



Study the Effect of Gold Nanoparticles Prepared by Green Synthesis and Exposed to Laser Pulses on the White Blood Cells Count and Liver Functions and Tissues in Male Albino Rats

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Abstract

Gold nanoparticles (AuNPs) act as protective agents for liver health by inhibiting oxidative stress factors, and laser treatment combined with green synthesis enhances their protective properties. In this study, AuNPs were prepared by green synthesis using an aqueous mint leaf extract (5 ml of aqueous mint extract was added to 10 ml of a gold salt solution). After that, the produced AuNPs were exposed to laser pulses from a 532 nm crystal with energy from 500 to 1300 mJ to determine the toxicity of AuNPs exposed and unexposed to laser pulses on hematological and biochemical parameters and liver tissues. 40 Adult male albino rats were divided into four groups: group 1 was control, group 2 was intraperitoneally injected with an aqueous extract of mint, group 3 was injected with AuNPs unexposed to laser pulses, and group 4 was injected with AuNPs exposed to laser pulses. After the end of the experimental duration, blood was collected, and a white blood cell, lymphocyte, and differential count were performed. Hepatic function enzyme assessments of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) and a histological section of the liver were performed and examined. The results showed the AuNPs exposed to laser pulses group did not show a significant difference compared to the control group and showed significant differences compared to the extract and AuNPs group unexposed to laser pulses groups.

Keywords: AuNPs, green synthesis, laser pulses, WBCs, ALT, AST, ALP.

1. Introduction

Mint has antibacterial, antioxidant, anti-inflammatory, immunomodulatory, anti-cancer, anti-aging, and analgesic characteristics and may be useful in the treatment of a variety of conditions, including gastrointestinal diseases. Furthermore, mint possesses therapeutic advantages for dental health, urinary retention, skin, and wound healing and may enhance memory. This is attributed to the presence of three primary categories of phytochemical constituents: essential oils (predominantly menthol), flavonoids (including hesperidin, eriodictyol, naringenin, quercetin, myricetin, and kaempferol), and nonflavonoid phenolcarboxylic acids¹. Menthol is a naturally occurring cyclic terpene alcohol that constitutes the primary component of mint essential oils derived from mint. Menthol and its derivatives are extensively utilized in pharmaceuticals, cosmetics, and food products; the molecular structure of menthol enables a more precise interaction with the thermoreceptors in the skin and mucous membranes². However, there is some evidence that shows that despite its beneficial effects, spearmint has some toxic and adverse effects. Severe histopathological changes in kidney, liver, and uterus tissue^{3,4}. Nanotechnology possesses extensive applications in medicine and biomedical

engineering, including tissue engineering, diagnostics, and therapy⁵. The novel and distinctive characteristics of gold nanoparticles (AuNPs), such as their low cytotoxicity, biocompatibility, and optical qualities, make them promising for use in biomedical domains such as drug administration, biosensing, bioimaging, and cancer therapy. The application of AuNPs in radiation and photothermal therapy has established an innovative framework for primary diagnostics and cancer treatment. Due to the extensive surface area of AuNPs, chemical functional groups or biological molecules, such as drug molecules, can be affixed to the gold surface⁶. The plant-based synthesis of gold nanoparticles has gained significant interest owing to the many functions of biomolecules in guiding the physical and chemical properties of biosynthesized nanoparticles. Identifying the key bioactive compounds involved in the production of gold nanoparticles is crucial to controlling their tunable properties for potential applications. These key bioactive compounds contributed to the qualitative and quantitative production of gold nanoparticles utilizing aqueous solutions of plants. They demonstrated an involvement in the reduction of Au^{3+} and maintaining gold nanoparticle stability⁷. The green synthesis method is the environmentally sustainable and accurate purification methodology utilizing plant extracts as reducing agents and stabilizers for the synthesis of AuNPs⁸. Therefore, our study synthesized gold nanoparticles in an environmentally friendly method by using an aqueous extract of mint leaves as a reducing agent and stabilizer and exposure to laser pulses at different energies, leading to the generation of smaller gold nanoparticles that are biocompatible and non-toxic and investigate their toxicity on hematological and biochemical parameters and histological effect on the liver.

2. Materials and Methods

The hot aqueous extract of mint plant was prepared in the plant laboratory of the college of science by weighing 100g of dried ground mint (which was prepared from fresh mint after washing it well and drying it in a place with low humidity and good ventilation (**figure 1**). To this amount of 100g, 1000 ml of de-ionized water was added. The period of time 25-40 min, it was filtered using filter paper, then poured into Petri dishes and the dishes were dried in the oven for use in the experiment. The concentration of the mint that was prepared is 100mg per ml to be injected into laboratory rats.

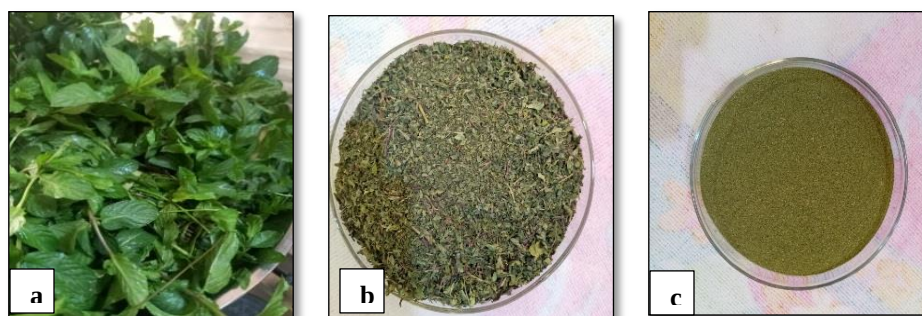


Figure 1. Mint plant a. Ground b. dried c. Fresh

2.1. Preparation of gold nanoparticle solution

The AuNPs solution was prepared through dissolution of 0.590 grams of HauCl_4 in 100 mL distilled water with continuous agitation on a magnetic stirring hot plate. The final AuNPs solution concentration is 10^{-2} mM. The stock solution was preserved in an opaque container within a refrigeration unit⁹. The color of AuNPs is similar to red wine^{10, 11}. In our current study, AuNPs were prepared using the other synthesis method, which is an environmentally friendly method. They were prepared by adding 5 ml of the prepared mint extract at a specific concentration of 100 mg/ml incorporated into 10 ml of the prepared AuNPs solution; the addition is done gradually using a Pasteur pipet. This process is done by continuous agitation on

a magnetic stirring plate for 45 minutes. We notice a gradual change in the hue of the AuNPs solution from yellow to wine red.

2.2. Spacemen's collection and processing

The total number of test animals was 40 adult albino rats; their weights range between 175 and 250 g, divided into four groups, each comprising ten rats: Group 1 (control), Group 2 (extract), Group 3 (AuNPs unexposed to laser), and Group 4 (AuNPs exposed to laser). Group 2 was injected intraperitoneally daily with 0.1 ml of a concentration of 100 mg/ml aqueous extract of mint. Group 3 was injected with a 0.5 ml solution of AuNPs without a laser, and Group 4 was also injected with a 0.5 ml AuNPs solution with laser¹². The experiment lasted 35 days. Blood was then collected for examination of comprehensive and differential leukocyte counts, as well as hepatic function enzyme levels, and tissue liver samples were preserved in 10% formalin for histological sections. This work adhered to all institutional and national regulations on the care and use of laboratory animals.

2.3. Laser energy's effects

A pulsed Nd:YAG laser has a wavelength of 532 nm and was used to irradiate the gold mass. Using laser energies of 500mJ, 700mJ, 900mJ, 1100mJ, and 1300mJ, five samples were produced while the other parameters remained consistent (P.R.R. at 1 Hz; the quantity of pulses was 1000). Increasing the number of laser pulses progressively colored the solution and changed faster for laser energy of 1100 mJ than 500 mJ¹³.

