PHYSIOLOGICAL EFFECTS OF TURMERIC (Curcuma longa L) IN THE FORM OF NANO AND CRUDE EXTRACT

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ABSTRACT

Turmeric (Curcuma longa L.) is well-known as a medicinal plant with many physiological effects on the human body. This study compares the physiological effects of nano-extract and crude turmeric extract on blood, kidney, liver, and blood serum. The phytochemical was qualitatively screened using thin-layer chromatography. The antioxidant capacity was evaluated through the 2,2-diphenyl-1-picrylhydrazyl method. In this study, 45 Mus musculus were divided into nine groups, each comprising five Mus musculus. The first group was the control group to compare the group given the nano extract and the crude extract (group C). The other 40 musculus were divided into two large groups, where four groups were given nano extract with various doses (group N). The other four groups were given crude extract with varying quantities (Group E). The dose variation between the nano and the crude extract is the same. For example, N1 and E1 are given 500 mg/kg bw, and N2 and E2 are given 1000 mg/kg bw. Then up to N4 and E4 with an increase of 500 mg/kg bw. The results show that the nano-extract's inhibitory concentration antioxidant activity was 9.87 μg/mL, more than the ethanol extract (64.39 μg/mL). In addition, the nano-extract significantly increased the number of blood cells and improved the liver's physiology to an average level. The conclusion that can be drawn here is that the efficacy of nano extracts was better than crude extracts in improving the physiological effects.

Keywords: Hematology, Kidney, Liver, Nano-extract, Turmeric.

INTRODUCTION

It has been known that herbal medicine has fewer side effects than synthetic drugs. However, there are several drawbacks to using herbal medicines, for instance, the lengthy process of material handling and the particle size of the crude extract. The larger particle size may hinder the penetration of bioactive compounds into the target cells. In addition, the efficiency of herbal medicines decreases during their formulation due to the heating process. In Indonesia, the practical utilization of medicinal plants is generally accomplished by decoction. Commercial medicinal plants are processed in the industry at high temperatures in each stage to reduce water content and extend their shelf life as commercial drugs. An emerging phytochemical disruption results in low concentrations of flavonoids due to their instability during industrial processing. The main components of the essential oil of turmeric are curcuminoid groups, sesquiterpenes, phenylpropane turmerone groups, kurlon, kurkumol, atlanton, bisabolene, zingiberene, and humulene. The rhizome extraction also found several carbohydrates, minerals, and diferuloylmethane. Furthermore, turmeric has been tested for pharmacological activity to exert anti-inflammatory, anti-immunodeficiency, anti-cancer, anti-viral, anti-bacterial, anti-fungal, neuroprotective, anti-diarrhea, antioxidant, anti-carcinogenic, and anti-infective effects. The safe dose of turmeric extract is 800 mg/kg. This quantity may improve hepatocyte cell count, lower death cell count, degrade organ parenchyma, and strengthen renal diameter. The research findings above show that researchers focused on pharmaceutical effects. To the best of the authors' knowledge, no study has been conducted on the physiological effects of turmeric by evaluating the blood system. Blood circulation is directly affected when exposed to new substances because blood transports these substances throughout the body. The liver is the first organ to respond to recognition when a new substance enters. The liver releases enzymes to degrade these poison effects.
substances if it is considered poison. As a result, blood test results can be utilized to evaluate. Furthermore, the circulatory system will execute the drug residue to the kidneys and excrete it through urine. This study aims to assess the physiological effects of turmeric in the blood system on the liver, kidneys, and blood. Two types of turmeric extract will be used: a crude ethanolic extract of turmeric (EEKR) and the turmeric nano-extract (NKR). The results will supply information on promoting turmeric as an advanced herbal medicine.

EXPERIMENTAL

Turmeric Preparation and Assessment
2.5 kilograms of coarse turmeric flour was milled into nanoparticles utilizing a method known as high-energy milling (HEM) with a stimulant solution consisting of HCl 2 M (Tokyo, Japan). The turmeric nano-extract (NKR) size was measured using a particle size analyzer (PSA). The outer layer shape of NKR was investigated through a scanning electron microscope (SEM) with an enlargement of 5000. For ethanol extract of turmeric (EEKR), approximately 2 kg of coarse turmeric powder was macerated using the cold extraction method and screening of phytochemicals and proximate characteristics to test its feasibility.

