Wound Drug Preparations For Hemophilia Sufferers Using Tea-Tehan Leaf Extract and AgNPs

D Saputra[1], A P Anggiani[2], D R H Hakim,[1] and N Aznam[2]

[1]Department of Biology Education, Universitas Negeri Yogyakarta, Yogyakarta, Indonesia
[2]Department of Chemistry Education, Universitas Negeri Yogyakarta, Yogyakarta, Indonesia

E-mail: dian.ckj@gmail.com

Abstract. In this study the preparation of wound preparations was carried out for hemophilia patients to treat external wounds. The innovation offered from the research lies in the basic ingredients of the drug consisting of silver nanoparticles (AgNp) and teh-tehan leaf extract. Thus, this study aims to (i) find out the effect of adding silver nanoparticles (AgNPs) on tea-tehan leaf extract (Acalypha siamensis) and (ii) find out the correct formulation on the addition of silver nanoparticles (AgNPs) to tea leaf extract - tehan (Acalypha siamensis) to the time of wound healing in hemophilia patients.This research is an experimental research. The work steps in this study can be explained as follows: namely making tea-tehan leaf extract, making AgNps, making formulations. Then characterization (UV-Vis, FTIR, and TEM) was performed on AgNps and formulations. Next is the conditioning of the test animals in the form of mice that are modeled as hemophilia mice, then testing the formulations against test animals.The results of this study indicate that i) drug preparations have UV-Vis peaks at wavelengths of 437 nm and 224.5 nm, each of which indicates that AgNP synthesis was successfully carried out, ii) FTIR results show functional groups in AgNP in the form of C = C and OH with wave number 1635.7 cm^{-1} and 3319.66 cm^{-1}.

1. Introduction

Hemophilia is a hereditary disease in the form of bleeding disorders due to lack of blood clotting factors that make it difficult for blood to clot. Based on data from the World Federation of Hemophilia (WFH) there are around 400 thousand people with hemophilia in the world. In Indonesia alone, it is estimated that there are around 20 thousand to 25 thousand people with hemophilia. However, based on data from the Hemophilia and Blood Transfusion Association (PHTDI) only 1,025 patients were noted to have hemophilia. The difference between predictions and those with hemophilia is recorded, consisting of several factors, one of which is possible for people with hemophilia to die before being diagnosed. For example when circumcision or continuous bleeding during surgery then dies. Or it could be a small number [1,2].

So far hemophilia is considered an incurable disease but if you get good treatment and are well managed, people can live normally. Blood clots in patients with hemophilia if a wound takes a long time, this can cause sufferers to lose a lot of blood. In handling hemophilia patients, it is very

necessary to have a blood component / therapy as a replacement / substitution treatment for several components of blood.

One antibacterial that can work in inhibiting bacterial growth is silver nanoparticles. Silver nanoparticles are also susceptible to clumping or aggregation [3]. This problem can be overcome by adding tea-tehan leaf extract (Acalypha siamensis) based on a study conducted by [4] that tea-tehan plants can be used as wound healing because they have antibacterial properties that are located in chemical compounds such as 2-pyridinepropanoic, 1,2,3-Benzenetriol, 9-Octadecenal in tea-tehan leaf extract, this compound inhibits the activity of Staphylococcus aureus bacteria.

With the combination of silver nanoparticles with tea-tehan leaf extract (Acalypha siamensis), it is expected to accelerate the occurrence of blood clots and still pay attention to their antibacterial properties. Therefore the authors took the initiative to conduct the study: Preparations for Wounds for People with Hemophilia Using Tea-Tehan Extract and AgNPs.

2. Experimental section

2.1. Material

$AgNO_3$, sodium citrate, tea-tehan leaves, ethanol, distilled water, alcohol, anhadrat were the materals in this research. While the instrumentation that used were UV-Vis, FTIR, TEM, evaporator, oven,grinding machine, syringe, beaker, cutter, glass funnel, aluminum foil, filter paper, analytical scale, pipette volume, vortex, small bottle / vial, erlenmeyer, petri dish , and cotton.

