this investigation are all displayed in Fig. 1.

SD rats were treated at 24-hour intervals for a duration of 42 days. For those receiving AuNPs, the dosage was set at 450 mg/kg per day. In the case of the group administered with olive oil, the chosen dosage was 10 ml/kg per day. Following the conclusion of the treatment period, the rats were first weighed. Subsequently, they were anesthetized using diethyl ether. Post-anesthesia, dissection was performed, during which the liver was extracted, weighed, and its volume determined using the immersion method [26]. After the extraction, the livers were thoroughly rinsed in normal saline. To preserve the liver tissues for further histological examination, they were then immersed in Neutral Buffered Formalin (NBF) fixative for a duration of seven days.

After the liver tissues were fixed in NBF, isotropic uniform random (IUR) sections were prepared. This involved a two-step randomization process using ϕ and θ coordinates. Firstly, each liver lobe was placed on the ϕ coordinate system, represented as a clock face. A random number between 0 and 9 was selected from a numerical table to determine the cutting angle. Based on this number, the liver lobe was bisected, resulting in two separate pieces. The first piece of each lobe was then aligned on the θ clock, ensuring the cut surface was parallel to the 0-0 axis of this coordinate system. Another random number was chosen, and the tissue was sliced again along this second number. Subsequent cuts were made parallel to this second cut.

For the second piece of each liver lobe, it was rotated 90 degrees to align the cut surface tangentially with the 0-0 axis on the θ clock. A random number was selected again, guiding parallel cuts along this axis. These IUR slices were then placed into baskets, ensuring the tissue was oriented correctly for microtome cutting from the second cut surface. Following this, the slices underwent a tissue processing cycle, which included embedding in paraffin. The embedded tissues were then sectioned using a microtome into slices of 5- and 15-microns thickness. These sections were placed on slides and subsequently underwent staining using Hematoxylin and Eosin (H&E) method, which involved placing the slides in special staining baskets for coloration.

In this study, Alkaline Phosphatase (ALP) levels in rat liver samples were determined using the p-nitrophenyl phosphate method [27]. This method involves the hydrolysis of p-nitrophenyl phosphate by ALP in an alkaline solution to form p-nitrophenol. The concentration of p-nitrophenol, which is directly proportional to ALP activity, was measured at a wavelength of 450 nm using a microplate reader. For the measurement of Gamma-glutamyl Transferase (GGT) activity, a blood test was employed [28]. Blood samples from rats were collected and analyzed for GGT activity. Elevated levels of GGT in the bloodstream are indicative of liver damage or disease, providing crucial information about the hepatic health of the rats. Lactate Dehydrogenase (LDH) levels in rat

Table 1. Comparison of the mean body weights (g) and liver weights (g) in different groups of adult male SD rats after a 42-day treatment period with AuNPs, and olive oil.

Groups	Average baseline body weight (g)	Average post-treatment body weight (g)	Average liver weight (g)	Average relative liver weight (g)
Control	26.44 ± 1.63a	29.27 ± 2.4a	2.39 ± 0.22a	7.81 ± 0.46a
AuNPs	26.70 ± 2.35a	30.67 ± 2.18a	2.21 ± 0.22a	6.85 ± 0.45b
Olive Oil	27.50 ± 2.55a	30.02 ± 3.59a	2.32 ± 0.17a	7.48 ± 0.86ab
Combined	27.04 ± 2.91a	29.07 ± 1.47a	2.38 ± 0.17a	7.77 ± 0.24a

Note: The mean values are accompanied by their respective standard deviations. Distinct letters signify statistically meaningful distinctions among groups ($p<0.05$).

liver samples were assessed using the colorimetric method with tetrazolium salts [29]. This method involves monitoring the absorbance in the range of 430–550 nm, as various tetrazolium salts are reduced to formazan. The intensity of the color, proportional to the amount of LDH in the samples, was measured to determine the enzyme activity, which is indicative of cellular respiration or tissue damage in the liver.