2.4. Optical Characterization of Gold Nanoparticles

2.4.1. Ultraviolet-Visible Spectrophotometry (UV-Vis)

The AuNPs absorption with aqueous mint extract was measured by an ultraviolet-visible spectrophotometer radiation-advancing spectrophotometer for ultraviolet/visible radiation. Amersham Biosciences Corporation provides the Ultraspectro 4300 Pro for this work project. The test spanned wavelengths from 190 nm to 1100 nm¹⁴.

2.4.2. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR investigations of the potential biophysical molecules responsible for the stability and reduction of the Au⁺ ions, as well as the capping of bio-reduced gold nanoparticles produced by pulse laser extraction, and to identify the effective aggregates of AuNPs-mint extract. The wavelength range is between 400 - 4000 cm⁻¹. The FTIR type is Nicolet Is50, supplied from the company Thermo Fisher Scientific (USA)¹⁵.

2.4.3. Gas Chromatography–Mass Spectrometry (GC-MS)

Gas chromatography is used to examine a broad variety of organic and inorganic chemicals such as nitrates and phosphates. When combined with mass spectrometry, GC-MS can be utilized to segregate intricate mixes and quantify analytes, detect unknown peaks, and analyze trace levels of contamination (MS). This study's GC-MS equipment was comprised of an Agilent 6890N Gas Chromatograph and an Agilent 5973N Mass Selective Detector (MSD) (Agilent Technologies, Palo Alto, California, U.S.A.). On DB-WAX, one microliter of the extract was injected. The gas was utilized as the carrier gas Helium, at a steady flow rate of 1 mL/min. The temperatures of the injector and ion source were measured at 150 and 220 °C, respectively.

2.5. Morphological Characterization

2.5.1. Field Emission Scanning Electron Microscope (FESEM)

SEM (ZEISS ULTRA 60, USA) was employed to determine the size, shape, and surface of particles in the gold nanoparticle-mint extract. SEM gives topographical information at magnifications of 10000X to 100000X, with nearly infinite depth of focus. It belongs to various energies that were employed to create the wavelength used. When operating at various energies, utilized a wavelength of 532 nanometers and a particle size of up to 45 nanometers.

2.5.2. Transmission Electron Microscopy (TEM)

TEM images were obtained using a Philips Model CM10 Transmission Electron Microscope to analyze the AuNPs. A 314-square copper grid, including a 3 mm diameter and 200 mesh, was employed with nanoparticle dispersions on a carbon-coated TEM copper grid. A colloidal

solution was utilized to absorb the energy, which was then used to test the consistency of the produced AuNPs.

2.5.3. Energy-Dispersive X-ray (EDX)

Utilizing the Scanning Electron Microscope, the compositional difference resulting from various atomic number elements and their distribution will be displayed by the backscattered electron picture. This will allow one of them to determine what those elements are and their relative quantities through the use of Energy Dispersive Spectroscopy (EDS) (atomic percent, for example). The EDS was being used to examine the data (ZIESS ULTRA 60 USA).

2.6. Structural Characterization

2.6.1. X-Ray Diffraction (XRD)

The concentration of AuNPs in solution was measured using a Bragg angle function. The specimen was prepared by positioning nano-gold particle solutions on glass slides using the Shimadzu XRD 6000 X-ray diffractometry system used. A Cu (K) radiation source exhibiting a certain wavelength of 1.5405 was used in this experiment. Using a fixed period of 0.15 seconds, the scanning angle 2θ was varied in the range of 20-80 degrees with speed 8 (degrees/min). We can determine the mean size of large Au NPs by indexing the diffraction pattern of a sample and assigning a Miller index (hkl) to each peak, the fcc structure of Au NPs could be seen in all Miller (hkl) indicators.

2.7. Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) application (2019) was employed to analyze the influence of various groups on study parameters. The Least Significant Difference (LSD) test was utilized to conduct a significant comparison of means in this study¹⁶.

3. Results

3.1. Gold nanoparticles Characterization Results

To investigate the impact of laser pulses on the AuNPs of plant extracts, the absorption spectra of samples of green-leaf extracts were studied at 500–1300 nm with and without laser energy. **Figure 2** depicts the absorption spectra of mint green-leaf extract; the absorption peak was enhanced by using a pulse laser and without laser pulses.

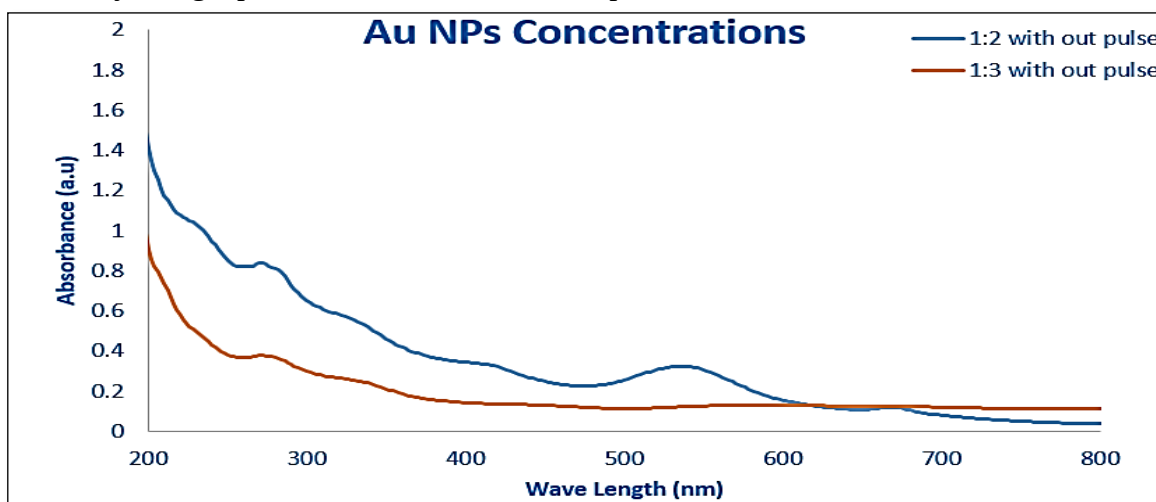


Figure 2. Ultraviolet-visible absorption spectra of mints extract AuNPs exposed & unexposed to laser pulses

The absorption spectra of gold colloids, such as gold foil, for different energies for this type of crystal, 532 nm, are shown in **(Figure 3)**, which is fragmented by continuous influence.

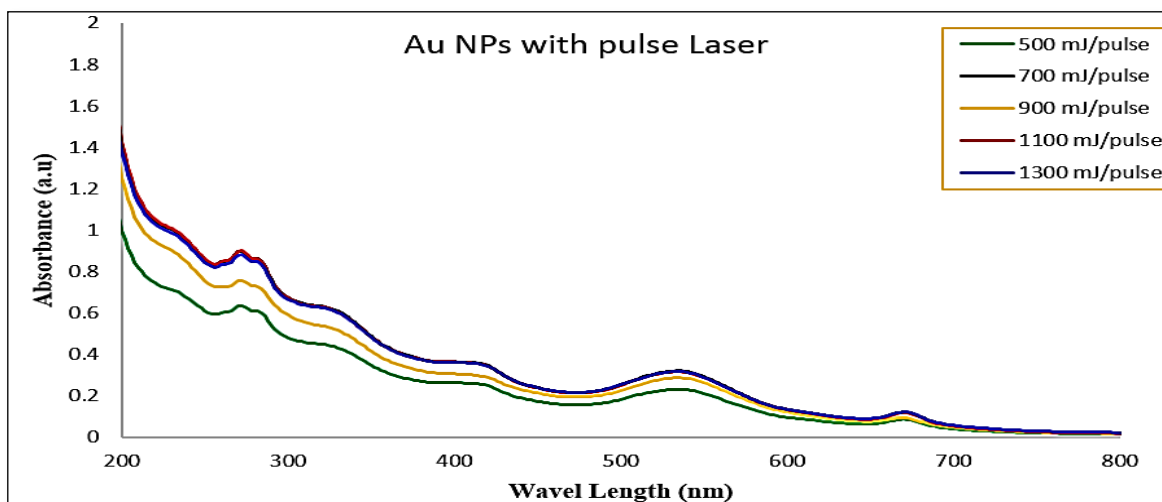


Figure 3. Gold Colloid Absorption Spectrum primed at different pulse energies by distinct laser influence

FTIR spectroscopy characterization of the sample spectra of stabilized AuNPs produced from mint leaf broths in (Figure 4) shows absorption peaks situated at about 3436.70, 2067.67, 1636.33, 1083.44, and 684.87.

