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Synthesis of Gold Nanoparticles Using Ascorbic Acid as a Bioreductor and the Effect of Uric Acid

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Abstract: Gold nanoparticles (AuNPs), ranging in size from 1 to 100 nm, represent one of the most significant advancements in metal-based technologies in recent years. Their application in the medical field, especially as drug delivery agents, is particularly noteworthy and demonstrates their transformative potential. This study aims to synthesize AuNPs using HAuCl4 as a precursor and ascorbic acid as a bioreductant at a concentration of 20 ppm with a bottom-up method. The result of the synthesis process yields a striking burgundy-colored solution, signifying the successful formation of gold nanoparticles. The results of UV-Vis characterization provided insightful data on the transformation of the HAuCl4 solution. Initially recorded at a wavelength of 314.60 nm, the solution underwent a notable shift to 523.50 nm after the synthesis process, demonstrating an absorbance value of 0.323. Furthermore, the transmission electron microscopy (TEM) analysis illuminated the unique characteristics of the synthesized nanoparticles, revealing an average particle size of 6.23 nm with the highest particle frequency in the range of 6-8 nm. Moreover, the result of clinical trials conducted with ten randomly selected volunteers, who received 20 ppm of AuNPs over a five-week period, demonstrated that seven out of ten participants experienced a significant reduction in uric acid levels, underscoring the therapeutic potential of gold nanoparticles. Microscopic observations following the injection revealed that volunteers with normal uric acid levels exhibited fewer leukocytes than those with elevated uric acid levels, who were noted to have leukocyte counts up to three times higher. In conclusion, the synthesis of AuNPs using ascorbic acid is marked by a wine-red solution and a significant absorption wavelength shift to the 500–600 nm range. The positive results of the clinical trial reflect the ability of AuNPs to lower uric acid levels, thereby paving the way for innovative nanotechnology-based therapeutic strategies in the medical field.

Keywords: Ascorbic Acid; Characterization; Gold Nanoparticle; Injection; Uric Acid.

Introduction

Uric acid is a substance that our body produces when it breaks down purines, which are found in the cell nucleus [1]. Normally, our kidneys remove excess uric acid, which we then eliminate through urine. However, some people may have high levels of uric acid due to a diet high in purines or problems with kidney function [2]. When uric acid levels are too high, this condition is called hyperuricemia. A low level of uric acid in the blood is called hypouricemia [3]. Hyperuricemia can cause health issues like gouty arthritis, kidney stones, and may also lead to other problems, such as chronic kidney disease, heart disease, and diabetes [4]. Whereas, hypouricemia can be linked to genetic disorders like Parkinson's and Alzheimer's diseases [5]. A person is considered to have hyperuricemia if their uric acid levels are above 7.0 mg/dl for men and 6.0 mg/dl for women [6]. This condition is a significant global health issue, and the World Health Organization reported that 39.3% of people had hyperuricemia in 2020. The rise in cases is often due to diet, obesity, and genetics [7].

People with hyperuricemia may experience chronic joint inflammation. Symptoms include joint stiffness, swelling, and deformities, often affecting the knees, shoulders, ankles, and even the ears [6]. Managing inflammation can be done through two methods: medication and non-medication approaches. One non-medication option is acupressure therapy, which can help improve kidney function and lower uric acid levels [8]. For medication, doctors typically prescribe drugs like allopurinol, febuxostat, and pain relievers. However, these can cause side effects, such as stomach irritation, skin reactions, and kidney issues [5].

Recently, researchers have been exploring nanoparticles, particularly gold nanoparticles (AuNPs), as a potential treatment for gout. Gold nanoparticles are small particles with unique properties that can be utilised in various medical applications. They resist oxidation and corrosion, and when taken, they do not harm the body as they break down slowly over time [9]. AuNPs have been studied for their use in treating cancer, diabetes, skin diseases, herpes, and other inflammatory conditions [10,11,12,13]. Some studies have shown that AuNPs can help lower uric acid levels and reduce gout symptoms in mice. For example, one study found that 4 nm gold nanoparticles effectively reduced gout symptoms after being injected intravenously for 24 hours at a specific dose, with no harmful effects on the kidneys [14]. Another study showed that gold nanoparticles sized between 5-10 nm could normalize uric acid levels after being injected for seven days at low doses, indicating that they are safe for treating gout [15].

