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THE IMPROVEMENT OF THE PHYSIOLOGICAL EFFECTS OF NANOHERBAL SIKKAM LEAVES (*Bischofia javanica*)

C.G.P. Rumahorbo[1], S. Ilyas[1,✉], S. Hutahaean[1], C.F. Zuhra[2], and P.C. Situmorang[1]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia, 20155

[2]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia, 20155

✉Corresponding Author: syafruddin6@usu.ac.id

ABSTRACT

Extracts of B. javanica have been used for years in traditional medicine and have been evaluated scientifically as well. Nano-sized drugs have a high ability to penetrate cells and can be manufactured without the use of heat. This study compared the physiological effect of nano herbal Sikkam leaves (NSL) to an ethanol extract of Sikkam leaves (EESL) in blood, kidney, liver, and urine. The NSL was created through high-energy milling. The chemical components of the NSL were determined using thin-layer chromatography. The 2,2-Diphenyl-1-picrylhydrazyl method was used to conduct antioxidant tests. By giving NSL and EESL at doses of 800, 1300, 2000, and 3200 mg/kg during 14 weeks, the mice's blood was collected for the hematological parameters, kidney and liver for the biochemical parameters, and urine for the electrolyte parameters. The diameter of the NSL was 188.8 nm ± 42, and the IC50 level was 28.24 µg/mL (very strong). NSL did not significantly change the number of blood cells, decreased the liver biochemical parameters, and decreased the number of electrolytes in urine better than EESL. NSL provides a better physiological effect compared to ethanol extract preparations.

Keywords: Nanoherbal, Bischofia Javanica, Kidney and Liver Biochemical, Hematology.

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INTRODUCTION

The side effects of herbal medicines are much smaller than synthetic drugs.[1] The issue with using herbal medicines is that the process is relatively lengthy due to the large size of the crude extract of herbal plants, which causes the penetration of the phytochemical compounds to be disrupted. In addition, the efficiency of herbal medicines is reduced due to their heating process. In Indonesia, medicinal plants are directly used by boiling the ingredients and then consuming the juice. Commercially grown medicinal plants are produced in factories with a heating process at each stage to reduce the water content and make them last longer. When the temperature was raised, the flavonoid content of plant extracts decreased.[2] Tannins, amyrins, betulinic acid, epifriedelanol, friedelan, luteolin, glucoside, quercetin, beta-sitosterol, stigmasterol, ursolic acid, chrysoberyl, fisetin, and triacontane have been isolated from Sikkam leaves.[3,4] Furthermore, Sikkam leaves have been tested for pharmacological activity as antiparasitic[5], anti-leukemic[6], antimicrobial[7], anti-inflammatory[8], anti-nociceptive[9], and anti-diabetic.[10,11] The safe dose of nano herbal Sikkam leaves is 1300 mg/kg. They increase the hepatocyte cell, decrease the necrosis cell, and decrease the parenchymal degeneration in the liver, lungs, and heart. The diameter of the tubules in the kidney increases in doses 800-2000 mg/kg, but the most effective is 1300 mg/kg.[12] The blood is part of the body that is directly affected by drug administration. As a transportation tool, blood will distribute the drug throughout the body. The liver is the first organ that reacts quickly to chemicals. Therefore, drug administration's positive and negative effects can be assessed from the hematological and hepatologic parameters. In addition, the residue of drug administration will be carried by the blood to the kidneys to be excreted through urine.

Therefore, our reason for assessing the parameters of changes in the body's metabolic pathways after administration of NSL to the liver, kidneys, and blood is an appropriate step to expand the reader's knowledge about the safe use of NSL as an advanced herbal medicine.

EXPERIMENTAL

NSL was produced based on patent S002022119511[13], and EESL was based on the instructions in the Indonesian Pharmacopoeia.[14] Forty-five mice were divided into nine groups of 5 mice each. Group C was the control, group N was given NSL (i.e., N1, N2, N3, N4), and Group E was given EESL (i.e., E1, E2, E3, E4) with the same dose. The NSL and EESL doses were given at a range of 800 (N1 and E1), 1300 (N2 and E2), 2000 (N3 and E3), and 3200 (N4 and E4) mg/kg during 14 weeks.[12] The presence of phytochemical compounds was confirmed using thin-layer chromatography. Ash levels, acid-insoluble ash content, water-soluble substance content, ethanol-soluble extracts, water content, and antioxidant testing through the 1,1-diphenyl-2-picryhydrazil method were assessed to determine the characterization of NSL and EESL based on the Indonesian herbal pharmacopoeia14. The hematological evaluations were measured using a hematology examiner (Brand: Mindray, Bc 2800). The Cobas 6000 analyzer was used to measure liver and serum biochemical parameters. The electrolyte parameters were measured using the Cobas b-221 system.[15,16]

RESULTS AND DISCUSSION

NSL and EESL Characterization

NSL resembled flour in appearance, as seen in Fig.-1A. A scanning electron microscopy examination at 5000 magnifications revealed that NSL looked like clots, as seen in Fig.-1B.