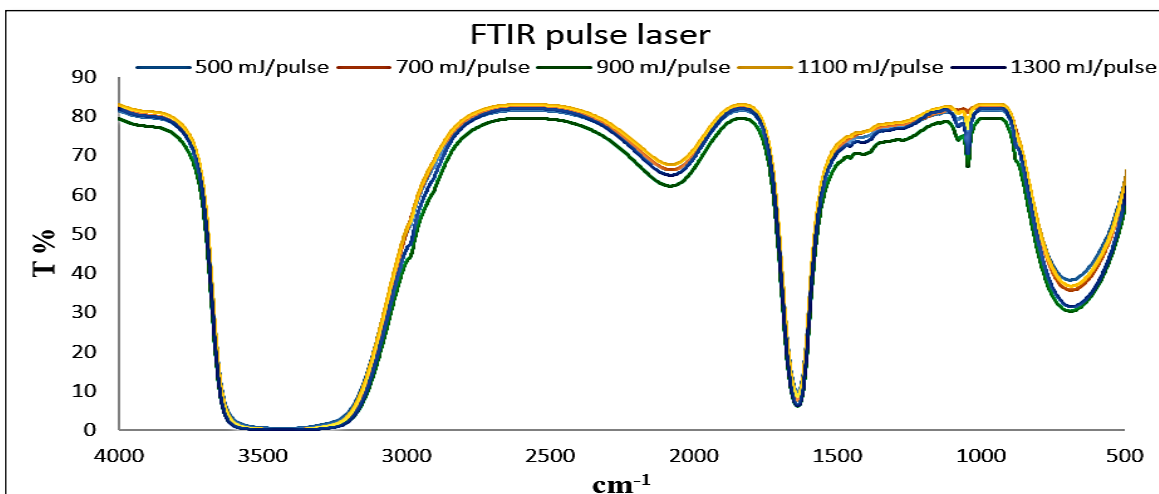


Figure 4. FTIR spectra of AuNPs synthesized using mint leaves' extracts

Figure 5 shows carvone constitutes 61% of the extract and interacts with the gold nanoparticles, whilst limonene comprises 54% of the extract.

Five samples may be identified as spinodal particles due to the appearance of a string of pearls in SEM (Figure 6 a, b, c, d and e), which illustrates the SEM morphology of Au NPs samples fragmented by laser pulse energy with a laser pulse energy of 1100 mJ/pulse for (E), which has a highly comparable ultrafine microstructure at the nanoscale but is reasonably isotropic at the 10s and 100s nm scale.

By examining (Figure 7 a and b), a portion of larger particles might be observed. In relation to the morphology of the AuNPs, the TEM images illustrate AuNPs in spherical and triangular configurations, as showing the differences in the image declaration between the preparation sample with and without laser pulse energy.

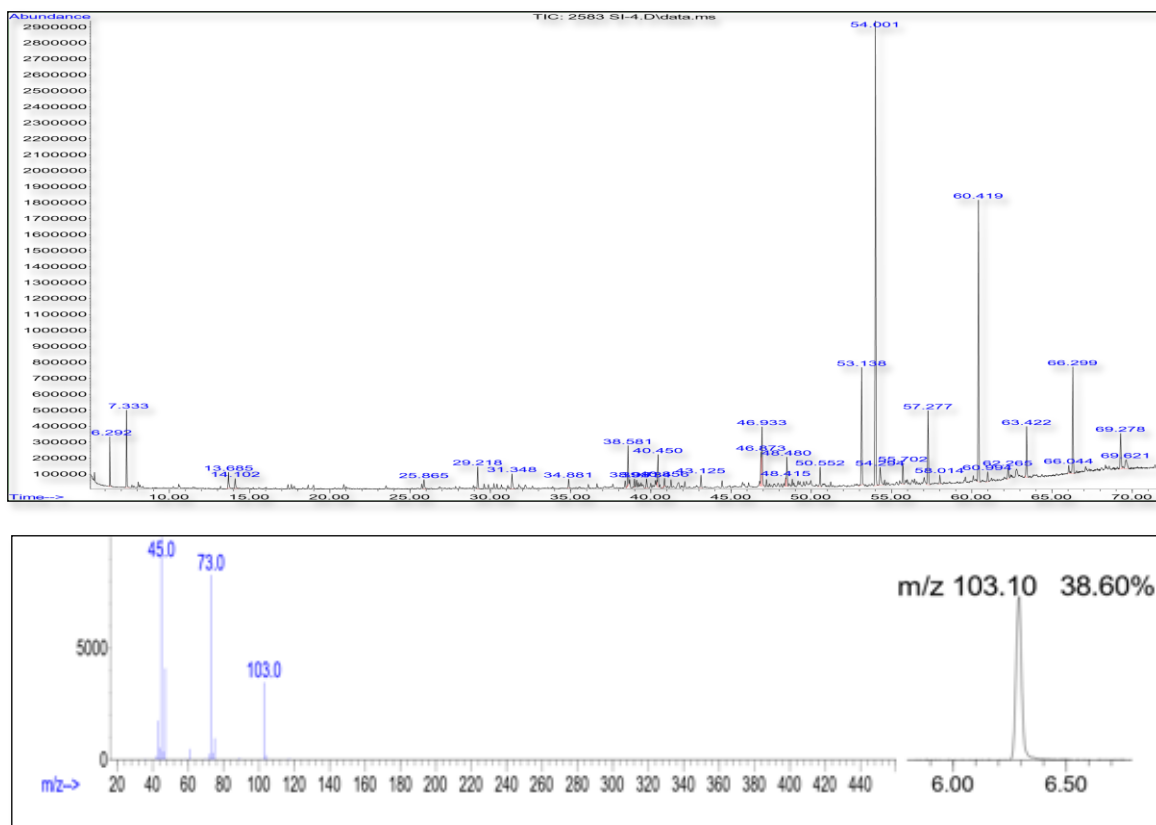


Figure 5. GC-MS chromatogram from mint with AuNPs and mass spectrum peak of the compounds

