

Synthesis of Gold Nanoparticles Using Ascorbic Acid Bioreductor and Its Effect on Human Blood Pressure

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Abstract: Blood pressure is an important factor in the circulatory system and should be within the normal range of 90-140/60-90 mmHg. Blood pressure imbalances, such as hypertension and hypotension, can be caused by an unhealthy lifestyle, poor diet, or chronic illness. These conditions can trigger oxidative stress, which is an imbalance between the production of free radicals and the body's ability to neutralize them. Oxidative stress can damage cells and tissues, including blood vessels. Therefore, antioxidant intake is needed to help prevent further damage and maintain stable blood pressure. One innovative approach in blood pressure management is the use of gold nanoparticles, which have antioxidant activity. This study aims to characterize gold nanoparticles (AuNPs) synthesized using ascorbic acid bioreductors and examine the effect of their injection on human blood pressure. Characterization was performed using UV-Vis and Transmission Electron Microscopy (TEM), showing a maximum wavelength of 523.50 nm and an average particle diameter of 6.45 nm. Gold nanoparticles were injected intravenously at a dose of 200 mL every week. The results of the study indicate that the administration of gold nanoparticles significantly lowers blood pressure, as shown by the Paired T-Test, which shows an average decrease of 12.6 mmHg (95% CI: 5.1–20.1) and a p-value of 0.004. Based on the blood pressure trend graph, it was found that hypertensive volunteers' blood pressure decreased to normal levels, hypotensive volunteers' blood pressure increased to normal levels, and volunteers with normal blood pressure remained stable. Microscopic analysis showed a higher number of white blood cells in hypertensive volunteers compared to volunteers with normal blood pressure.

Keywords: Antioxidant; Ascorbic Acid; Blood Pressure; Gold Nanoparticles; Oxidative Stress.

Introduction

Blood pressure is the pressure of blood flow in arteries [1]. Blood pressure is a very important factor in the circulatory system. Not all blood pressure readings are within normal limits, leading to blood pressure disorders. Blood pressure problems are conditions affecting cardiovascular health. The normal blood pressure range in humans is 90-140/60-90 mmHg, where the first number (systolic) reflects blood pressure when the heart contracts, and the second (diastolic) reflects blood pressure when the heart relaxes [2]. Low blood pressure (Hypotension) is a condition where blood pressure is less than 90/60 mmHg [3], causing dizziness, weakness, mild headache, shortness of breath, chest pain, irregular heartbeat, nausea, blurred vision, difficulty concentrating, and, in extreme cases, fainting can occur [4]. High blood pressure (hypertension) is defined as a blood pressure exceeding 140/90 mmHg [5]. According to the World Health Organization (WHO) (2021), hypertension or high blood pressure is a serious medical condition that significantly increases the risk of heart, brain, kidney, and other diseases.

Hypotension and hypertension can be caused by various factors, including unhealthy lifestyles, unbalanced diets, and chronic health disorders [6]. Hypotension is often caused by dehydration, hormonal disorders, and heart disease, while hypertension is related to a high-salt diet, obesity, stress, and lack of physical activity. In the elderly, namely those over 65 years of age, hypertension is often

affected. This is due to the blockage of blood vessels, which become stiff, accompanied by the narrowing and enlargement of plaques that hinder peripheral circulatory disorders. Slow blood flow increases the heart's workload, elevating blood pressure throughout the circulatory system [7]. Hypotension and hypertension conditions can trigger cell damage due to oxidative stress, which is an imbalance between the production of free radicals and the body's ability to neutralize them [8]. Therefore, antioxidant intake is needed to protect body cells from further damage, reduce inflammation, and help maintain vascular health, thus contributing to the prevention and management of abnormal blood pressure [9]. Antioxidants can be obtained through food consumption, such as fruits, vegetables, and certain supplements [10]. However, in reality, people with hypotension and hypertension often have to take drugs routinely every day to keep their blood pressure stable. This long-term treatment can pose challenges, including low patient compliance and unwanted side effects. Therefore, new, more efficient and safe technologies are needed to overcome this problem.