The collected data from the study were analyzed using SPSS software, version 23.0. Statistical significance was determined through a one-way ANOVA, followed by Tukey's post-hoc test to compare means between groups. A P-value of less than 0.05 was set as the threshold for statistical significance.

RESULTS AND DISCUSSION

Upon comparing the mean body weights of the SD rats and their liver weights post-treatment, no significant differences were noted among the various groups ($p>0.05$). However, the mean relative liver weight at the end of the treatment period in the group treated with AuNPs exhibited a significant decrease when compared to both the control group ($p<0.05$) and the group treated with olive oil ($p<0.03$) (Table 1).

At the conclusion of the treatment period, there was no significant difference observed in the average liver volume among the different groups ($p>0.05$). In the group treated with AuNPs, a notable decrease in the volume of the central vein was observed compared to the control group ($p<0.001$). However, in the group receiving a combination of AuNPs and olive oil, the central vein volume showed an increase compared to the group treated solely with AuNPs, though this increase was not statistically significant. The average volume of hepatocyte cells in the group treated with AuNPs exhibited a significant increase when compared to the control group ($p<0.002$). In contrast, the hepatocyte cell volume in the group treated with a combination of AuNPs and olive oil was comparable to that of the control group. Upon examining the hepatocyte nuclei volume across the different groups of rats, no significant differences were observed (Table 2).

The average total volume of hepatocytes across all groups did not exhibit any significant differences. In the group treated with AuNPs, there

Table 2. Variations in liver volume (mm^3), central venous volume (mm^3), and hepatocyte morphology (μm^3) in SD rats after a 42-day treatment period with AuNPs, and olive oil.

Groups	Average liver volume (mm^3)	Mean central venous volume (mm^3)	Average hepatocyte cell volume (μm^3)	Average volume of hepatocyte nuclei (μm^3)
Control	1532.00 ± 138.85 a	190.88 ± 15.69 b	5550.00 ± 885.28 ab	320.35 ± 46.85 a
AuNPs	1400.63 ± 133.08 a	129.23 ± 2.07 b	7547.80 ± 923.19 ab	351.36 ± 65.01 a
Olive Oil	1507.98 ± 103.06 a	146.03 ± 25.08 b	6820.40 ± 915.76 ab	281.42 ± 29.35 a
Combined	1543.23 ± 95.91 a	188.08 ± 22.56 b	6007.35 ± 579.85 ab	302.89 ± 56.54 a

Note: The mean values are accompanied by their respective standard deviations. Distinct letters signify statistically meaningful distinctions among groups ($p<0.05$).

Table 3. Comparison of mean volume of hepatocytes (mm^3), volume of sinusoids (mm^3) and volume of interstitial tissue (mm^3) in in SD rats after a 42-day treatment period with AuNPs, and olive oil.

Groups	Average volume of hepatocytes (mm^3)	Average volume of sinusoids (mm^3)	Average volume of interstitial tissue (mm^3)
Control	1098.27 ± 103.49a	95.30 ± 6.83a	24.20 ± 6.66a
AuNPs	1025.48 ± 82.29a	121.86 ± 17.13b	14.69 ± 2.24b
Olive Oil	1115.70 ± 63.11a	105.76 ± 21.15ab	18.45 ± 4.04a
Combined	1109.47 ± 63.83a	99.66 ± 14.15a	23.62 ± 6.54a

Note: The mean values are accompanied by their respective standard deviations. Distinct letters signify statistically meaningful distinctions among groups ($p<0.05$).

was a significant increase in the average volume of sinusoids compared to the control group ($p<0.02$). Additionally, there was a notable decrease in the average volume of the interstitial tissue in this group ($p<0.01$) (Table 3).

{insert Table 3}

The quantity of hepatocytes ($\times 10^8$) within the liver tissue of rats treated with AuNPs demonstrated a significant reduction when compared to both the control group and the olive oil group ($p<0.001$). However, in the group treated with a combination of gold nanoparticles and olive oil, there was a partial increase in the number of hepatocytes, but this did not reach the levels observed in the control group (Fig. 2).