Animals
In this study, 45 Mus musculus were divided into nine groups, each comprising five Mus musculus. The first group was the control group to compare the group given the nano extract and the crude extract (group C). The other 40 musculus were divided into two large groups, where four groups were given nano extract with various doses (group N). The other four groups were given crude extract with varying quantities (Group E). The dose variation between the nano and the crude extract is the same. For example, N1 and E1 are given 500 mg/kg bw, and N2 and E2 are given 1000 mg/kg bw. Then up to N4 and E4 with an increase of 500 mg/kg bw. The animal management and experiments were carried out by the ARRIVE principles (Animal Research: Reporting of In Vivo Experiments) and under the European Union Directive 2010/63/EU on the safeguarding of animals used for scientific research, therefore, The Animal Research Ethics Committee of Universitas Sumatera Utara has reviewed this study and recommended that it be approved (No. 0921/KEPH-FMIPA/2023, Jan 16, 2023).

Physiological Observations
The physiological observations are carried out using complete hematological analysis, liver biochemical test, kidney biochemical test, and electrolyte test. In the biochemical liver test, the examined parameters are total protein, albumin, bilirubin direct, Serum Glutamic Oxaloacetic Transaminase, Alkaline Phosphatase, Serum Glutamic Pyruvic Transaminase, and Serum Glutamic Pyruvic Transaminase Alkaline Phosphatase by using Cobas 6000 (Roche Diagnostic, Switzerland). In the kidney biochemical test, the urea, creatinine, and uric acid levels are measured in the blood. In addition, the sodium, chloride, and potassium levels are in the blood in the electrolyte test.

Statistical Analysis
The GraphPad Prism 9 software, version 26 (IBM, Chicago, IL, USA), is the tool for numerical, descriptive, and intervention analyses. To see if significant variations in physiological indicators existed, ANOVA was used. The data gathered was examined at a 95% confidence level, with significance defined as p less than 0.05. To ascertain what therapy was significantly different in comparison to the control, the difference with the lowest significance (LSD) test was used.

RESULTS AND DISCUSSION

Characteristics of EEKR and NKR
Figure-1 shows the physical appearance of the (A) turmeric powder and (B) SEM images at 5000 magnification power of nano-extract (NKR). Table-1 shows the result of phytochemical screening and emulsion characterization of EEKR and NKR. Figure-2 shows the distribution of particle size of turmeric for both (A) Crude Ethanolic extract and (B) Nano Extract. The particle size of EEKR is 1680.1 nm, and the NKR is 693.02 nm. The nano processing reduced the particle size of turmeric by an order of 58.74%. The antioxidant assessment was performed at the wavelength of 517 nm using ultraviolet (UV)-visible spectrophotometry. NKR's antioxidant activity (IC50) was 9.87 g/mL; on the other hand, the EEKR was 64.39 μg/mL, demonstrating that NKR has a more potent antioxidant property than EEKR.
Fig. 1: (A) Turmeric Powder and (B) SEM Image of NKR

Table 1: Phytochemical Screening and Proximate Characteristics of EEKR and NKR

<table>
<thead>
<tr>
<th>Components</th>
<th>NKR</th>
<th>EEKR</th>
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<tbody>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
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<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
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<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water content</td>
<td>2.0</td>
</tr>
<tr>
<td>Content of water-soluble substances</td>
<td>15.66</td>
</tr>
<tr>
<td>Extracts soluble in ethanol</td>
<td>13.38</td>
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<tr>
<td>Levels of ash</td>
<td>4.10</td>
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<tr>
<td>Insoluble in acid ash</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Figure 2 shows the distribution of particle size of turmeric for both (A) Crude Ethanolic extract and (B) Nano Extract. The diameter of the particles of EEKR is 1680.1 nm, as shown in the Figure. On the other hand, the NKR is 693.02 nm. This fact reveals that the nano processing reduced the particle size of turmeric by an order of 58.74%. The figure also shows that the milling process increases the polydispersity index from 0.262 to 0.69.