To develop new methods to treat hypertension, nanogold or gold nanoparticles have shown promising potential. Gold nanoparticles (AuNPs) are referred to as synthetic antioxidants because they are engineered nanomaterials designed to mimic or enhance the activity of natural antioxidants, with no known carcinogenic effects [11]. Their advantages over natural antioxidants include high

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stability, resistance to degradation, and longer-lasting activity in the body [12].

One innovative synthesis method is using ascorbic acid (Vitamin C) bioreductors. Ascorbic acid has the main advantage of being environmentally friendly and biocompatible, so it does not cause toxic waste or harmful side effects on the environment or its use in medical applications [13]. As a reducing agent, ascorbic acid effectively converts gold ions (Au^{3+}) into gold nanoparticles (Au^0) without the need for hazardous chemicals or extreme reaction conditions [14]. Ascorbic acid can also show the size of gold nanoparticles that are round and uniform. In the hydroxylation reaction, ascorbic acid acts as an antioxidant that has strong reducing ability [15]. In addition, ascorbic acid enables better control of particle size and distribution, which is crucial for developing nanoparticles with desirable properties for therapeutic applications, including as drug delivery agents. With its superior ability to produce stable, safe gold nanoparticles, ascorbic acid is a very attractive option for nanoparticle synthesis for various health applications, including the treatment of diseases such as hypertension and hypotension [15]. The use of ascorbic acid as a bioreductant in the synthesis of gold nanoparticles provides multiple benefits. Besides functioning as an environmentally friendly reducing agent, ascorbic acid also has strong antioxidant capabilities [16]. The combination of gold nanoparticles and ascorbic acid can provide more effective protection against oxidative damage, potentially improving vascular function in patients with hypotension and hypertension. Ascorbic acid will reduce Au^{3+} ions from HAuCl_4 solution to Au^0 ions, which are characterized by a yellow to ruby red color change [17]. The formation of gold nanoparticles is not only marked by a change in color, but also ensures that the gold nanoparticles that have been formed can be characterized using a UV-Vis spectrophotometer and Transmission Electron Microscopy (TEM). Characterization of gold nanoparticles using a UV-Vis spectrophotometer is done to analyze the wavelength. Gold nanoparticles have a wavelength range of 500-600 nm [18]. Characterization of gold nanoparticles can also be done using TEM, which aims to determine the size of gold nanoparticles that have been produced [14].

The synthesized gold nanoparticles that have been mixed with drinking water and consumed regularly can reduce blood pressure in people with hypertension [19]. Therefore, in this study, the synthesized results obtained will be tested intravenously to determine the effect of gold nanoparticles on human blood pressure. Blood pressure can be affected by blood viscosity [20]. The higher the blood viscosity, the greater the resistance to blood flow in the vessels, which can raise blood pressure. Conversely, low viscosity will reduce resistance, so blood pressure tends to be lower. Changes in blood viscosity can be caused by several factors, such as dehydration, which increases the concentration of red blood cells in the plasma; polycythemia, which increases the number of red blood cells; or anemia, which decreases blood viscosity due to fewer red blood cells. However, blood pressure can also be influenced by other factors, such as an unhealthy lifestyle, an unbalanced diet, and other health problems [6]. Therefore, a microscopic test is needed to compare the results of examinations between patients with low, high, and normal blood pressure. Based on the description above, it is necessary to conduct research to

determine the effect of gold nanoparticles that have been synthesized with ascorbic acid bioreductor on human blood pressure.