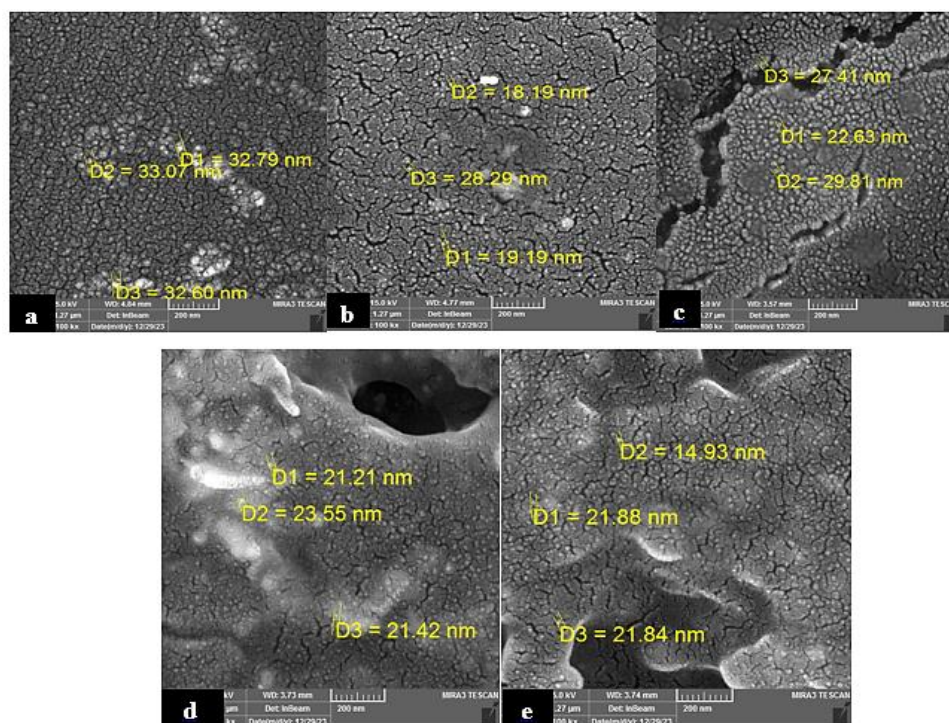


Figure 6. FESEM images for the samples of AuNPs by laser fragmentation with different energy; a. 500, b.700, c.900, d. 1100and e. 1300 mJ/pulse

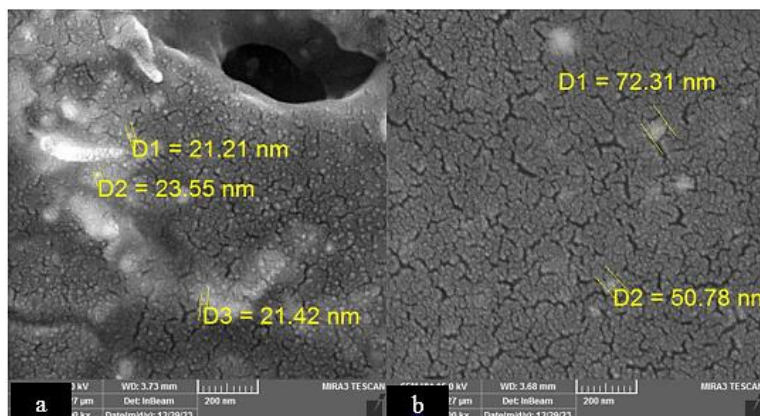


Figure 7. SEM images of particle production in the extract in the with and without the laser, a. with laser pulse and 1100 mJ/pulse energy & b. without laser pulse.

The variation in laser fragmentation efficacy is largely determined by the laser effect. To get a close approximation of the link, it is necessary to do repeated spot size testing. Fluence quality is directly proportional to pulse efficiency. The structure, as shown in the TEM images, is a spherical formation of AuNPs, with many twinning planes as a common feature. These images of Au nanoparticles illustrate the structure in an orientation with sizes ranging depending on the pulse energy utilized (**Figure 7 a, c, d and e**). TEM images reveal almost spherical particles (**Figure 9 a and b**).

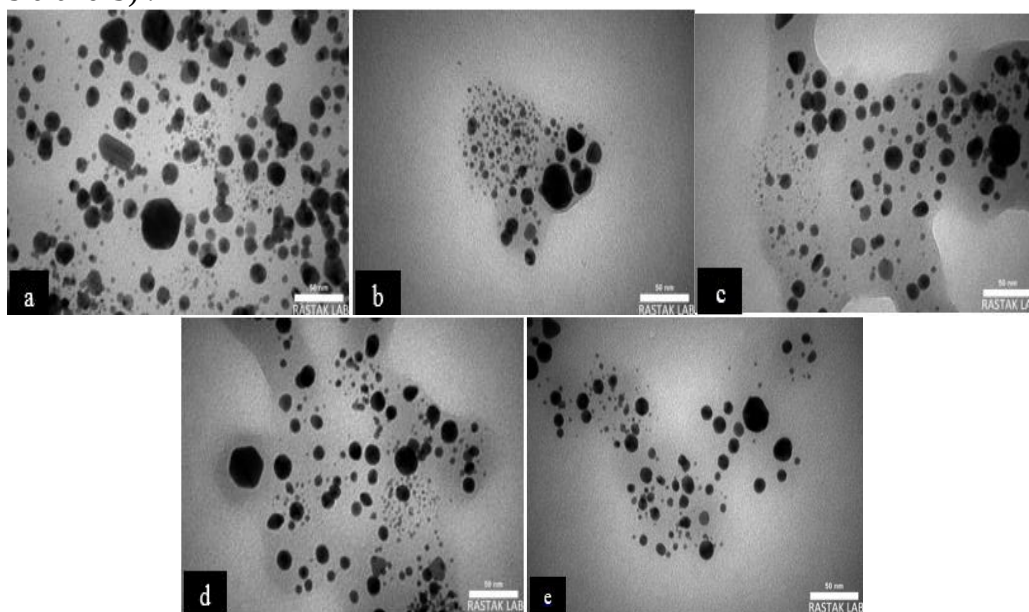


Figure 8 .TEM images for AuNPs 532 nm crystal and energies (a) 500, (b) 700, (c) 900, (d) 1100, and (e) 1300mJ/ pulse

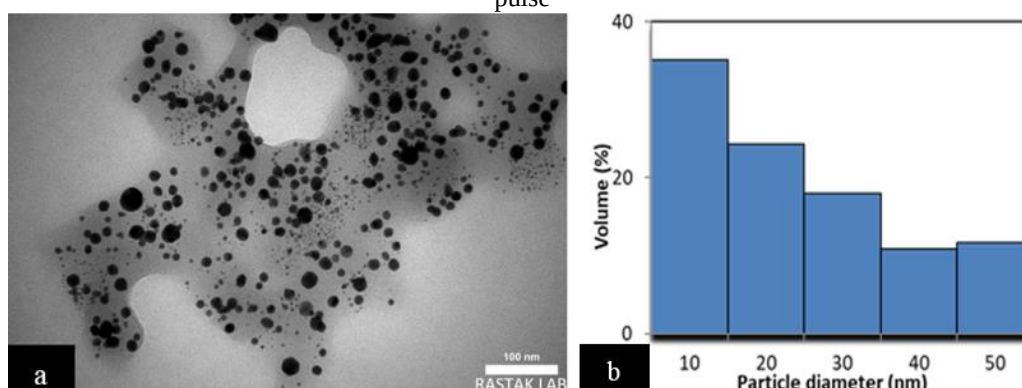


Figure 9. a. TEM pictures of AuNPs in colloidal solutions produced with 1100 mJ/ pulse (spherical particles), the TEM was set at 200 kV. B. Particle diameter and a percentage of volume

XRD characterization: the wavelength used is 532 nm, and according to the different energy pulses, the diffraction peaks of $2\theta = 32.420, 38.600, 53.620,$ and 63.410 can be used to identify the gold face-centered cubic crystal structure (fcc) (**Figure 10**). JCPDS No. 89-3722 (Joint Committee on Powder Diffraction Standards) JCPDS Nos. (111), (202), (220), and (311) planes of metallic gold are interpreted from XRD. After collecting the characteristics of the optimum energies utilized by the crystal, it was discovered that the energy of 1100 mJ/pulses provided the best data, as tests on the 532 nm crystal were carried out to identify the cubic crystal structure of metallic gold (**Figure 10**). The efficiency and qualities of the material were found to differ, with the extracted material exhibiting fewer characteristics than the laser-treated one, as evidenced by the X-ray diffraction analysis. This was detected even though the examination was conducted on a sample that had not been exposed to any laser radiation (**Figure 11**).