Research on nanoparticles covers both their applications and synthesis methods [16]. Gold nanoparticles can be synthesized using top-down methods (breaking larger

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particles into nanoparticles) or bottom-up methods (building nanoparticles from smaller particles). The bottom-up method is often preferred due to its ease of size manipulation [17]. A widely utilized bottom-up approach is the reduction-oxidation reaction, where gold ions (Au^{3+}) are reduced to metallic gold (Au^0) using a reducing agent [18]. Ascorbic acid is noted for producing uniform spherical gold nanoparticles and is recognized for its antioxidant, biodegradable, and biocompatible properties. It may also inhibit xanthine oxidase (XO), which produces uric acid, potentially aiding in the treatment of gout [18,19].

This study aims to assess the effectiveness of gold nanoparticles synthesized with ascorbic acid in lowering uric acid levels in patients with hyperuricemia. Gold nanoparticles were synthesized from $HAuCl_4$ with ascorbic acid and characterized using UV-Vis spectroscopy, revealing a ruby red color at 520 nm due to surface electron oscillations [20]. Transmission Electron Microscopy (TEM) was used to analyze the nanoparticle size, which was targeted to be under 8 nm for better cellular absorption [21]. Following characterization, clinical trials were conducted to evaluate the impact of these nanoparticles on uric acid levels and on leukocyte counts in patients with gout, positioning AuNPs as a potential alternative treatment for stabilizing blood uric acid levels.

Research Methods

Materials and Intrumentations

The materials used in this study include $HAuCl_4$, Ascorbic Acid, Water for Injection, Eosin and Methylene Blue Stain. Meanwhile, the equipment used in this study included 500 mL beaker glass, 10 mL measuring cup, 10 mL volumetric pipette, 1000 mL measuring flask, watch glass, spatula, dropper pipette, analytical balance, UV-Vis Spectrophotometer, Transmission Electron Microscopy (TEM), object glass, lancet, Olympus CX23 Microscope, tourniquet, spoeit, wing needle, and a uric acid level detector (Easy Touch GCU Meter).

Synthesis of Gold Nanoparticles

Nanogold is synthesized from a yellow-colored $HAuCl_4$ solution at a concentration of 1000 ppm. To prepare a 20 ppm $HAuCl_4$ solution, 10 mL of the 1000 ppm solution is diluted in 500 mL of sterile water for injection (aquabides). After dilution, 0.06 gr of ascorbic acid is incorporated into the mixture and homogenized thoroughly until a red wine color is achieved. Ascorbic acid functions as a bioreductant in the synthesis of gold nanoparticles (AuNPs), facilitating the process without the necessity for heating while ensuring high levels of biocompatibility [22]. The synthesis of gold nanoparticles is shown in Figure 1.

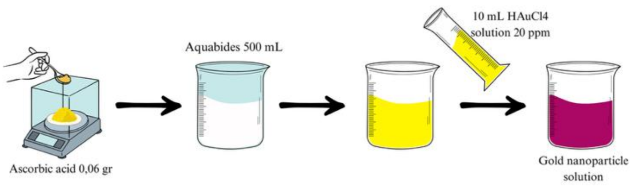


Figure 1. Synthesis of Gold Nanoparticles

Characterization of Gold Nanoparticles Using UV-Vis Spectrophotometry

Measurements were performed on the HAuCl4 solution and a 20 ppm solution of gold nanoparticles (AuNPs) obtained from the synthesis. The aim was to determine the maximum wavelength shift of the HAuCl4 solution, confirming the formation of gold nanoparticles. A 20 ppm HAuCl4 solution was placed in a cuvette and analyzed over a wavelength range of 200–800 nm. Similarly, the colloidal 20 ppm AuNPs from the synthesis were subjected to analysis in a cuvette, with measurements taken across a wavelength range of 400–800 nm [10].

Characterization of Gold Nanoparticles Using TEM

Figure 2 illustrates the characterisation of the nanoparticles using Transmission Electron Microscopy (TEM). A drop of colloidal gold nanoparticles (AuNPs) was placed on a copper grid, which was then dried under vacuum. After drying, the grid was mounted on a specimen holder and analyzed with the TEM instrument to determine the shape and size of the gold nanoparticles [23].