Table-1: Component and Emulsion Characterization of NSL and EESL

Components of NSL and EESL			Emulsion characterization test of NSL and EESL		
Components	Results		Parameters	Results	
	NSL	EESL		NSL%	EESL%
Flavonoids	+++	+++	Water content	7.81	6.65
Alkaloids	-	-	Water-soluble substance content	23.86	38.56
Steroids	+	++	Ethanol-soluble extracts	25.62	39.17
Glycosides	+	++	Ash levels	8.26	4.94
Saponins	++	++	Acid-insoluble ash	5.61	5.78
Tannins	++	++			

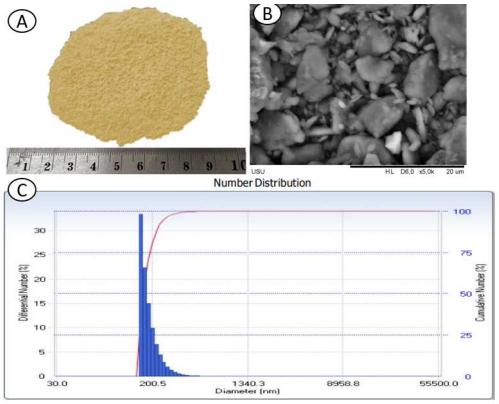

Fig.-1: (A) NSL Powder, (B) NSL In Scanning Electron Microscope (X5000), (C) Graph of NSL Particle Size Analysis Result

Based on the data obtained from the PSA test results, the grain size of Bischofia javanica before the nano process was 7084.8 nm, and after the nano process was 188.8 nm. The milling method reduced the size of Bischofia javanica leaves by 95.13%. In addition, the grinding process made the Bischofia javanica particle size more homogeneous, as indicated by the change in the PI value from 6.569 to 0.465, as seen in Fig.-1C. The characterization results in Table-1 revealed that NSL and EESL fulfilled the criteria and could be designed as drugs.[17,18,19] The antioxidant test was carried out using UV-visible spectrophotometry at 517 nm. IC50's NSL value was 28.24 g/mL (very strong), and EESL was 50.94 µg/mL (strong). In terms of antioxidative activity, NSL outperformed EESL. Based on those criteria, NSL was much more eligible to be produced as a developed medicinal herb in the upcoming years. It will be reinforced by comparing NSL

and EESL on mice simultaneously, as well as the results of parameters in blood, urine, kidneys, and liver, all of which are organs that respond quickly to chemical exposure.

Hematological Analysis

The variables calculated in the hematological analysis were Leucosyte (103/µL), erythrocyte (106/uL), Hb (g/dL), Hct (%), MCV (g/dL), MCH (g/dL), MCHC (g/dL), as seen in Fig.-2. The absence of significant changes in blood parameters after NSL administration was due to the exposure to phytochemicals in nano preparation that did not damage the balance of blood cell counts or other criteria for complete blood tests. All phytochemicals were metabolized entirely in the cells.

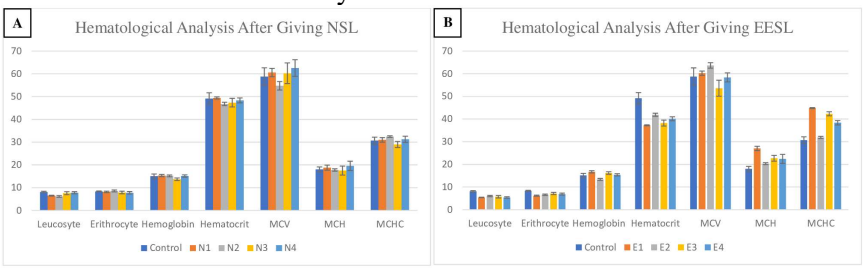

Fig.-2: Hematological Analysis Parameters

Sikkam leaves contain the active substance saponins, which can stimulate immunity.[20] Saponins can act as immunostimulatory by stimulating immune cells and increasing antibody formation.[21] Under stress conditions, there is a decrease in erythrocyte count, hematocrit, hemoglobin levels, and leukocyte.[22] Those situations occurred in the administration of EESL. Various sources of stress, including environmental factors such as temperature, light, and maintenance, as well as biological factors such as microorganism infection, will harm the physiology of mice. An increase in leukocytes indicates the body's ability to respond to infection or foreign objects is strong.[23] The high saponin concentration in nano herbal sikkam leaves can be used as a natural antibiotic. In this study, the increase was found in N4. Enhancement of leukocyte count can be caused by the active compounds contained in nano herbal sikkam leaves, such as saponins, which act as immunostimulants and can improve the immune system at a 3200 mg/kg dose.