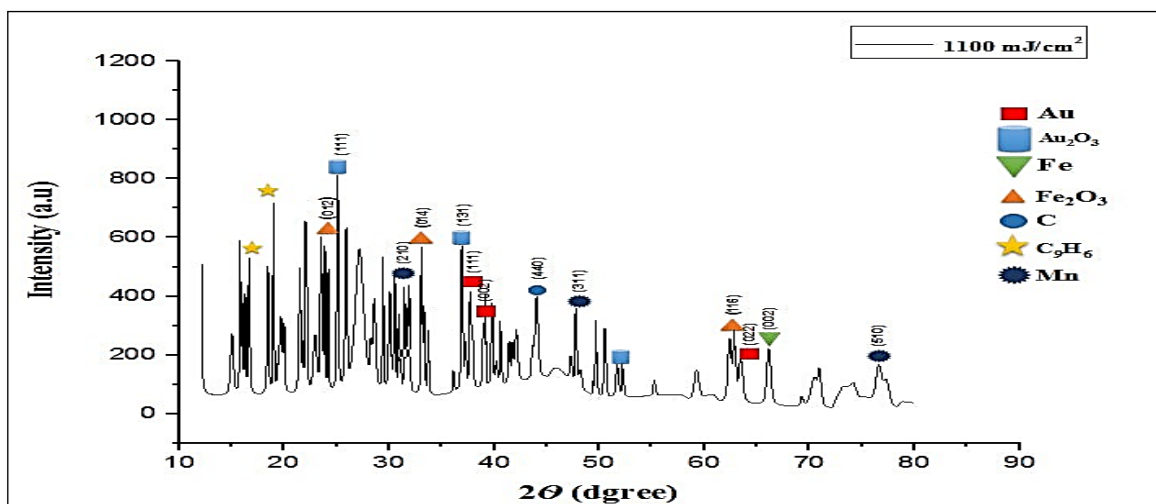


Figure 10. Showing the colloidal AuNPs depicts the synthesized AuNPs XRD pattern for 532 nm the energy of 1100 mJ/ pulses

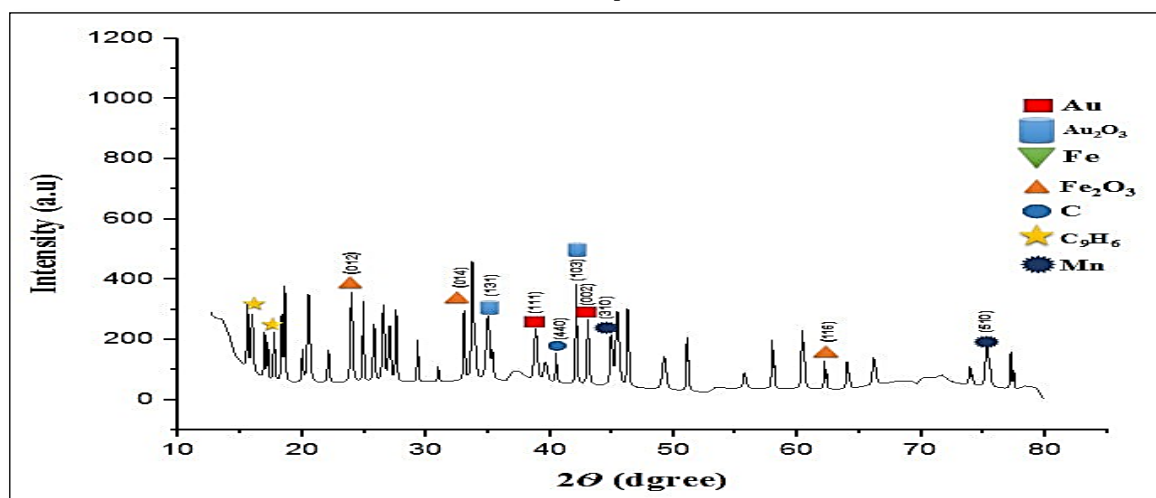


Figure 11. Showing the colloidal AuNPs depicts the synthesized AuNPs XRD pattern without any pulses of laser.

Figure 12. a,b,c,d and e, shows distribution of present elements in plant extracts with gold nanoparticles from mints collected by interaction with laser pulses and at predetermined energy at very high magnifications, especially under high beam currents.

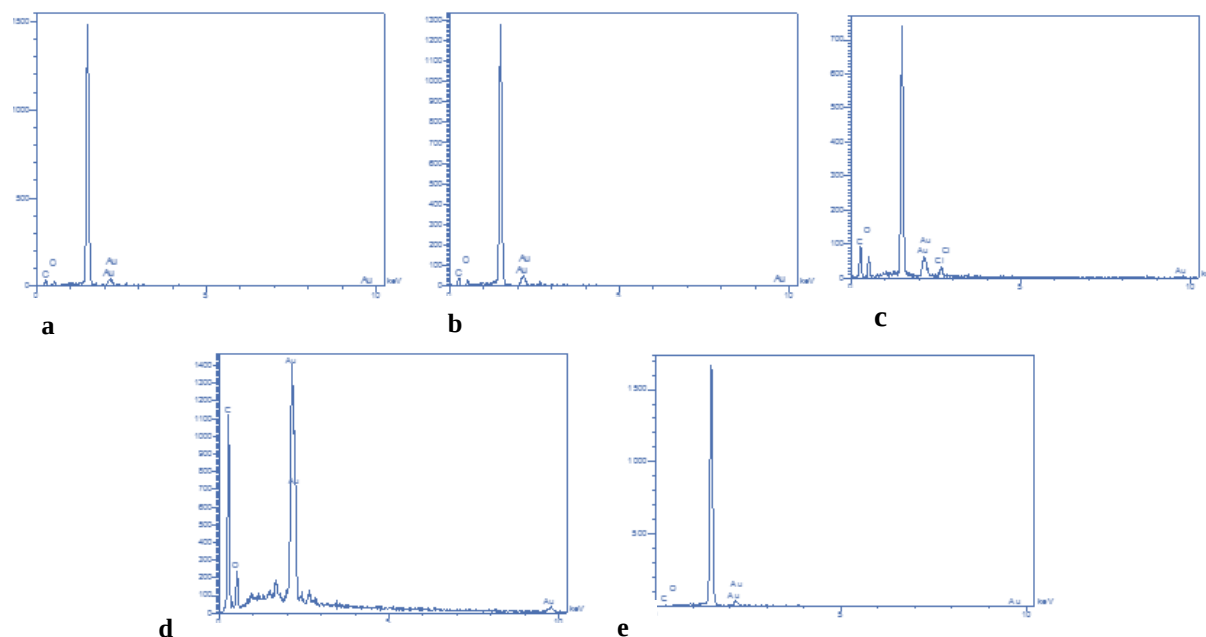


Figure 12. EDS images for the samples of plant extractions with different energy laser pulses, respectively

3.2. Hematological parameters results

The results of the current study indicated a significant difference ($P \leq 0.01$) in WBCs and lymphocytes in both groups that were injected intraperitoneally with the aqueous extract of mint leaves and the AuNPs unexposed to laser pulses compared to the group of AuNPs exposed to laser pulses and the control group, which showed normal results (**Figure 13 a and b**). **Table 1** shows a highly significant difference ($P \leq 0.01$) due to a decrease in the values of both neutrophils and monocytes for both the extract and AuNPs unexposed to laser pulses groups compared to the AuNPs exposed to laser pulses and control groups (**Figures 14 a and b**). **Table 1** also showed no significant differences in the outcomes of basophils for all groups compared to the control group (**Figure 15 a**). Based on the results of low eosinophil counts, **Table 1** and (**Figure 15 b**) showed a high significant difference ($P \leq 0.01$) in the AuNPs group unexposed to laser pulses compared to the other treated groups and the control group. **Table 2:** The results of liver function enzymes represented by ALT, AST, and ALP showed an increase in these enzymes and had a highly significant difference ($P \leq 0.01$) in both the group of aqueous extract of mint leaves and the group of AuNPs unexposed to laser pulses (**Table 2**), but the ALT enzyme in the group of AuNPs unexposed to laser pulses showed an increase of more than in the extract group. While the AuNPs group exposed to laser pulses showed no significant difference in the elevations of these enzymes that were recorded in the previous groups (the extract group and the AuNPs group unexposed to laser pulses), similar to the results of the control (**figures 16 a, b, and c**).