2.2. Making tea-tehan leaf extract

Tea-tehan leaf extract is done by picking tea-tehan leaves, weighing the leaves as much as 3 kg, then washing, drying in the oven and grinding so as to produce 650 grams of tea-tehan leaf powder. Furthermore, weighing 125 grams of tea-tehan leaf. Then do maceration by dissolving the powder in 1 liter of ethanol for 24 hours. Furthermore, macerate was evaporated for 3 hours.

2.3. AgNPs synthesis [5,6,7]

AgNp is synthesized by reducing silver nitrate ($AgNO_3$) with sodium citrate. The synthesis step of AgNp was carried out by weighing 0.5 grams of $AgNO_3$ powder. Then $AgNO_3$ is dissolved in 500 mL of distilled water. Take 50 mL of $AgNO_3$ solution and heat it to boil. Lift the $AgNO_3$ solution and add 3 drops of sodium citrate with a concentration of 1%. Reheat until the sample is yellowish brass.

2.4. *Preparation of drug preparations*

The preparation of the drug dosage formulations was carried out by mixing each 5mL of tea extract with a variation of the volume of addition of AgNP by 0 mL; 0.1 mL; 0.5 mL and 1 mL. Then shaking using the vortexer for about 30 seconds. Then the solution is stored in the vial.

2.5. Characterization of Samples

After the sample is formed, the sample is tested using 4 instruments, namely the initial stage using UV-Vis with the liquid suspension phase, then testing FTIR and TEM.

2.6. Making a Hemophilia Model

The test animals were injected intravenously into the rat's tail using 5mL of warfarin with 100 units / mL of 0.2 mL or the equivalent of 20 USP units. This is based on an adult dose of warfarin which is 75 units / kg. Furthermore, the test animals were observed for bleeding time.

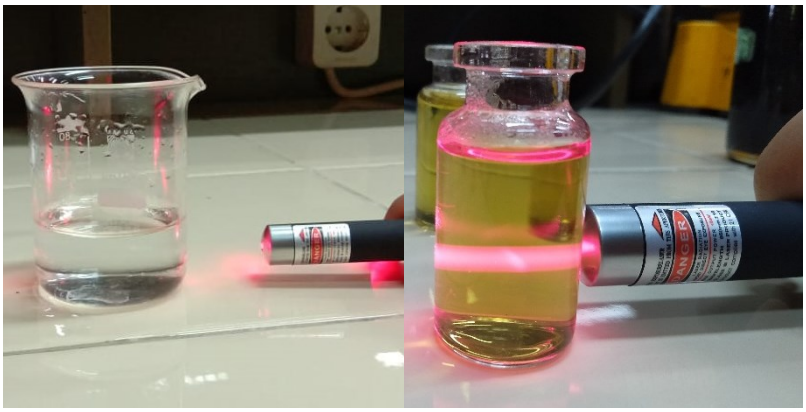
2.7. *Test on test animals*

The test animal that has been used as a model of hemophilia is injured (slashed) as long as 5 cm with a depth of 0.2 cm and the wound is left for 3 minutes. Furthermore, the wound is smeared with the medicine that has been made in point 3.5.4 b as much as 2 times. The wound is rubbed twice a day and observed changes in the wound. For controls used normal mice that have been injured with a length of 5 cm incision with 0.2 cm depth.

3. Discussion

3.1. Results of AgNP synthesis

The synthesis of a brownish AgNP solution that shows the existence of a light path when shot using a UV red laser (Figure 1).



(a) Aquades (b) AgNPs solution

Figure 1. Difference between aquades and AgNP when shot by a UV red laser

3.2. Characterization of silver nanoparticles with a UV Vis spectrophotometer

The results of AgNP synthesis were then followed by UV-Vis characterization. UV-Vis characterization is done to determine the absorbance pattern at a certain wavelength. The results of the characterization of AgNP solution with distilled water solvent are as follows (Figure 2).