Research Methods

This type of research is a single case study. The materials needed for this study include HAuCl_4 , ascorbic acid, water for injection, eosin, and methylene blue. Meanwhile, the equipment used in this study includes a 500 mL beaker, a 10 mL measuring cup, a 10 mL volumetric pipette, a 1000 mL measuring flask, a watch, a spatula, a dropper pipette, an analytical balance, a UV-Vis spectrophotometer, a Transmission Electron Microscope (TEM), a glass slide, a lancet, an Olympus CX23 microscope, a tourniquet, a sponge, a winged needle, and an Omron Hem 8712 tensiometer. Gold nanoparticles were synthesized from a yellow HAuCl_4 solution with a concentration of 1000 ppm. To prepare a 20 ppm HAuCl_4 solution, 10 mL of the 1000 ppm solution was diluted in 500 mL of sterile water for injection (aquabides). After dilution, 0.06 g of ascorbic acid was added to the mixture and thoroughly homogenized until a red wine color was obtained. Ascorbic acid serves as a bioreductant in the synthesis of gold nanoparticles (AuNPs), facilitating the process without heating while maintaining high biocompatibility [14]. The gold nanoparticles are then characterized using a UV-Vis spectrophotometer to determine the maximum wavelength. A 20 ppm HAuCl_4 solution was analyzed in the wavelength range of 200-800 nm. Meanwhile, AuNPs 20 were measured in the wavelength range of 400-800 nm [21]. Furthermore, analysis was performed using a transmission electron microscope (TEM) to determine the cluster diameter of the gold nanoparticles.

Gold nanoparticles were then injected at a dose of 200 mL into 10 volunteers. The injections were administered intravenously to 10 volunteers aged 23-58 years over 5 weeks. The injection process was carried out by qualified professionals who had obtained ethical approval from the relevant authorities to perform the procedure. Blood pressure was measured using an Omron HEM-8712 tensiometer. Blood pressure was measured before and after gold nanoparticle injection every week. The results obtained were presented in graph form.

Observations were made using an Olympus CX23 microscope and Optilab Viewer 2.2 software to compare blood cell images from volunteers with low, normal, and high blood pressure. Blood samples were collected from volunteers' fingertips using a sterile lancet. Thin smears were prepared on glass slides, allowed to dry, 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 blood pressure

Results and Discussion

Synthesis and characterization of gold nanoparticles

The process of synthesizing gold nanoparticles begins with the preparation of an HAuCl_4 solution. Preparation of HAuCl_4 mother liquor begins with dissolving gold metal with king's water (aqua regia). The aqua regia solution was

made by mixing two types of strong mineral acids, namely hydrochloric acid (HCl) 12N as much as 6 mL and nitric acid (HNO₃) 14N as much as 2 mL, in a ratio of 3: 1. The mixing stage is carried out carefully by pouring HNO₃ first into HCl where the mixing process should not be done upside down. This is important to note to avoid excessive exothermic reactions and explosive properties. In the preparation of HAuCl₄ solution, a redox reaction occurs where the uncharged Au ion (Au⁰) is oxidised to trivalent Au (Au³⁺), which then produces a tetrachloroaurate (III) complex [AuCl₄]. The reaction equation is as follows:



Based on the reaction that occurs, NO₂ and H₂ gases are formed, so heating is needed to remove these gases and evaporate the remaining acid in the solution. The heating process is continued until brown gas and small pops appear, indicating that almost complete evaporation has occurred. During dissolution, the mixture is left open in a fume hood until all the gold is completely dissolved and a clear yellow solution is produced. After the solution is formed, it is cooled and diluted to 1000 mL with distilled water in a volumetric flask, yielding a 1000 ppm HAuCl₄ solution, which is used as a parent solution for the synthesis of gold nanoparticles [17].

In this study, we used a 1000 ppm HAuCl₄ mother solution, which is already available at the Analytical Laboratory of the Chemistry Department of Universitas Negeri Surabaya. The use of ascorbic acid as a bioreductor in this study was chosen because it effectively converts gold ions (Au³⁺) into gold nanoparticles (Au⁰) without the need for hazardous chemicals or extreme reaction conditions [14]. The hydroxyl group on ascorbic acid will play a role in reducing gold(III) ions (Au³⁺) to the uncharged gold (Au⁰) metal. Negatively charged ions from ascorbic acid will attach to the surface of gold nanoparticles. The negative charge repels the gold particles, preventing aggregation or clumping. Ascorbic acid has two hydroxyl groups at the enediol position, which are highly reactive and easily oxidised. In this process, the enediol group is oxidized to a diketone group, releasing electrons, which then reduce trivalent gold ions (Au³⁺) to neutral gold metal (Au⁰) [15]. Positively charged Au³⁺ ions repel each other due to electrostatic forces. However, upon reduction to Au⁰, the gold atoms become neutral, allowing them to approach one another and form metal-metal bonds, forming nanoscale clusters [22]. One of the characteristics of the formed gold nanoparticles is a change in the color of the solution to wine red, as shown in Figure 1.