Table 1. Comparison between treatment and control groups in WBCs differential count

Group	Means $\pm$ SE					
	WBCs ($10^3/\mu\text{L}$)	Lymphocyte (%)	Neutrophil (%)	Monocyte (%)	Basophil (%)	Eosinophil (%)
Control	4.94 ± 0.12 b	57.22 ± 0.74 d	26.92 ± 1.79 a	7.60 ± 0.13 a	0.260 ± 0.09	1.74 ± 0.36 a
Extract	9.92 ± 0.35 a	69.65 ± 0.96 b	17.03 ± 0.72 b	4.48 ± 0.18 b	0.210 ± 0.05	0.88 ± 0.12 b
Nano unexposed to laser	10.27 ± 1.16 a	73.40 ± 1.30 a	16.12 ± 0.80 b	4.79 ± 0.31 b	0.320 ± 0.06	0.080 ± 0.03 c
Nano exposed to laser	5.00 ± 0.24 b	60.77 ± 1.80 c	25.59 ± 1.84 a	7.34 ± 0.10 a	0.220 ± 0.07	1.32 ± 0.29 a
L.S.D.	0.893 **	3.816 **	4.003 **	0.571 **	0.205 NS	0.695 **
P-value	0.0001	0.0001	0.0001	0.0001	0.6940	0.0002

Means having with the different letters in same column differed significantly ** ($P \leq 0.01$), NS: Non-Significant

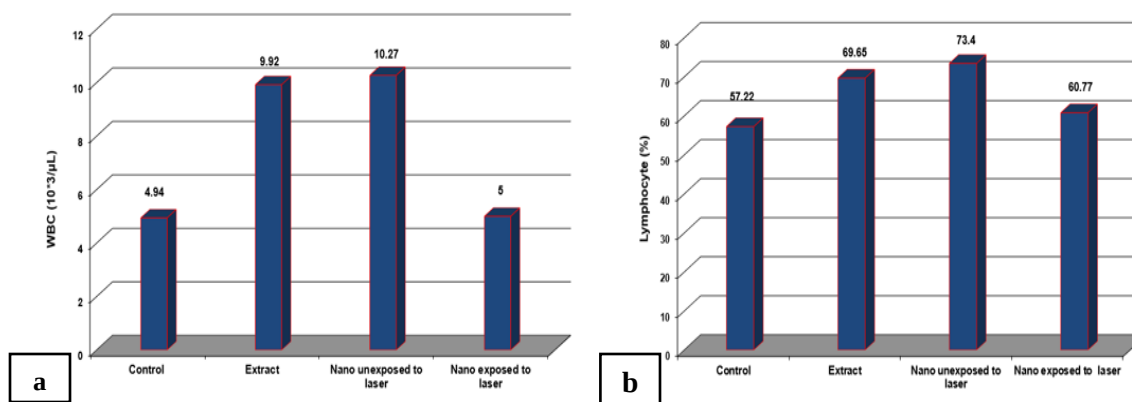


Figure 13. Comparison between treatment and control groups in (a) WBCs, (b) Lymphocyte

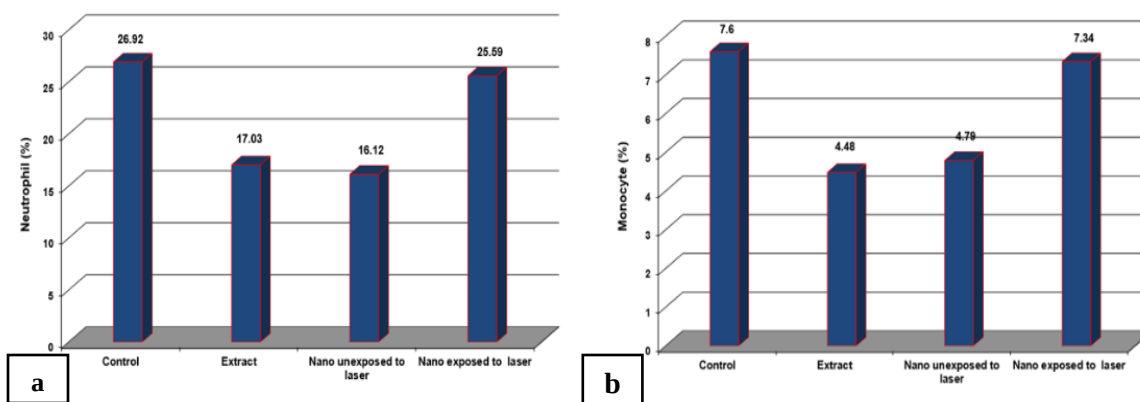


Figure 14. Comparison between treatment and control groups in (a) Neutrophil, (b) Monocyte

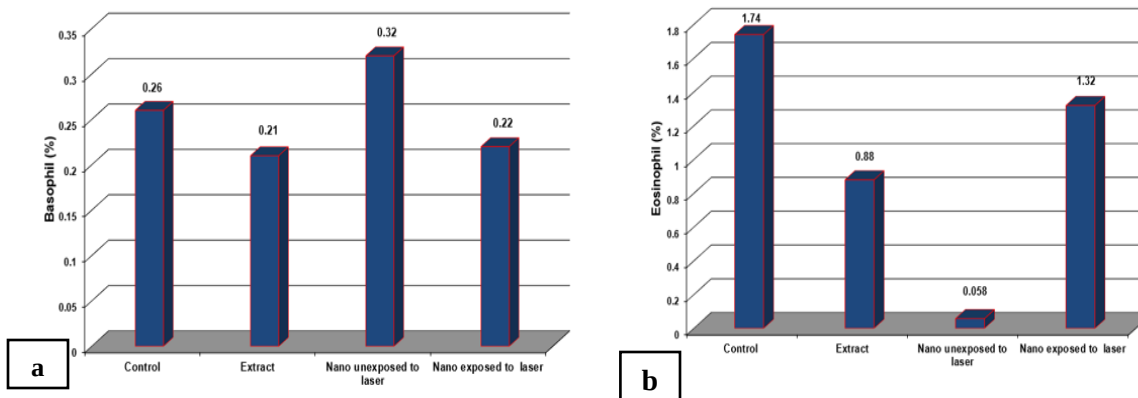


Figure 15. Comparison between treatment and control groups in (a) basophil and (b) eosinophil

Table 2. Comparison between treatment and control groups in liver enzymes

Group	Means ±SE		
	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Control	29.80 ±0.78 c	52.00 ±1.05 c	92.70 ±1.38 b
Extract	85.90 ±3.04 b	173.80 ±7.56 b	109.50 ±1.93 b
Nano unexposed to laser	102.10 ±1.81 a	278.60 ±20.26 a	139.60 ±12.93 a
Nano exposed to laser	29.90 ±0.98 c	51.50 ±0.86 c	95.90 ±0.76 b
L.S.D.	5.396 **	31.073 **	18.897 **
P-value	0.0001	0.0001	0.0001

Means having with the different letters in same column differed significantly ** ($P \leq 0.01$), NS: Non-Significant.