The concentration of LDH in the group treated with AuNPs exhibited a significant increase compared to the control group ($p<0.001$), the olive oil group ($P < 0.004$), and the AuNPs plus olive oil group ($p<0.001$). In the group receiving a combination of AuNPs and olive oil, the LDH concentration decreased slightly, aligning more closely with that of the control group. Furthermore, the level of ALP in the group treated with AuNPs was significantly higher compared to the control group ($p<0.006$). This increase was also significant when compared to the group treated with a combination of AuNPs and olive oil ($p<0.02$), as well as the group receiving only olive oil ($p<0.001$) (Table 4).

This study explored the protective effects of olive oil against hepatotoxicity induced by AuNPs in male SD rats. The results revealed several notable findings with implications for understanding the biocompatibility and toxicity of AuNPs, as well as the protective potential of natural antioxidants like those found in olive oil.

The rats treated with AuNPs exhibited a significant decrease in mean relative liver weight compared to the control and olive oil groups (Table 1). This aligns with findings in Ref. [12] who reported hepatic stress and damage in mice exposed to AuNPs due to their accumulation in vital organs. There was no significant difference in liver volume among the groups, which contrasts with [17], who observed liver volume alterations in rats exposed to nanoparticles.

The central vein volume decreased significantly in the AuNPs group but increased in the group receiving AuNPs and olive oil, indicating a protective effect of olive oil. This is consistent with the hepatoprotective role of antioxidants reported in Ref. [18]. The average hepatocyte cell volume increased in the AuNPs group, suggesting cellular stress or damage, a phenomenon observed in studies like Ref. [15] on nanoparticle-induced

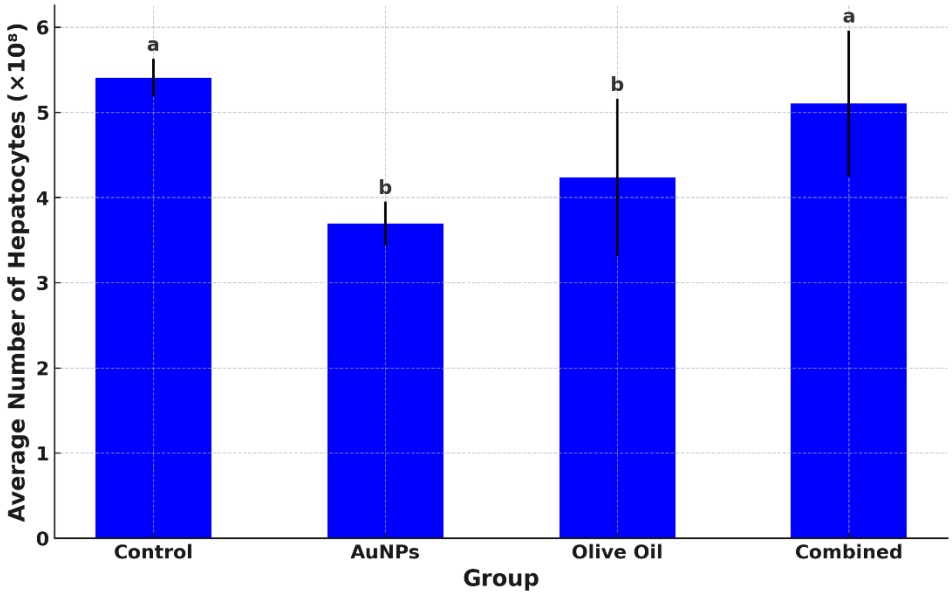


Fig. 2. Comparison of the average number of hepatocytes ($\times 10^8$) in SD rats after a 42-day treatment period with AuNPs, and olive oil. Different letters (a, b) indicate statistically significant differences between groups ($p<0.05$).

Table 4. Comparison of the mean Alkaline Phosphatase (ALP, U/L), Gamma-glutamyl Transferase (GGT, U/L), and Lactate Dehydrogenase (LDH, U/L) in SD rats after a 42-day treatment period with AuNPs, and olive oil.