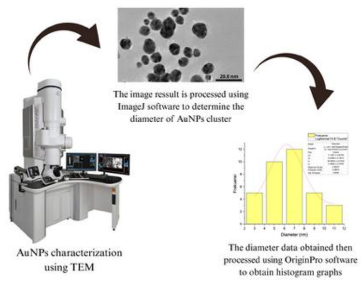


Figure 2. Characterization of AuNPs Using TEM

Injection Intravenous

The injection process was carried out using 20 ppm gold nanoparticles synthesized and injected through a vein at a rate of 200 cc per injection. This study was conducted in the Gununganyar area of Surabaya over a period of five weeks, from May to June 2025, involving 10 volunteers of varying ages. The procedure of intravenous injection successfully reduced uric acid levels toward normal ranges [23].

The injection process was performed by qualified professionals who secured ethical approval from the appropriate authorities to execute the procedure. Volunteers were randomly selected based on specific criteria: they were over 20 years old, in good health as confirmed by initial examinations, and were enthusiastic about participating and fully committed to the five-week research program.

Uric Acid Blood Test Using Easy Touch GCU Meter

The procedure begins by taking a blood sample from the volunteer's fingertip using a sterile lancet to prevent contamination. The blood is applied to a special test strip attached to the Easy Touch GCU Meter, which checks the uric acid level. The device calculates the uric acid level and shows the results on a screen [24].

Peripheral Blood Smear Using a Microscope

This test used an Olympus CX23 microscope and Optilab Viewer 2.2 software to compare blood cell images from volunteers with high and normal uric acid levels. Blood samples were collected from the volunteers' fingertips using a sterile lancet. A thin smear was created on a glass slide, allowed to dry, and then fixed with methanol and stained with Eosin and Methylene blue. The specimens were first examined under low magnification for orientation, followed by higher magnification to observe differences in blood cells related to uric acid levels [25]. The procedure of peripheral blood smear is shown in Figure 3.

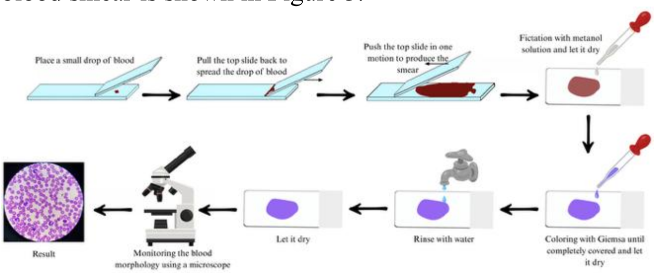


Figure 3. Peripheral Blood Smear Preparation Using a Microscope

Results and Discussion

Synthesis of Gold Nanoparticles

Gold nanoparticles are made using two main methods: physical (top-down) and chemical (bottom-up). Several techniques exist for creating gold nanoparticles, including chemical, thermal, electrochemical, and sonochemical methods. A common way to synthesize gold nanoparticles is to reduce a gold hydrochlorate solution with sodium citrate at 100°C. This method was first introduced by Turkevich in 1951 [26]. In this study, we used a stock solution of 1000 ppm $HAuCl_4$, ascorbic acid, and water for injection. To prepare the $HAuCl_4$ stock solution, we dissolved gold metal in aqua regia. Aqua regia is made by mixing two strong acids: 6 mL of 12N HCl and 2 mL of 14N HNO_3 in a 3:1 ratio. It is important to add HNO_3 to HCl, not the other way around, to prevent dangerous reactions and explosions. When creating the $HAuCl_4$ solution, a redox reaction happens. In this reaction, uncharged gold ions (Au^0) change into trivalent gold ions (Au^{3+}), which form a tetrachloroaurate (III) complex $[AuCl_4]^-$. The equation for the reaction that takes place is as follows:

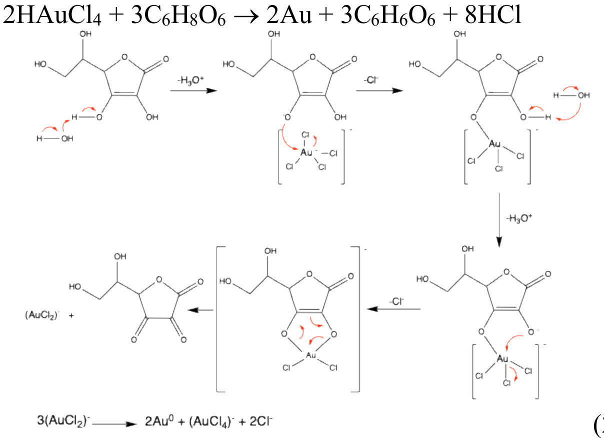
$$Au_{(s)}+HNO_{3(aq)}+4HCl_{(aq)}\rightarrow HAuCl_{4(aq)}+NO_{2(g)}+H_{2(g)}+H_2O_{(l)}$$

The synthesis process begins by producing gold nanoparticles at a concentration of 20 ppm, as determined by research [27]. They found that 20 ppm provided the best stability and the highest number of nanoparticles compared to concentrations ranging from 2.5 to 20 ppm. The next step is dilution. To create a 20 ppm solution of $HAuCl_4$, mix 10 mL of a 1000 ppm $HAuCl_4$ solution with 500 mL of water for injection. This creates a colorless solution. Then, add 0.06 grams of ascorbic acid to the 20 ppm $HAuCl_4$ solution. [28] Explained that increasing the amount of bioreductant helps reduce more Au^{3+} ions to Au^0. This happens because more collisions occur between the bioreductant and Au^{3+} ions. As you add more bioreductant, the absorbance increases, indicating that more gold nanoparticles are produced. The addition of ascorbic acid leads to a maximum absorbance wavelength of 523.50 nm and an absorbance value of 0.323.

Figure 4. Gold Nanoparticle 20 ppm Solution

The characteristics of gold nanoparticle formation can be observed physically through a color change in the solution, transitioning from colorless to wine red as shown in Figure 4. This color change is produced by surface plasmon resonance (SPR), which results from the collective oscillation of free electrons on the surface of gold nanoparticles when they are excited by light [28]. Additionally, the strong antioxidant properties of ascorbic acid help neutralize free radicals (ROS) and serve as a reducing agent [29]. The active group in ascorbic acid that functions as a reducing agent must have certain requirements, one of which is the presence of a lone electron pair (PEB) [30]. Furthermore, as a potent reducing agent, ascorbic acid reduces Au^{3+} to Au^0 through electron transfer, as demonstrated in the following equation:

$$2HAuCl_4 + 3C_6H_8O_6 \rightarrow 2Au + 3C_6H_6O_6 + 8HCl \quad (1)$$



(2)

Figure 5. Reaction Mechanism of Gold Nanoparticles with Ascorbic Acid

Based on Figure 5. shows that ascorbic acid has two hydroxyl groups (-OH) at the enediol position, which is very easily oxidized. This enediol group will then be oxidized to a diketone group, which releases electrons and reduces trivalent gold metal ions (Au^{3+}) to uncharged metal (Au^0) [18]. Next, the other OH- group will be deprotonated and form a complex compound. The reduction of gold nanoparticles is carried out in two stages: the first stage, Au3+ is reduced to Au+, and the second stage is reduced again to Au0, resulting in the final product in the form of Au0, dehydroascorbic acid (DHA), and HCl, as in equations 1 and 2 [31]. When in ion form (Au^{3+}), they will repel each other due to the influence of similar charges, but after being reduced to Au^0, the Au atomic charge becomes neutral, allowing the Au atoms to approach each other and interact with each other through intermetallic bonds to form a nano-sized cluster [32]. A solution of gold nanoparticles at 20 ppm, made with ascorbic acid, appears wine-red. This color change from colorless to wine-red shows that gold nanoparticles have formed. The color changes during the synthesis suggest that the clusters are getting larger. When gold atoms do not interact, the solution stays colorless. As the amount of gold clusters increases, they first turn dark blue and then red. Once the clusters reach the nanoscale size, they change to wine-red [33].