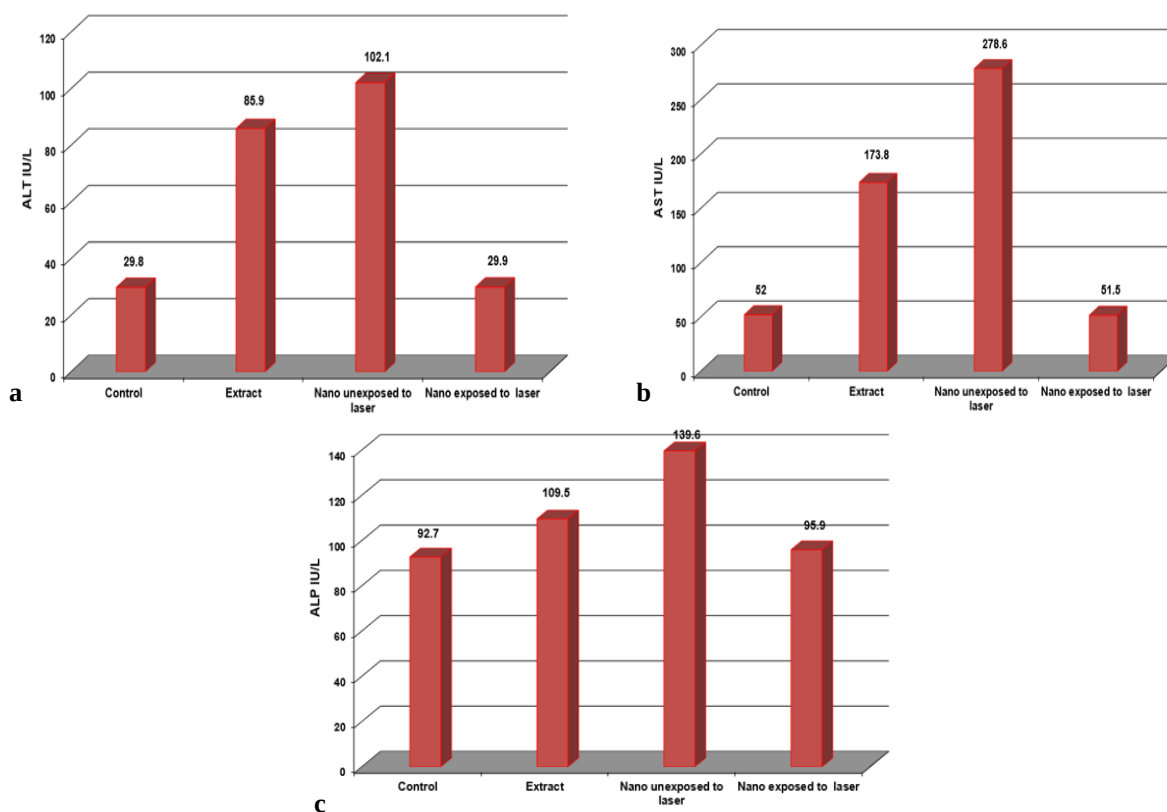


Figure 16. Biochemical parameters for liver enzymes between treatment and control groups in (a) ALT, (b) AST and (c) ALP

3.3. Histological Results

As for the results of the tissue sectioning, they showed the following: for the group treated with the aqueous extract of mint, histopathological figures of the liver showed congestion of the central vein, and the hepatic cords showed mild cellular swelling of hepatocytes (**Figure 17**). The group of gold nanoparticles unexposed to laser pulses: the histopathological figures of the liver showed that the central veins revealed intravascular hemolysis while the parenchyma revealed multiple focal necrosis of hepatocytes with tissue depletion (**Figure 18**). As for the group of AuNPs unexposed to laser pulses, the histopathological figures of the liver showed that the central veins revealed intravascular hemolysis, while the parenchyma revealed multiple focal necroses of hepatocytes with tissue depletion (**Figure 19**), histopathological figures of the liver of AuNPs that were exposed to laser pulses, showing the normal appearance of the central vein, normal hepatic cords with normal hepatocytes, and sinusoids).

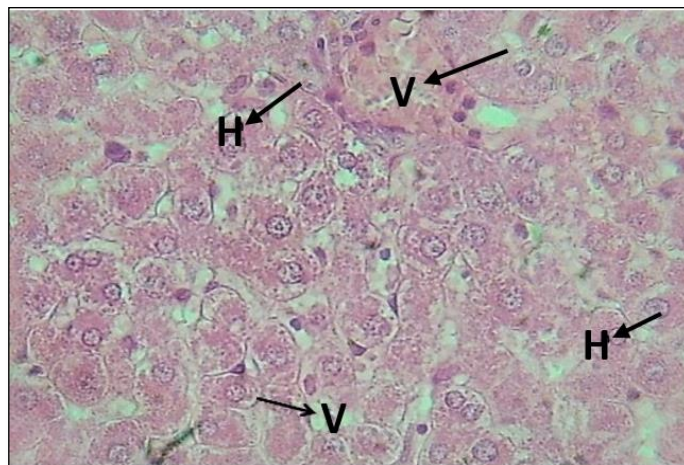


Figure 17. Section of liver. The extract group, the arrow points to V (V=vein) and the arrow points to H (H=hepatocytes). H&E, 40x

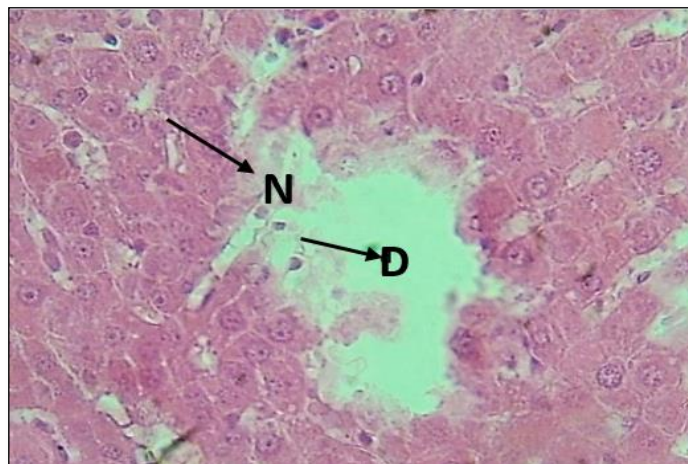


Figure 18. Section of liver, AuNPs unexposed to laser group , the arrow points to N (N=Necrosis) and the arrow points to D (D=Depletion). H&E.40x

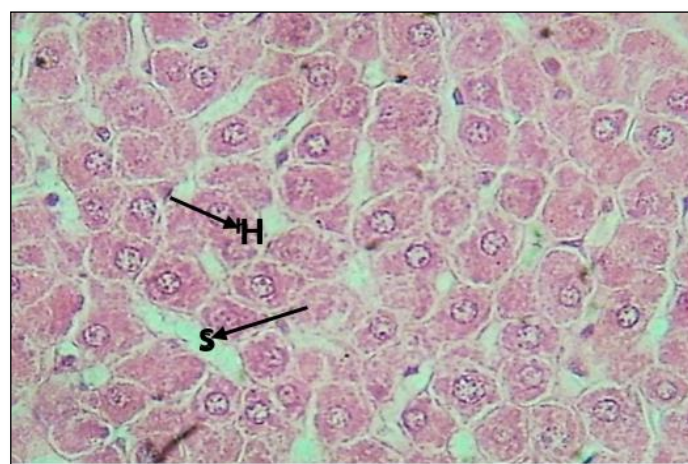


Figure 19. Section of liver, AuNPs exposed to laser group show normal hepatocytes, the arrow points to H&S (H =Hepatocytes & S=Sinusoids). H&E, 40x