Serum Biochemical Parameters

Normal creatinine levels in mice are 0.06–2.72 mg/dL, normal urea levels are <30 mg/dL and normal uric acid levels are 1.5–6.0 mg/dL.[24] In NSL, those parameters in each treatment group still showed normal levels. However, in EESL administration, the urea levels showed a significant difference compared to the standard group, as seen in Fig.-3. There was no impairment of kidney function with NSL administration instead of EESL. Also, the creatinine and uric acid parameters showed different results in the NSL and EESL administrations. While in the NSL group, all parameters showed no significant difference from the control, and the EESL group increased the level in all parameters.

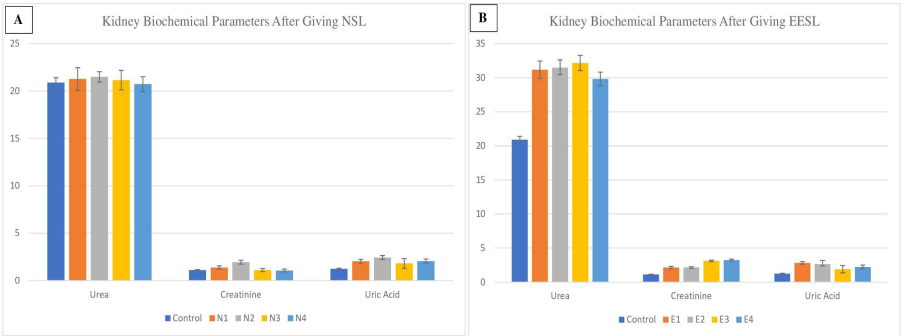

Fig.-3: Biochemical Kidney Parameters

The kidney's concentration of urea, blood urea nitrogen (BUN), creatinine, and ammonia are closely related to the food and drink consumed. In E1 and E2, elevated creatinine and uric acid levels were caused by impaired kidney function during the glomerular filtering process.[25] The large particle size of EESL caused the glomerulus to work slower than with NSL treatment, even though the results of the EESL measurement were not categorized as kidney failure.

Liver Biochemical Parameters

Evaluation of abnormal liver function tests is needed to ascertain whether the nano herbal sikkam leaves affect the liver through parameters such as Total protein (g/Dl), Albumin (g %), Bilirubin direct (mg/dL), SGOT (U/L), SGPT (U/L), ALP (U/L). All those parameters were assessed in blood drawn from all groups on day 14, as seen in Fig.-4. Total protein, SGOT, SGPT, and ALP parameters differed significantly ($p<0.05$) both in NSL and EESL.