Characterization of Gold Nanoparticles

Characterization Using UV-Vis Spectrophotometer

The formation of gold nanoparticles can be visually confirmed by the change in solution color to red wine, caused by Surface Plasmon Resonance (SPR). In addition to visual observation, gold nanoparticles are also characterized using a UV-Vis Spectrophotometer and Transmission Electron Microscopy (TEM). Characterization using a UV-Vis Spectrophotometer aims to determine the wavelength and absorbance of a 1000 ppm $HAuCl_4$ solution and gold nanoparticles synthesized using ascorbic acid. This characterization was performed using a Shimadzu 1800 UV-Vis Spectrophotometer.

The results of gold nanoparticle characterization using a UV-Vis spectrophotometer showed a high absorbance value of 3.953 in the $HAuCl_4$ solution, indicating that the Au^{3+} ions had not yet been reduced or that gold metal was still present in the solution. After the synthesis process, the absorbance value decreased drastically to 0.323, indicating that most of the gold ions have been successfully reduced to gold nanoparticles or Au^0.

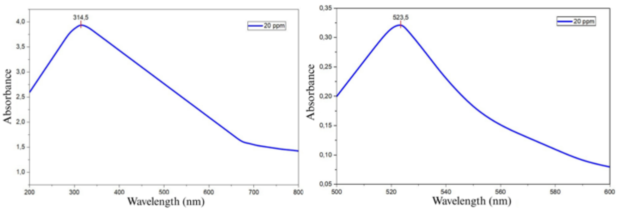


Figure 6. UV-Vis Spectrophotometer Graph

Based on the characterization results in Figure 6, there is a wavelength shift from 314.60 nm to 523.50 nm. This wavelength shift indicates that new gold nanoparticles have formed within the range of 500–600 nm [34], which aligns with the characterization results of gold nanoparticles added with ascorbic acid, yielding a wavelength of 523.50 nm and producing a ruby red color. Thus, changes in wavelength, absorbance values, and solution color serve as qualitative indicators that the reduction reaction catalyzed by ascorbic acid successfully formed gold nanoparticles effectively.

Characterization Using Transmission Electron Microscope (TEM)

The formation of gold nanoparticles is not only seen from their wavelength and absorbance values, but also observed from their particle size. The particle size of these gold nanoparticles was characterized using Transmission Electron Microscopy (TEM) JEM-2100Plus JEOL, which produced scales of 100 nm and 20 nm.

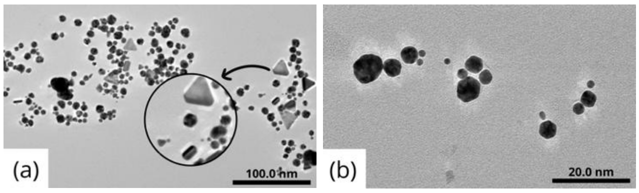


Figure 7. Result of TEM Testing of Gold Nanoparticles (a) 100 nm (b) 20 nm

Based on Figure 7, it can be observed that the TEM test results reveal various shapes of gold nanoparticles, including spherical, triangular, and rod-like shapes, at both 100 nm and 20 nm scales. The differences in particle shapes may be attributed to variations in temperature and pH during the synthesis process. The TEM testing results were further analyzed using OriginPro and ImageJ to determine the size of the particles formed.

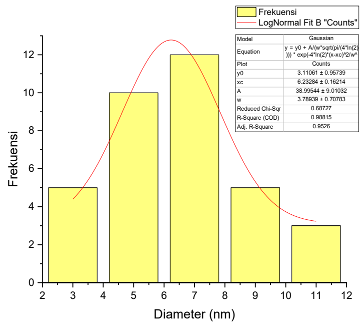


Figure 8. Histogram Results of OriginPro

Based on the analysis results in Figure 8, it was found that the distribution of the smallest gold nanoparticle cluster diameter ranged from 2 to 3 nm, and the largest cluster diameter ranged from 11 to 12 nm, with the highest frequency of cluster size in the range of 6 to 8 nm. According to the above data, the average diameter of the gold nanoparticle cluster was found to be 6.23 nm. It is essential for the average diameter of the gold nanoparticles to be ≤ 8 nm, as smaller AuNPs can more easily penetrate cells and have enhanced absorption in the body. The observed TEM cluster diameter aligns with existing theory; for instance, a study by Sun et al., (2022) reported a cluster diameter of 4.5 nm [14]. Additionally, other research indicates that the diameter of TEM clusters suitable for injection ranges from 5 to 10 nm [15]. The TEM results also indicate that most of the gold nanoparticles are uniformly spherical, although some exhibit other shapes, such as triangles and rods.