4. Discussion

The characterization of gold nanoparticles exposed and unexposed to laser pulses showed fragmentations' performance and absorbance were enhanced and converted to higher energies by increasing the fragmentations' energy from 500 to 1300 mJ per pulse. As a result, gold nanoparticles do not curd and are stable at high energy, the GC-MS chromatogram from mint with AuNPs identified two primary components, menthol and mintne, in mint essential oil, present at concentrations of 10%–63% and 12%–76%, respectively, consistent with existing literature¹⁷. To describe the optical characteristics of AuNPs, the morphology of SEM pictures and the categorization of ultrafine particle sizes may be used of gold particle production in the extract with and without the laser. We assert that our samples exhibit a restricted concentration of triangles. AuNPs by laser fragmentation, the characteristic unique spherical point shape of AuNPs was found independent of energy concentration¹⁸. The particle shape was unaffected by the wavelength of the fragmentation light. Regardless of the fragment light's wavelength. As a result, particle accumulation should result in the plasmon band's width increasing aggregation of gold particles, according to¹⁹. The findings of the XRD coefficients show that the average particle size decreases with the rise in energy of the wavelength utilized according to the wavelength of the crystal. When it comes to the energy utilized by each crystal, it has a little effective role. The average crystallite size dropped as the crystal's wave length increased; this is due to decreased particle agglomeration¹⁸. In this study, hematological results showed an increase in white blood cells (WBCs), this result was consistent with another study that showed mint extract can enhance

the immune system at a dosage of 200 mg/kg by increasing the count of white blood cells²⁰. Increase in leukocyte count due to mint extract for these reasons: Increase in mitotic division via influencing stem cells (pluripotent, myeloid multipotent, and lymphoid) due to increased levels of mitotic division in primitive cells²¹. The findings of our study also indicated a substantial increase in lymphocytes in the group that was injected with the mint extract only, and these results are consistent with another study that showed the aqueous extract of mint increased lymphocytes and WBCs²². In a study supplemented with a concentration of 0.8% of mint extract, higher bursa chicken percentages were shown (the bursa, an essential lymphoid organ, facilitates the differentiation of B lymphocytes in birds and their response to immunological stimuli), and the same study showed supplementing mint extract with a concentration of 0.6% resulted in greater lymphocyte % and reduced monocyte % compared to other treatments; the meat-type chicken supplemented with 0.6 % had a significantly higher WBCs²³. A study that indicates an improved immune response, given that lymphocytes play a vital role in adaptive immunity, this study is also consistent with the findings of our research, which indicated a reduction in monocytes in the aqueous extract of mentha group²⁴. So it is probable that liver cell destruction induced a physiological insult that contributed to the observed increases in WBCs²⁵⁻²⁷. The research results also showed an increase in the levels of ALT, AST, and ALP for the group that was treated with the aqueous extract of mint leaves, and this result was consistent with results of a study that showed giving 500, 1000, and 1500 mg/kg of mint extracts to rats for 28 days showed a high dose-dependent increase in ALT, ALP, and other toxicological parameters in the sub-acute toxicity test; a significant elevation in AST levels was noted in rats administered *Mentha spicata* extract²⁸. In our current study, we used AuNPs prepared by green synthesis with aqueous mint extract, which were exposed to laser pulses with different energies, and the sample exposed to laser pulses with an energy of 1100 mJ was the best and was used to treat a group of adult male rats to determine its effect on hematological parameters and liver function enzymes and prove the difference between it (AuNPs exposed to laser pulses) and the toxic effect of AuNPs unexposed to laser pulses when injected intraperitoneally on the immune system. The results of our research showed an increase in the numbers of white blood cells and lymphocytes. These results are also consistent with a study, that showed WBCs and lymphocytes count a significant increase in the group that received repeated injections of AuNRs compared to the group that received a single injection²⁹. The results of our current study also showed a decrease in the percentage of neutrophils, monocytes, and eosinophils for the group of AuNPs unexposed to laser pulses. The results of our study also showed agreement with the results of other studies, Abdelhalim and Moussa, which showed treatment by intraperitoneal injection of two groups of male albino rats with two variations in size of gold nanoparticles, 10 nm and 50 nm, also decreased the number of neutrophils, monocytes, and eosinophils³⁰. The results of basophils in our study showed normal results for all treated groups, which was incompatible with the study²⁹, where basophils indicated an increase in the group that received repeated injections of AuNRs. Also, the results of our research showed an increase in liver function enzymes in the group treated with gold nanoparticles that were unexposed to laser pulses, and these results were consistent with a study that showed the dose-dependent effects on liver enzymes, and elevated levels of ALT and AST have been observed, reflecting hepatocellular damage and impaired liver function³¹. An increase in ALT and AST serum level shows significant hepatic cellular damage due to multiple causes. The liver serves as the principal site for the metabolism of both endogenous chemicals and foreign molecules, such as medications and poisons. Consequently, the increase in serum ALT activity is sensitive to measuring hepatocellular damage³². Another study indicated that high doses of gold nanoparticles cause further damage to liver cells and a decline in liver function, which is due to AuNPs being able to cause reactive oxygen species (ROS) to be produced, resulting in oxidative damage to cellular macromolecules and activation of pro-inflammatory pathways. Additionally, AuNPs may disrupt mitochondrial function, trigger apoptotic pathways, and stimulate hepatic stellate cell activation, contributing to liver fibrosis

and tissue remodeling³³. Understanding the dose-dependent histological effects of AuNPs on rats' livers is essential for evaluating their safety profile and optimizing their therapeutic applications. While AuNPs offer tremendous potential in biomedicine, careful consideration of dose, size, surface chemistry, and route of administration is necessary to alleviate potential toxicity and maximize therapeutic efficacy³⁴. These results demonstrated that AuNPs exposed to laser pulses had a non-toxic effect and acted as a protective factor compared to AuNPs unexposed to laser pulses. This is due to differences in the efficiency and quality of the material; exposure to laser energy pulses has an effect on the number and size of AuNPs and the production of biocompatible molecules. The protective effect of gold nanoparticles exposed to laser pulses on hematological and tissue parameters is achieved by reducing or preventing the oxidative stress and inflammation. Another study showed that numerous factors influence nanoparticle size development during laser ablation, with crucial determinants including physical characteristics such as laser fluency, pulse duration, irradiation time, wavelength, and repetition rate; the wavelength of 532 nm is predominantly employed in the synthesis of nanoparticles with ideal sizes and morphology³⁵. In a study, the antibacterial efficacy of chromium nanoparticles synthesized via pulsed laser ablation was evaluated against *Staphylococcus aureus* and *Escherichia coli*. The quantity of killed bacterial colonies exhibited variation at distinct laser energy levels ranging from 400 to 700 mJ. However, *E. coli* was completely eliminated, particularly at a laser energy level of 700 mJ, and another study demonstrated the effect of laser energy in the water ablation technique on the antibacterial activity of *Escherichia coli* and *Staphylococcus aureus*³⁶.

A study, was consistent with our study, as it showed the impact of laser wavelengths on silver nanoparticle size; the average particle diameter diminished from 27 to 8 nanometers with a reduction in laser wavelength, this was characterized by the fragmentation of colloidal particles due to the self-absorption of laser pulses and produced AuNPs suitable for biomedical uses¹³. Our study's results are also consistent with a previous study whose results showed the effectiveness of fractionation is greatly influenced by the spontaneous absorption of laser beam radiation by colloidal particles. As a result, the wavelength is an important parameter for synthesis, studying and controlling the behavior of AuNPs, facilitating their development in a variety of applications³⁷.

5. Conclusion

The present study showed that AuNPs prepared by green synthesis with aqueous extract of mint leaves and exposed to laser pulses of different energy. The best of these energies was 1100 mJ, based on the results of characterization (optical, structural, and morphological characterization). They showed their effectiveness in forming protective blood cells and protecting the liver from elevated levels of liver function enzymes. The gold nanoparticles exposed to laser pulses did not show any pathological histological changes in male albino laboratory rats. Therefore, gold nanoparticles can be used in many biological application fields.

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Conflict of Interest

Authors declare that they have no conflict of interest.

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Ethical Clearance

The experiment was conducted in accordance with the approval of the research ethics committee of the Ministry of Higher Education and Scientific Research, No. CSEC/1023/0118, on October 30, 2023.

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