Kidney Biochemical Parameters

Figure 3 shows kidney biochemical profiles of the experimental results. The figure shows that the average Creatinine concentrations in mice range between 0.06-2.72 mg/dL, while the expected concentration of urea and uric acids are <30 mg/dL and 1.5–6.0 mg/dL, respectively. The kidneys are considered imbalanced when the creatinine concentration hits below or above the average concentration. There is an increase in the concentration of urea, creatinine, and uric acid compared to the standard group, but these values were still categorized as usual increases. These facts suggest that administering NKR and EEKR does not cause kidney damage. Urea in urine, BUN, creatinine, and ammonia levels in the renal system are closely linked to the food and fluids consumed. This could be due to turmeric metabolism in the cells, as evidenced by residues excreted through the kidneys.
Hematological Analysis

Figure-4 shows the hematological profile of experimental results from mice. A leukocyte count of 5-15 $10^3/\mu l$ is generally considered normal.\textsuperscript{22,23} It was found that both NKR and EEKR in all doses decreased the number of leukocytes (<15,000 $10^3/\mu L$), regarded as leukopenia.\textsuperscript{24} Based on the average hemoglobin count of 12–18 g/dL, both EEKR, and NKR did not cause thrombocytosis and thrombopenia, but EEKR increased the hemoglobin better than NKR. The performance of erythrocytes and hemoglobin is synergistic. This study found that the hematocrit volume also increased as the number of hemoglobin and erythrocytes increased.

![Fig.-3: Biochemical Kidney Results](image)

The average value of mean corpuscular volume (MCV) is 80–86 femtoliters (fL), while that of mean cell hemoglobin (MCH) is within the range of 27–31 picograms (pg).\textsuperscript{23} Therefore, the increase in MCV and MCH occurred following the administration of EEKR rather than NKR. The mean cell hemoglobin concentration (MCHC) test evaluates whether the erythrocyte is filled with the optimum hemoglobin concentration to indicate a good health condition.\textsuperscript{23}

![Fig.-4: Hematological Analysis Results](image)

Liver Biochemical Parameters

Figure-5 shows the biochemical parameters resulting from the experiments. The figure shows that the usual total protein concentrations range from 6.0 to 8.3 g/dL, albumin is 3.5 to 5.0 g/dL, and bilirubin is 0–0.4 mg/dL.\textsuperscript{25} As the dose of NKR and EEKR increased, the amount of total protein increased, contrary to the albumin concentration. The low albumin may be due to the poor absorption of nutrients.\textsuperscript{26} The mice in the group E4 treatment significantly reduced the decrease in albumin concentration. The bilirubin analysis shows that the higher EEKR and NKR doses, the higher the bilirubin concentration in the liver, but still within the normal range. Measurement of SGOT and SGPT values showed a fluctuating trend within each
treatment. The ALP concentration increased dose-dependent after the administration of EEKR and NKR. The increase in SGPT following NKR administration was inversely related to the increase in hepatocytes following the same NKR treatment. When there is liver damage, all parameters typically rise due to the liver's importance in compound metabolism. Turmeric and curcumin, two chemical compounds in this plant, have been shown to have hepatoprotective properties.26

Electrolyte Parameters
Figure-6 depicts the parameters of minerals. Potassium levels in the blood range from 3.6 to 5.3 mmol/L, while sodium levels average 137-146 mmol/L, and chloride levels range from 95 to 105 mmol/L.27 When the electrolyte concentrations were compared, it was evident that EEKR performed better than NKR. The body requires electrolytes for organs to function normally.

CONCLUSION
Two types of turmeric extracts, NKR and EEKR, have been examined and tested in the blood system of mice. The results show clear evidence of the benefit of using nano drugs based on their physicochemical properties and physiological effects. Turmeric particle size reduction to the nanoscale improves adhesion, facilitates the penetration of nanoparticles into cells, and enhances the function of the quantum dot structure.
Furthermore, the outer layer of drugs is critical to successfully diffusing drugs to the target cell receptor. The conclusion that can be drawn here is that NKR has more potential than EEKR.

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CONFLICT OF INTERESTS
The authors of this article claim that they have no competing interests.

AUTHOR CONTRIBUTIONS
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