Figure 1. Nanogold solution

In this study, characterization was carried out using a UV-Vis spectrophotometer, where the formation of gold nanoparticles can be characterized by a shift in the maximum wavelength of the HAuCl₄ solution [21]. The maximum wavelength of the HAuCl₄ solution was 314.60 nm, with an

absorbance of 3.953. This is consistent with previous studies, which reported a maximum wavelength of 315 nm for HAuCl₄ solutions (Figure 2) [21]. The resulting gold nanoparticles (AuNPs) exhibited a maximum wavelength of 523.50 nm, as shown in Figure 3. In this study, the maximum wavelength of the HAuCl₄ parent solution with the maximum wavelength of gold nanoparticles (AuNPs) synthesized with ascorbic acid bioreductor was obtained from 314.60 nm to 523.50 nm. There is a shift in the wavelength, indicating the formation of gold nanoparticles.

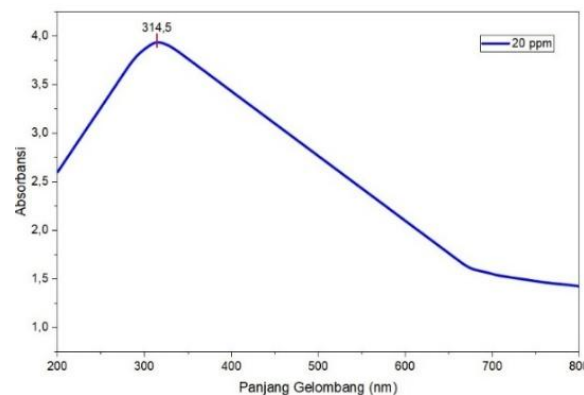


Figure 2. Results of HAuCl₄ characterization analysis using a UV-Vis spectrophotometer

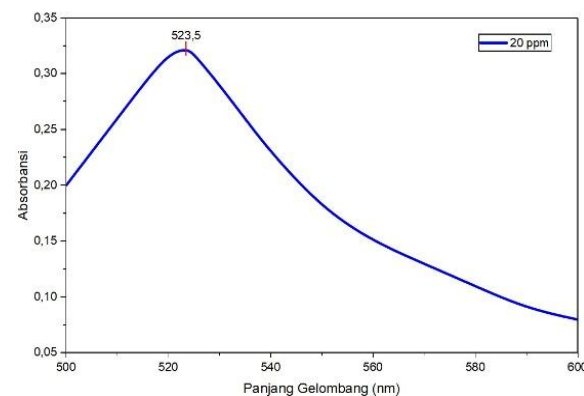


Figure 3. Results of gold nanoparticle (AuNPs) characterization analysis using a UV-Vis spectrophotometer

The formed gold nanoparticles also need to be tested by TEM to determine the diameter of the resulting nanoparticle cluster. The results of characterization using TEM show that the majority of gold nanoparticles have a uniform round morphology, accompanied by variations in other shapes such as triangles and rods, as shown in Figure 4. These morphological variations are influenced by several factors, especially pH and temperature during synthesis. Changes in the pH of the medium have a direct impact on particle shape: at pH 2 and 3-4, rod-shaped particles of different sizes are formed; at pH 8 produces spherical, triangular, and polyhedral shapes are produced; at pH 9, the spherical shape dominates; at pH 10, rod-shaped; while at pH 11 forms a nanowire structure is formed. In addition to pH, reaction temperature also affects morphology, with temperatures between 25 and 90°C producing triangular, pentagonal, hexagonal, and spherical particles. Therefore, the variation in the shape of gold nanoparticles observed through TEM is most likely due to the uncontrolled pH and temperature during the synthesis process [23].