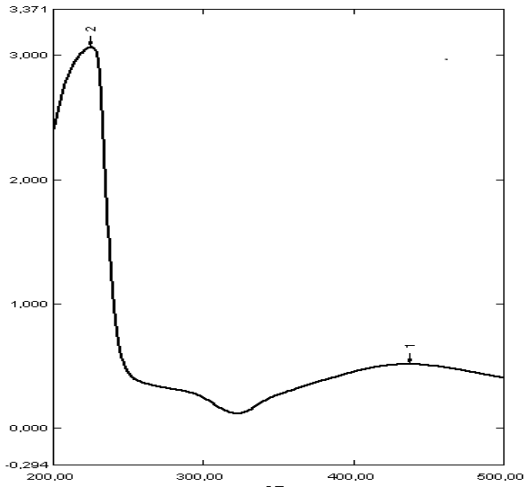


Figure 2. AgNP UV-Vis test results with distilled water solvent

Based on the spectra of AgNP solution with distilled water solvent above, showed that in AgNP solution 2 absorbance peaks were obtained, namely at wavelengths of 224.5 nm and 437 nm. Based on

the references obtained, AgNP is formed in the wavelength range of 400-450 nm. This shows that AgNP synthesis has been successfully carried out.

3.3. Characterization of AgNPs and formulations using the FTIR test

Based on the FTIR test results (Figure 3) qualitatively shows the functional groups contained in AgNP in the form of C=C and OH with wave numbers 1635.7 cm^{-1} and 3319.66 cm^{-1}.

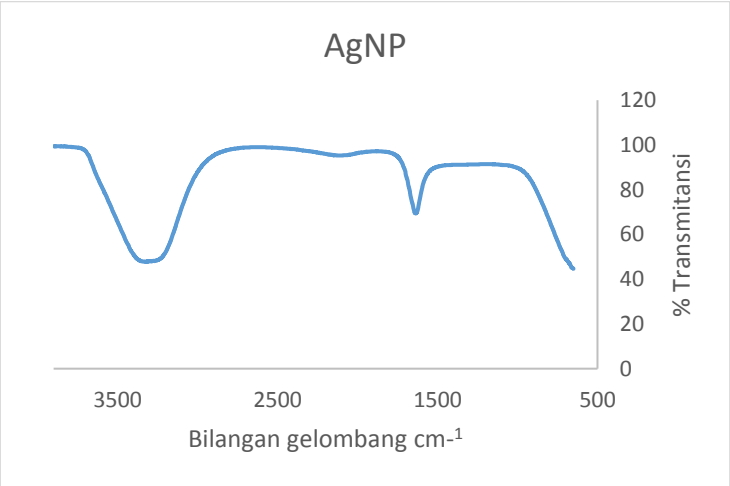


Figure 3. The results of AgNP characterization using FTIR

3.4. Observation of bleeding time test on test animals (mice)

In this study, test animals (mice) were modeled as hemophilia by intravenous injection of warfarin in mice via tails. The results obtained are as Figure 4.

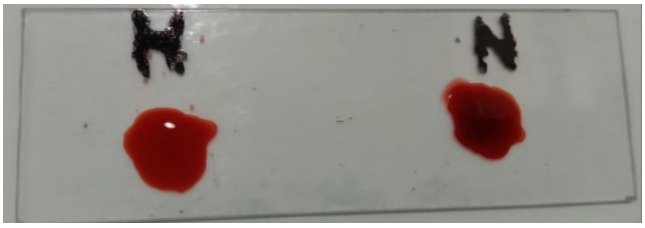


Figure 4. Blood color comparison of hemophilia and normal model animals

From the picture above shows that the test animals that have been injected with warfarin have relatively thinner blood and are red in color (blood with the H mark), while the normal test animals (without treatment) have thick and thick red blood (blood with an N sign). The bleeding time was then performed on the test animals using the Duke method adaptation. The results of bleeding time can be seen through Table 1.

Table 1. Hemophilia model animals.

Rat Condition	Treatment	Repeat			Average
		1	2	3	
Normal	Injured and left	181	156	167	168.0
Hemophilia Model	Injuredand left	242	342	278	287.3

* time in seconds

Based on Table 1 above shows that hemophilia model animals have a longer bleeding time compared to normal test animals. This is due to the presence of warfarin in hemophilia model animals which causes blood in the test animals to be runny and difficult to freeze.

4. Conclusion

Based on research and data analysis that has been done, it can be concluded as follows: - wound healing time for five days with marked scarring and no bleeding, - the most optimum formulation is formulation A which is with 5 ml of tea-tehan extract and 0 l AgNPs.

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