The variations in the shapes of the resulting nanoparticles can be influenced by several factors, including pH and temperature. The shape of gold nanoparticles changes with variations in the pH of the medium: at pH 2, they appear large and rod-shaped; at pH 3-4, they are smaller and rod-shaped; at pH 8, they appear as spherical, triangular, and polyhedral forms; at pH 9, they revert to spherical shapes; at pH 10, they take on a rod-like shape; and at pH 11, they become nanowire-shaped. Additionally, the synthesis temperature also affects the nanoparticles' shape; at temperatures between 25-90°C, various shapes such as triangular, pentagonal, hexagonal, and spherical are produced. Consequently, the shape variations observed in the TEM results are likely due to uncontrolled pH and temperature conditions during the gold nanoparticle synthesis process [35].

The Effect of Gold Nanoparticles on Uric Acid Levels After Intravenous Injection

Intravenous injection of gold nanoparticles aims to determine their effect on uric acid levels in volunteers, as indicated by a decrease or increase in uric acid toward normal levels. Uric acid levels were checked using an Easy Touch GCU Meter on 10 volunteers. Uric acid level data were collected six times, with the first measurement taken in the first week as baseline data prior to the administration of gold nanoparticles. The subsequent five measurements were obtained after administering 200 cc of gold nanoparticles over five consecutive weeks. The results of uric acid levels from volunteer 1 to volunteer 10 are presented in Figure 9.

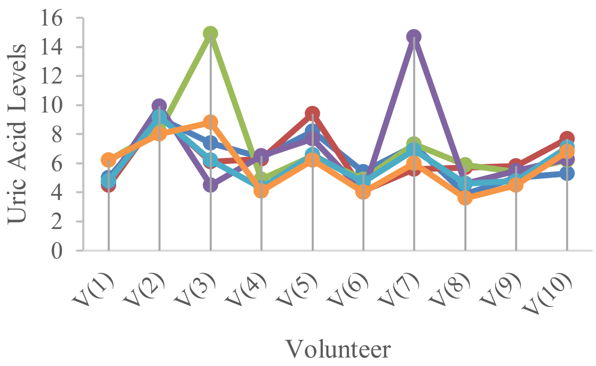


Figure 9. Graph of Uric Acid Levels in Volunteers 1-10

*Remarks: The horizontal line represents the number of volunteers who received the nanogold injection, while the vertical line shows the measured uric acid levels.

Figure 9 shows that the uric acid levels of 10 volunteers decreased in most cases. Seven volunteers experienced a decrease in uric acid levels to normal levels, as indicated by the downward trend in the graphs for volunteers 2, 3, 4, 5, 6, 8, and 9. Meanwhile, three other volunteers experienced an increase, as indicated by the upward trend in the graphs for volunteers 1, 7, and 10.

Intravenous injections of gold nanoparticles at a dose of 200 cc were given for five weeks to assess their ability to lower uric acid levels in volunteers. Certified professionals administered the injections to ensure safety and effectiveness. The aim was to see if gold nanoparticles could normalize uric acid levels. Studies suggest that they may help reduce inflammation and oxidative stress associated with uric acid production [36]. However, volunteer 1, aged 57, experienced a rise in uric acid levels, indicating that the nanoparticles do not work the same for everyone. Age affects how the body processes substances. Older adults often experience reduced kidney and liver function, which can result in higher uric acid levels [37]. Additionally, changes in biology may limit the effectiveness of nanoparticles in older adults. High uric acid levels can also result from a diet rich in purines, which are found in organ meats, nuts, and red meat [38]. In volunteer 1's case, the nanoparticles did not significantly lower uric acid levels, emphasizing that treatment success relies on individual health and lifestyle factors. In contrast, volunteer 2, aged 58, exhibited a slight decrease in uric acid levels, reaching 9.9 mg/dL, which is above the normal level of 7.0 mg/dL for men. This volunteer also struggled with dietary habits, including a high intake of purines from tempeh. While aging affects regenerative capacity, those in their late 50s can still respond reasonably well to treatments. Small gold nanoparticles can pass through the kidneys and interact with the uric acid transport system [39]. Thus, the decrease in uric acid for volunteer 2 was influenced by their kidney condition, lifestyle, and the body's response to stress, with gold nanoparticles potentially improving kidney function in older adults who still have some metabolic ability.