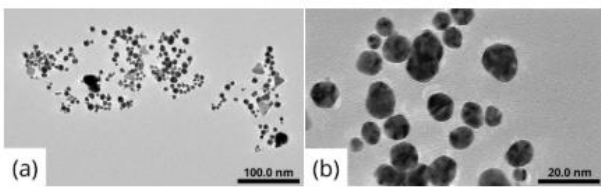


Figure 4. Characterization results of gold nanoparticles with a scale of 100 nm (a) and a scale of 20 nm (b)

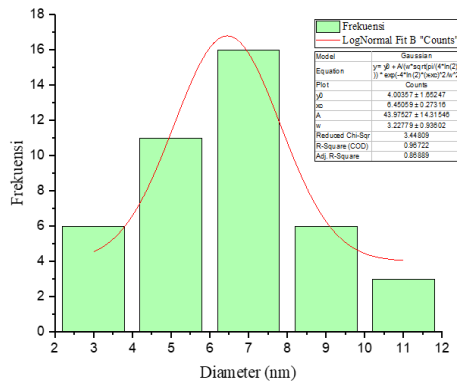


Figure 5. Histogram of OriginPro results

Based on Figure 5, the average diameter of the gold nanoparticle cluster is 6.45 nm. This shows that gold nanoparticles were successfully synthesized, with a cluster diameter range of 1-100 nm. In addition, the average diameter of the gold nanoparticle clusters produced was not significantly different from previous studies, in which gold nanoparticles ranged in size from 3.76 nm to 8.56 nm and had an average diameter of 6.58 nm [20]. Nanoparticles that are injected into the body are at least 8 nm in size. This is because smaller gold nanoparticles are more easily taken up by cells and show greater absorption in the body [24]. Based on the average cluster diameter of the gold nanoparticles produced, they can be injected into the human body, as their average cluster diameter is 6.45 nm. Gold nanoparticles are administered intravenously at a dose of 200 mL for 5 consecutive weeks. The injection procedure is performed by certified professionals to ensure safety and increase the bioavailability of nanoparticles in the bloodstream. The purpose of intravenous injection of gold nanoparticles is to determine the effectiveness of gold nanoparticles on human blood pressure.

Volunteer blood pressure results

Based on the systolic blood pressure graph of the volunteers, as shown in Figure 6, it is evident that the average systolic blood pressure of the volunteers is normal. This indicates that injecting gold nanoparticles into volunteers with normal blood pressure will maintain normal blood pressure. This is also supported by previous research showing that gold nanoparticles can maintain the elasticity of human blood vessels. Volunteers 2 and 3 had high systolic blood pressure, which then returned to normal [25]. This decrease in blood pressure is believed to be related to the intravenous injection of gold nanoparticles. Gold nanoparticles can help reduce plaque buildup on blood vessel walls, thereby preventing narrowing or blockage. In addition, gold nanoparticles also play a role in increasing blood vessel elasticity, which in turn helps stabilize blood

pressure. This is also related to the systolic blood pressure of volunteers 2 and 3 during the third to sixth weeks, which remained within the normal range and remained stable. Meanwhile, volunteer 9 had low blood pressure, based on his statement that he had a history of hypotension. This condition is also consistent with the volunteers' relatively low body weight. In addition, volunteers tend to have unhealthy lifestyles, such as frequently skipping breakfast, infrequent eating, and insufficient fluid intake. These factors contribute to a tendency for blood pressure to be below normal. Dehydration and unhealthy lifestyles can cause a person to experience hypotension.

