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Protective Effect of Curcuma zedoria Against Copper in Rats' White Blood Cell

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Abstract

Copper, an essential trace element, is essential for the human body to maintain homeostatic balance. Higher levels of copper in the human body can cause damage to the liver, kidney, and brain due to increased generation of ROS (reactive oxygen species). As an alternative solution, natural resources which can be used to reduce the impact of copper excess is *Curcuma zedoria* due to the presence of curcumin. This study was aimed to explore the protective effect of *Curcuma zedoria* against copper in rats using the WBC as an indicator. This study was performed using 15 rats that were divided into 5 groups consisting of a negative group, a positive group, and three experimental groups which were given ethanol extract of *Curcuma zedoria* in various dosage. Each group of rats was fed 0.36 mg/kg b.w of $CuSO_4$ solution on the 10^{th} to 14^{th} day of the experiment. The results showed that the level of band neutrophil, lymphocyte, and monocyte increased following the reduction of the dosage of *Curcuma Zedoria* that given to the rats. However, the levels of monocyte in each group were within normal range except the negative group. Based on the higher levels of WBC, all of the experiment groups suffered chronic active inflammation. *Curcuma zedoria* had a protective effect against overexposure to copper in rats, but it didn't completely protect the rats from the effects of overexposure.

Keywords: Curcuma zedoria; copper; CuSO4; curcumin; WBC .

1. Introduction

Environmental pollution in the world is the focus on environmental problems. Some heavy metals which are considered as major pollutants include lead, mercury, arsenic, aluminum, copper, nickel, tin, antimony, bromine, bismuth, and vanadium [1].

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Studies in many countries have shown that many sources of water have been polluted by high levels of metal. For instance, a study in Adelaide showed that rainwater collected in the sample tank had high levels of lead, zinc, cadmium, and copper. Similarly, the Ganga River in India had high levels of Ferrum, zinc, lead, nickel, manganese, and copper at various collection points. Likewise, some studies in Indonesia have indicated high levels of metal pollution in different bodies of water; for example, the Bone and Citarum River were contaminated [2].Copper is an essential trace element; nevertheless, the amount of copper need to maintain homeostasis of the body remains unclear. Several studies have shown that the normal range of Cu Intake value is around 0.8 mg/day-2..4mg/day. While the impact copper excess in the body is still unclear, several studies have shown that there is no relationship between high Cu levels and the risk of diseases such as cardiovascular disease, cognitive decline, arthritis, or cancer [3]. There were several studies investigated the toxic effects of copper. A report by Bugata and his colleagues (2019) showed that acute and sub-acute dosage of CuO-NP (copper oxide nanoparticle) caused damage in the liver, kidney, and brain due to increased generation of ROS [4]. Based on the studies above, copper is a pollutant that is harmful to various organs. As an alternative solution, several natural resources can be used to reduce the impact of copper. One of the natural resources which have potential protection against heavy metal pollutant due to the presence of curcumin as a bioactive compound is *Curcuma zedoria*. Some of the curcumin functions include anti-inflammatory, antioxidant, and inhibitor of cancer cell growth [5,6]. This study was aimed to explore the protective effect of *Curcuma zedoria* against copper in rats using the WBC as an indicator.

2. Methods

2.1. Material

Curcuma zedoria, ethanol 96%, aquadest, $CuSO_4.5H_2O$ (Cupric Sulfate pentahydrate), Giemsa, EDTA (*Ethylenediamine Tetraacetic Acid*), Buffer phosphate (pH 6.4), methanol

2.2. Preparation of Sample

Curcuma zedoria was collected from UPT Materia Medica Batu, Jawa Timur. It was cleaned, dried, and meshed to get the fine ingredients. Then the ingredients were macerated using ethanol 96% as a solvent; every 24 hours it was filtered, and the filtrate was collected. Then the residue was macerated again. This was repeated for 3 days. All of the filtrates were evaporated, using a rotary evaporator at 50^oC, until the filtrates became concentrated.