Groups	LDH	ALP	GGT
Control	4.14 ± 0.12ab	75.84 ± 10.41ab	157.85 ± 12.66ab
AuNPs	6.99 ± 0.49c	120.96 ± 34.28c	175.59 ± 20.77b
Olive Oil	5.22 ± 1.38b	84.39 ± 20.19b	153.58 ± 6.02a
Combined	3.17 ± 0.49a	49.4 ± 5.47a	151.05 ± 6.99a

Note: The mean values are accompanied by their respective standard deviations. Distinct letters signify statistically meaningful distinctions among groups ($p<0.05$).

oxidative stress.

A significant reduction in the number of hepatocytes was observed in the AuNPs group compared to controls, with partial recovery in the olive oil and AuNPs combined treatment group (Fig. 2). This partial recovery emphasizes the antioxidative potential of olive oil in countering nanoparticle-induced liver damage. The concentration of LDH, ALP, and GGT significantly increased in the AuNPs group, indicating liver damage (Table 4). These findings are in line with Refs. [27,28], who associated elevated levels of these enzymes with liver dysfunction. The combined treatment group exhibited a decrease in these enzyme levels, further substantiating the protective role of olive oil, as suggested in Ref. [29] on antioxidant therapies.

Compared to previous studies, our findings highlight the nuanced role of olive oil as a protective agent. While it did not completely reverse the effects of AuNPs, it significantly mitigated the impact on liver weight, enzyme levels, and hepatocyte count. The antioxidant properties of olive oil, particularly its polyphenols and vitamin E content, appear to offer a buffer against oxidative stress and cellular damage caused by AuNPs, a result that echoes the findings in Ref. [23] on olive oil's liver-protective capabilities.

In conclusion, this study demonstrates that while AuNPs induce notable hepatotoxicity in rats, as reflected in altered liver weights, hepatocyte volume, and elevated liver enzyme levels, the concurrent administration of olive oil shows a marked protective effect. These findings contribute to the growing body of literature on nanoparticle toxicity and the potential of natural antioxidants in mitigating these effects, thus offering valuable insights for both nanotechnology applications and nutritional interventions in toxin exposure. Further research should explore the mechanisms underlying olive oil's protective effects and investigate its potential in human models of nanoparticle exposure.

CONCLUSION

This study presents significant insights into the hepatoprotective effects of olive oil against gold nanoparticle-induced toxicity in male SD rats. Our findings reveal that while AuNPs contribute substantially to hepatotoxicity, as evidenced by altered liver weights, increased hepatocyte volume, and elevated liver enzyme levels, the concurrent administration of olive oil demonstrates a marked protective effect. Notably, the olive oil treatment notably mitigated these adverse effects, bringing various parameters, including liver weight, enzyme levels, and hepatocyte count, closer to control levels. These results underscore the potent antioxidative properties of olive oil, particularly its rich content of polyphenols and vitamin E, in combating oxidative stress and cellular damage induced by AuNPs.

The relevance of this study extends beyond its immediate findings. It contributes to the growing body of literature on nanoparticle toxicity, highlighting the potential of natural antioxidants in mitigating these effects. This is particularly crucial in the context of increasing use of nanoparticles in various industries, including medicine and environmental applications. Our research also opens avenues for exploring dietary interventions as a practical approach to reducing the risks associated with nanoparticle exposure.

Looking forward, further research should delve into the specific mechanisms underlying the protective effects of olive oil. There is a pressing need to investigate whether these findings can be extrapolated to human models, considering

the increasing human exposure to nanoparticles. Such studies could provide valuable insights for both nanotechnology applications and nutritional strategies to counteract toxin exposure. Additionally, exploring the interaction between different types of nanoparticles and various natural antioxidants could yield comprehensive strategies for mitigating nanoparticle-induced toxicity.

In conclusion, this study not only enhances our understanding of the biocompatibility and toxicity of AuNPs but also positions olive oil as a potential agent in reducing nanoparticle-induced liver damage. This paves the way for further research and development of preventive strategies against nanoparticle-induced toxicity, leveraging the natural protective properties of dietary components.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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