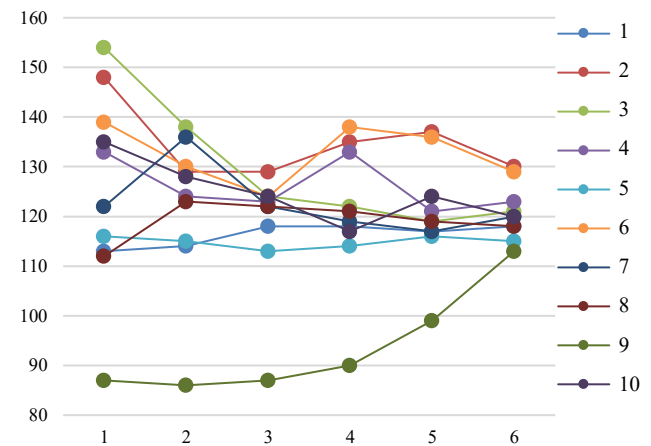


Figure 6. Systolic blood pressure of volunteers 1-10

In the fourth to sixth weeks, the blood pressure of volunteer 9 increased and then fell into the normal range. This is a positive indication of improved blood pressure, even though the volunteer's lifestyle has not changed significantly. This increase is believed to be related to the effect of gold nanoparticle injections, which can help stabilize blood pressure in individuals with a history of hypotension. This change was observed after two injections, when the initially hypotensive blood pressure began to increase gradually. This shows that gold nanoparticles not only have the potential to lower high blood pressure, but can also help increase low blood pressure. Intravenously injected gold nanoparticles are known to increase blood vessel elasticity and improve blood flow, thereby increasing blood supply to vital organs and helping to stabilize blood pressure. In addition, their antioxidant properties can improve endothelial function, which plays an important role in the physiological regulation of blood pressure.

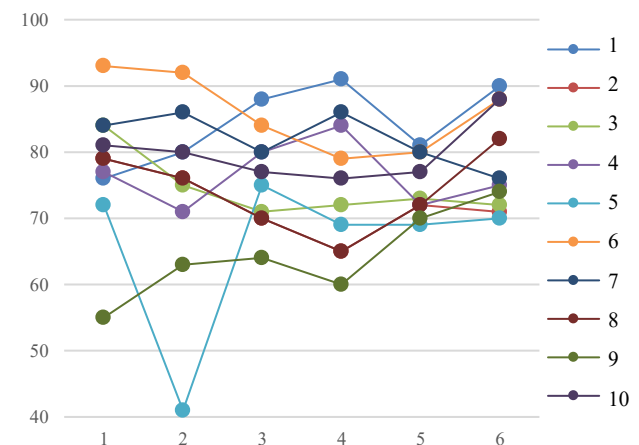


Figure 7. Diastolic blood pressure of volunteers 1-10

Based on the diastolic blood pressure graph of the volunteers, as shown in Figure 7, it can be seen that the average diastolic blood pressure of the volunteers is also within the normal range. Meanwhile, volunteers 5 and 6 had elevated diastolic blood pressure, but after receiving gold nanoparticles twice, their diastolic blood pressure gradually decreased to normal levels. This decrease is thought to be due to the weekly injections of gold nanoparticles. Gold particles can lower blood pressure through an antioxidant mechanism that protects blood vessels from oxidative stress and increases blood vessel elasticity. In addition, the volunteers reported that they had begun changing their diet to a healthier one during the study period. Therefore, the combination of the therapeutic effects of gold nanoparticles and dietary improvements is believed to have contributed to the decrease in blood pressure.

Based on the graph, the weekly administration of gold nanoparticles showed positive effects on all research

volunteers. Volunteers with a history of hypertension experienced a decrease in blood pressure to within the normal range. Meanwhile, volunteers with a history of hypotension experienced an increase in blood pressure to within the normal range. Volunteers with normal blood pressure from the outset experienced stable blood pressure throughout the administration period. After Shapiro–Wilk testing, the data were found to be normally distributed. This is because the p-value generated was greater than 0.05. According to the Paired t-test data shown in Table 1, there was a significant difference in systolic blood pressure before and after gold nanoparticle administration. This shows that administering gold nanoparticles can significantly affect blood pressure. In general, gold nanoparticles are known to have antioxidant properties that are beneficial to the body, namely protecting blood vessel walls from oxidative stress-induced damage, maintaining blood vessel elasticity, and inhibiting the formation of atherosclerotic plaques [26].