2.3. Study Design (Treatment)

Male Wistar rats were used in this study. They were housed in separate cages according to their treatment group. In this study, there were 15 rats which were divided into 5 groups. These groups are shown in the table below.

Table 1: Study Design

Group	**Treatment**
Negative	Feed normally 0.36 g/kg b.w of $CuSO_4.5H_2O$ solution in 10^{th}-14^{th} day
Positive	Feed normally 45 mg/kg b.w of ethanol extract of *Curcuma zedoria* once a day for 14 days
Ethanol Extract of *Curcuma zedoria*-I	Feed normally 45 mg/kg b.w of ethanol extract of *Curcuma zedoria* once a day for 14 days 0.36 g/kg b.w of $CuSO_4.5H_2O$ solution in 10^{th}-14^{th} day
Ethanol Extract of *Curcuma zedoria*-II	Feed normally 67.5 mg/ kg b.w of ethanol extract of *Curcuma zedoria* once a day for 14 days 0.36 g/ kg b.w of $CuSO_4.5H_2O$ solution in 10^{th}-14^{th} day
Ethanol Extract of *Curcuma zedoria*-III	Feed normally 90 mg/ kg b.w of ethanol extract of *Curcuma zedoria* once a day for 14 days 0.36 g/ kg b.w of $CuSO_4.5H_2O$ solution in 10^{th}-14^{th} day

2.4. Hematologic Analysis.

The blood sample was analyzed using the improved Neubauer chamber counting method. Peripheral blood smear was made and stained with Giemsa (5 drops of Giemsa in 1 ml of buffer phosphate). The number of each type of WBC was then expressed in percent.

2.5. Data Analysis

The number of each type of WBC was expressed in Mean ± SD as percentages (%) and analyzed by One Way ANOVA. Furthermore, the analysis was followed by Tukey HSD as post hoc test. The confidence level of these analyses was 95% ($\alpha = 0.05$).

3. Result

The number of WBC for each group is shown in figure 1, and the analysis for each type of WBC is shown in table 2 below.