Volunteer 3 is a 59-year-old individual who experienced significant changes in their uric acid levels during treatment. One time, their uric acid level spiked to 14.9 mg/dL, which is much higher than the normal level for women (6.0 mg/dL). They mentioned that they had eaten beef liver, which contains a high amount of purines. This may have caused their high levels. Gold nanoparticles might help reduce inflammation and improve kidney health. But how well they work can depend on a person’s overall health. Volunteer 3 experienced a slight drop in uric acid levels after a few weeks, but then their levels returned to normal. This suggests that other factors, such as diet and hydration, are also crucial. On the other hand, Volunteer 4, who is 61 years old, experienced a steadier decrease in uric acid levels, although there was a slight increase in the fourth week. This suggests that everyone’s body reacts differently. Volunteer 4’s body seemed to handle the gold nanoparticles better, helping to lower their uric acid levels over time. Volunteer 5, who is 63, had a similar pattern but with more ups and downs. They first saw an increase in uric acid, followed by a significant drop. This may occur because the body is adjusting to the presence of gold nanoparticles. It's also important to remember that as people age, their liver and kidneys don't function as well, which can affect how the body processes purines.

Volunteer 6 experienced a steady and gradual decrease in uric acid levels, a positive sign after using gold nanoparticles. This means the nanoparticles worked well for them. Volunteer 6 responded quickly to the treatment, demonstrating that these particles can help not only with very high uric acid levels but also in maintaining normal levels. Gold nanoparticles help lower uric acid in the body. They can fight off harmful substances that can hurt the kidneys, which helps with uric acid removal. They also stop an enzyme called xanthine oxidase from making too much uric acid. Volunteer 7 is 43 years old and had big ups and downs in uric acid levels. At one point, their levels shot up high because they ate a lot of nuts, which have a lot of purines. When the body breaks down purines, it turns into uric acid. Eating too many nuts can make uric acid levels too high for the kidneys to handle. Even though volunteer 7 is an adult and should have a good kidney function, their eating habits affected their health more than their age. This means the gold nanoparticles might not work well if they keep eating too many high-purine foods. On the other hand, volunteer 8, who is 47, showed a more even pattern with uric acid levels. Even when their levels went up a little, they eventually came down because of the gold nanoparticles. Uric acid comes from breaking down purines in our food, and humans can't turn uric acid into a form that is easy to remove like some animals can. Gold nanoparticles can help the kidneys function more efficiently and remove uric acid more effectively. For volunteer 7, the high levels happened in the third week but then started to improve. This suggests that gold nanoparticles are most effective for individuals with uric acid levels closer to normal.

Volunteer 9 is 21 years old and had lower uric acid levels. This means that the gold nanoparticles helped him eliminate uric acid effectively. Young people typically have healthy bodies that function more effectively in removing waste, such as uric acid. Uric acid can affect blood flow. If there is too much uric acid, it can cause problems in our blood vessels, making it harder for blood to flow, which is not good for our hearts. Volunteer 10 is 67 years old and had higher uric acid levels. This means the gold nanoparticles did not help him. He has diabetes, which can make it hard for his body to work properly. As a result, his body was unable to eliminate uric acid effectively, even with treatment. In the study, most volunteers experienced lower uric acid levels after several weeks of treatment, while a few showed higher levels. This indicates that gold nanoparticles were beneficial to some individuals.

Observation of White Blood Cells with a Microscope

The purpose of observing white blood cells using this microscopic technique is to compare white blood cells in volunteers with high and normal uric acid levels. This observation process was carried out using an Olympus CX23 microscope with 40x and 100x magnification. Additionally, a staining process is performed prior to observation. The staining process utilises Giemsa stain, a mixture of eosin and methylene blue solutions. The staining process resulted in purple coloring on the white blood cells or leukocytes and pink coloring on the red blood cells. Figure 10. shows the microscopic results of blood cells in patients with high and normal uric acid levels at 40x magnification.