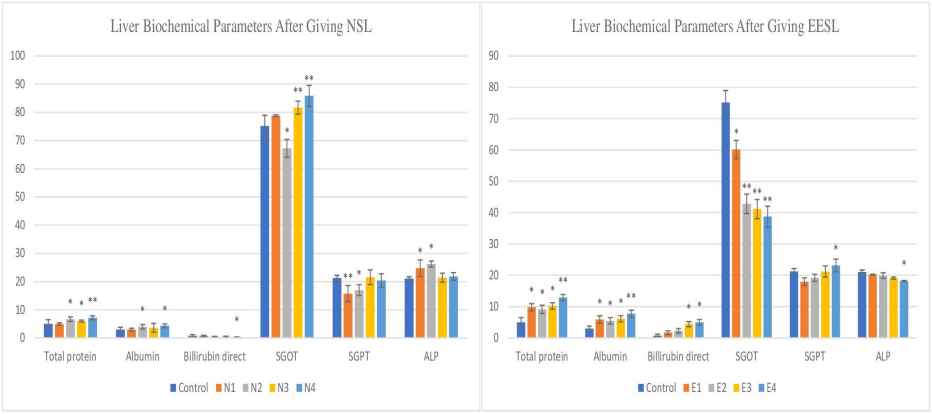

Fig.-4: Biochemical Liver Parameters

Total protein, albumin, bilirubin direct, and SGPT in the EESL administration were higher than in the NSL administration, while SGOT and ALP in the nano were higher in the NSL. The way these two herbal preparations work while entering the metabolic process still needs to be explored more specifically. Meanwhile, the suspicion that total protein, albumin, and direct bilirubin increased in the liver during the 14 days of EESL administration was due to the complicated metabolic pathway associated with its relatively large particle size. As a result of the increase in these three substances, the liver will be overworked, and AST will leak and enter the blood, which causes the AST protein content in the blood to be higher than usual. The increase in ALT on NSL administration was directly proportional to the increase in the number of hepatocytes in the liver with the same treatment as NSL.[12] ALT is an enzyme produced by liver hepatocytes in response to liver damage. The increase in the activity of the aminotransferase enzyme is caused by oxidative stress conditions, which cause an increase in production and an increase in the concentration of oxidants or free radicals. The increase in free radicals is excessive, and the number of cellular antioxidants remains constant or decreases, preventing antioxidants from facing free radical attacks.[26] All the parameters typically rise when there is liver damage. It is because the liver is a vital organ in the metabolism of compounds. Additionally, SGPT is more sensitive than SGOT to liver damage.[27] This study showed that the SGOT parameter increased dramatically in the N4 group compared to the control group. However, the SGPT parameter only increased in the N1 and N2 groups, as seen in Fig.-4. The chemical compounds from this plant, namely salidroside analogs, have been proven to have hepatoprotective properties.[8,28] Further testing is needed to determine the hepatoprotective properties of sikkam.

Electrolyte Parameters

Sodium, potassium, and chloride levels are used to identify and treat aldosteronism (excessive secretion), diabetes insipidus (severe excretion of large amounts of dilute urine affected by the hormone aldosterone), adrenal hypertension (followed by extreme thirst), Addison's disease (caused by the loss of the sodium channel in the brain), electrolyte imbalance, exaggerated antidiuretic hormone secretion, or other adrenal gland diseases.[29] All parameters showed a significant difference ($p<0.05$) in both the administrations of NSL and EESL, as seen in Fig.-5. If we compare the electrolyte in both NSL and EESL, it is higher in EESL than in NSL. B. javanica was high in calcium, phosphorus, potassium, sodium, iron, zinc, copper, and magnesium, according to the preliminary data from the previous study.[20,30] Although those components overflow in sikkam leaves, if our body's size and osmoregulatory system are out of balance, then all of those components would be excreted through the excretory system, like urine, as also happened with the

administration of EESL. The pattern of changes in the concentration of these electrolytes is also erratic. Electrolytes are needed by the body to keep the organs functioning normally. Some of the body's functions that are affected are heart rhythm, muscle contraction, and brain function. In this study, nano herbal sikkam leaves did not affect blood electrolyte levels. In addition, despite the plant's high mineral content, administering nano herbal sikkam leaves to test animals for 14 days had no significant effect on the animals' blood sodium and potassium levels.

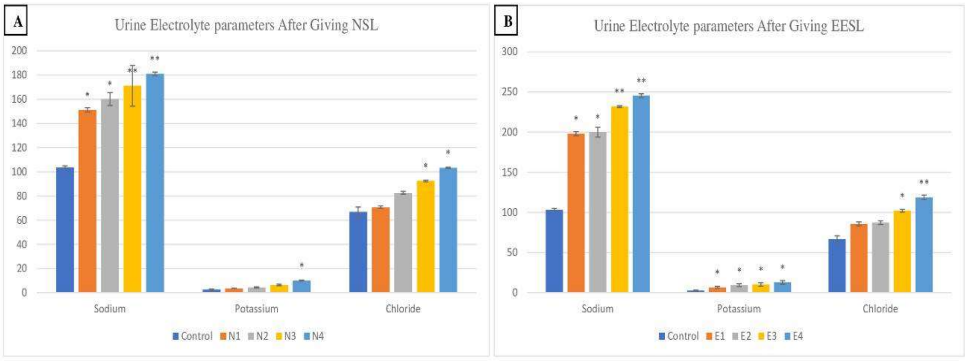

Fig.-5: Electrolyte Parameters(*Units: mmol)

This plant's nano-sized particles improve solubility, bioactivity, and consistency. They also improve therapeutic properties, increase tissue macrophages, and increase resistance to physical and chemical degradation.[31,32] Because of their nano-size and physical and chemical properties, they have a substantial benefit. Modifying the particle size of sikkam leaves to nano size will improve adhesion, allow nanoparticles to enter the cell, and improve the functioning of its quantum dots (QDs).[19] The out-layer property of herbs is critical to the drug's passage to the target cell receptor.[17-19] Based on the results of this study, NSL has more potential than EESL.

CONCLUSION

Modifying sikkam leaves to be nano-sized increases their antioxidant activity compared to ethanol extract preparations. Furthermore, sikkam leaves in nanoparticle form have a better physiological effect than ethanol extract preparations.

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CONFLICT OF INTERESTS

The authors confirm that no conflicts of interest and this research was thoroughly carried out with the same vision and mission and a close personal relationship between the authors.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

C.G.P. Rumahorbo https://orcid.org/0000-0002-9548-7418
S. Ilyas https://orcid.org/0000-0003-2760-2901
S. Hutahaean https://orcid.org/0000-0002-1490-3513
C.F. Zuhra https://orcid.org/0000-0002-5387-9085
P.C. Situmorang https://orcid.org/0000-0002-9895-1853

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