Table 1. SPSS Paired Sample Test Result

	Paired Differences						t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference					
				Lower	Upper				
Pair 1	12.60000	10.50079	3.32064	5.08818	20.11182	3.794	9	.004	

Results of microscopic analysis of volunteers' blood

Based on Figure 8, a significant difference in white blood cell count is evident. Volunteers with high and low blood pressure have higher white blood cell counts than volunteers with normal blood pressure. Based on microscopic observations, volunteers with low blood pressure had 13 white blood cells per microscope field of view, consisting of two types of leukocytes: neutrophils and lymphocytes. Volunteers with normal blood pressure had 5 white blood cells per microscope field of view, consisting of two types of leukocytes: neutrophils and lymphocytes. Meanwhile, in volunteers with high blood pressure, 15 leukocytes were observed in one microscope field of view, comprising three types: neutrophils, lymphocytes, and eosinophils. Based on this description, it appears that volunteers with high or low blood pressure have higher leukocyte counts than those with normal blood pressure. This condition indicates a systemic inflammatory response that may be triggered by oxidative stress and endothelial damage in blood vessels, which often accompany hypertension. When high blood pressure persists, the body responds by activating the innate immune system, particularly phagocytic cells such as neutrophils and macrophages, via the NLRP3 inflammatory pathway. Activation of this pathway triggers the production of various proinflammatory cytokines, including IL-1 β , IL-6, and TNF- α , which help maintain homeostasis and protect against tissue damage [27]. The increase in leukocyte count reflects the body's physiological response to stress and inflammation caused by hypertension. Conversely, in volunteers with normal blood pressure, no increase in leukocyte count was observed, indicating no excessive immune system activation. Thus, the link between hypertension or hypotension and immune system activation is further strengthened, as abnormal blood pressure conditions can trigger inflammatory processes, as evidenced by increased white blood cell counts in the bloodstream.

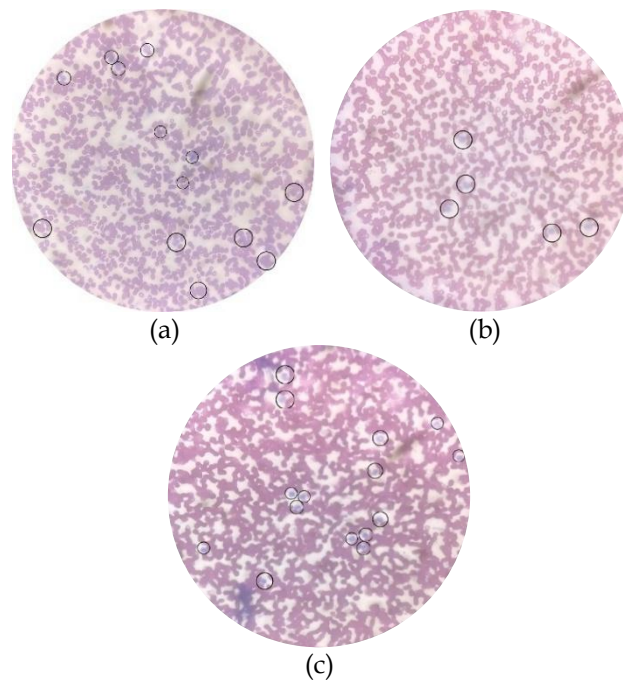


Figure 8. The results of observations of blood smears of volunteers microscopically at 100x (a) low blood pressure, (b) normal blood pressure and (c) high blood pressure

Conclusion

The synthesis of gold nanoparticles using ascorbic acid bioreductors at a concentration of 20 ppm produced a red wine-colored solution with a maximum wavelength of 523.50 and an average gold nanoparticle diameter of 6.45 nm. The results of the study indicate that the injection of gold nanoparticles synthesized using ascorbic acid bioreductors had a significant effect on lowering blood pressure in volunteers. This is demonstrated by an average decrease of 12.6 mmHg (95% CI: 5.1–20.1) and a p-value of

0.004 in the Paired t-test, thus concluding that the administration of gold nanoparticles via intravenous injection can affect human blood pressure.

Author's Contribution

A.C.D Rahmadhani: conducting research, analyzing, collecting data, and writing articles. T. Taufikurohmah: responsible person and article compiler.

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