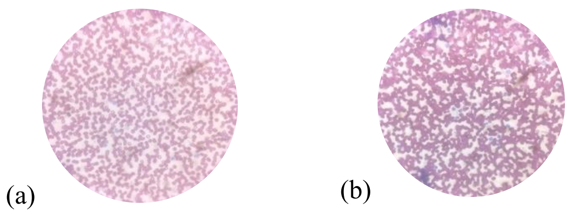


Figure 10. Microscopic Blood Results (a) Normal Uric Acid Levels (b) High Uric Acid Levels

In Figure 10 a significant difference in the number of white blood cells can be observed between the two groups. Volunteers with low uric acid levels tend to have fewer white blood cells, while those with high uric acid levels have three times more white blood cells compared to normal uric acid levels. In volunteers with normal uric acid levels, only two types of white blood cells are observed: neutrophils and lymphocytes. In contrast, in volunteers with high uric acid levels, three types of white blood cells are identified: neutrophils, lymphocytes, and eosinophils, with neutrophils and lymphocytes being the most dominant types in microscopic observations.

Our observations revealed that volunteers with normal uric acid levels had an average of five white blood cells per field of view under a microscope. In contrast, those with high uric acid levels had 16 white blood cells per field. In the blood smears from individuals with normal uric acid levels, we found only neutrophils and lymphocytes. However, in smears from those with high uric acid, we detected neutrophils, lymphocytes, and eosinophils [41]. Neutrophils act as the body's first defense against infections, while lymphocytes help in long-term immune responses. Eosinophils are usually involved in allergic reactions or chronic inflammation [40]. The higher leukocyte count in individuals with high uric acid levels may be attributed to inflammation caused by monosodium urate (MSU) crystals in the body. These crystals trigger the immune system, particularly affecting neutrophils and macrophages, and activate inflammatory pathways that produce proteins such as IL-1α, IL-6, and TNF-α [42]. This increased leukocyte count indicates the body's response to inflammation caused by the crystals, unlike those with normal uric acid levels, who typically have standard leukocyte counts. This finding underscores the relationship between elevated uric acid levels and immune system activation, characterized by increased white blood cell counts.

Conclusion

Based on research into the synthesis of gold nanoparticles using ascorbic acid and their impact on uric acid levels, we can conclude that. We successfully created gold nanoparticles using ascorbic acid. This is shown by the solution changing color from yellowish to ruby red and by an absorption peak appearing at 523.50 nm in UV-Vis spectrophotometer tests. Additionally, Transmission Electron Microscopy (TEM) showed that the gold nanoparticles averaged about 6.23 nm in size, with most of them being spherical. Injecting gold nanoparticles into volunteers resulted in a gradual decrease in uric acid levels, as observed in 7 volunteers who experienced a decrease in uric acid levels. Those who started with levels above the normal range of 6-7 mg/dL saw a decrease in their uric acid levels over five weeks after the injection. Microscopic examinations revealed that volunteers with high uric acid had three times as many leukocytes compared to those with normal levels. The limitations of this study are primarily characterized by a small sample size of only 10 volunteers, which constrains the statistical power and generalizability of the findings. Furthermore, detailed statistical analyses were not performed, rendering it impossible to confirm the statistical significance of the observed differences. Additionally, individual factors, including diet, physical activity, and the unique physiological conditions of each volunteer, may have impacted the measurements of uric acid levels. These considerations highlight the necessity for further research involving a larger sample size and a more rigorous experimental design. Such an approach would enhance the validity of these findings and facilitate a more comprehensive understanding of the therapeutic effects of gold nanoparticles (AuNPs).

Author's Contribution

Yulle Rachmawati: contributed to the conception and planning of the experiments, the collection and processing of the data utilized in this study, the execution of the experiments, the analysis of the data, and the writing, revision, and editing of the manuscript. Titik Taufikurohmah: contributed to the oversight of the research project and provided essential feedback to enhance its overall quality.

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