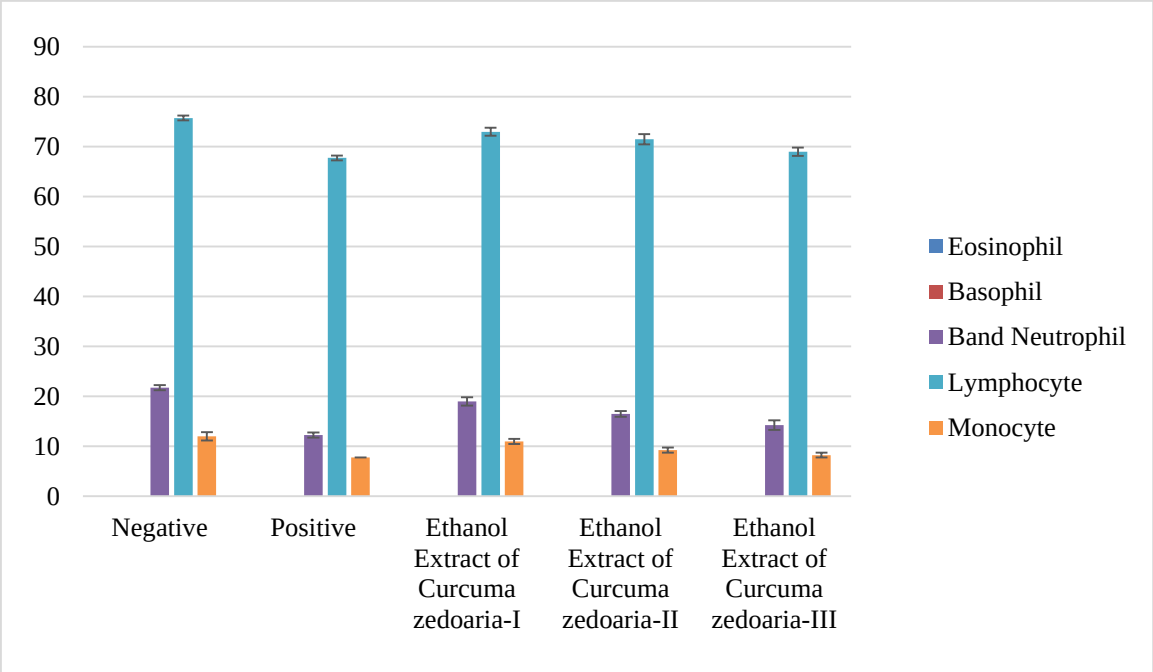

Figure 1: Comparison Level of Rats' WBC

Table 2: Analysis of Tukey HSD for Each type of WBC

Group	**Type of WBC Level (%) [Mean ± SD]**				
	Eosinophil	**Basophil**	**Band Neutrophil**	**Lymphocyte**	**Monocyte**
Negative	0	0	21.75 ± 0.50^{a}	75.75 ± 0.50^{a}	12.00 ± 0.82^{a}
Positive	0	0	12.25 ± 0.50^{b}	67.75 ± 0.50^{b}	7.75 ± 0.50^{b}
Ethanol Extract of *Curcuma zedoria*-I	0	0	19.00 ± 0.82^{c}	73.00 ± 0.82^{c}	11.00 ± 0.00^{a}
Ethanol Extract of *Curcuma zedoria*-II	0	0	16.50 ± 0.58^{d}	71.50 ± 1.00^{d}	9.25 ± 0.50^{c}
Ethanol Extract of *Curcuma zedoria*-III	0	0	14.25 ± 0.96^{e}	69.00 ± 0.82^{b}	8.25 ± 0.50^{b}

Data were presented as mean ± standard deviation. Different small letters in the same column are significant at $P < 0.05$.

Based on the table above, no basophil or eosinophil was found in the blood samples of the rats, but other types of WBC were present. The negative group had the highest level of band neutrophil, lymphocyte, and monocyte in the blood sample. On the other hand, the positive group had the lowest level of those three types of WBC. According to the analysis performed by the Tukey HSD test, there was a statistically significant difference between the positive group and treatment groups in the level of band neutrophil and lymphocyte in the samples. Similarly, there was a statistically significant difference between the positive group and Ethanol Extract of *Curcuma zedoria*-I and II in the level of monocyte.

4. Discussion

Copper is an essential micronutrient and co-factor for a wide range of enzymes. The balance of this micronutrient is tightly regulated because higher serum levels of copper can trigger oxidative stress which activates inflammatory responses [7]. Inflammation is a local response of living mammalian tissues involving host cells, blood vessels, and protein; it aims to eliminate the cause of cell injury like necrotic cells and to initiate the repairing process [8,9]. One cause of inflammation is chemical agents. These agents will alter the membrane permeability, osmotic homeostasis, or the integrity of an enzyme or co-factor [9]. Based on the defense capacity of the host and duration of the response, inflammation can be divided into acute and chronic inflammation. Acute inflammation is a short-term inflammation (lasting less than 2 weeks) which resolves quickly and is usually followed by healing. The main features of acute inflammation are the accumulation of fluid and plasma at the affected site, intravascular activation of platelets, and polymorphous nucleated neutrophil as the main type of inflammatory cells. If the causative agent for acute inflammation persists for a longer period, it can cause chronic inflammation. The main features of chronic inflammation are the presence of chronic inflammatory cells such as lymphocyte, plasma cells, and macrophages, granulation tissue formation, and in a specific situation as granulomatous inflammation. As a variant of chronic inflammation, there is chronic active inflammation, acute exacerbation of chronic inflammation[8]. Leucocytes or white blood cells migrate from blood vessels to tissues to perform the various activity. There are 2 types of leucocyte based on its cytoplasmic granules and the shape of its nuclei—polymorphonuclear granulocytes and mononuclear agranulocytes [10]. Granulocytes possess 2 types of granules include specific granules and azurophilic granules. Neutrophil as an inflammatory cell during acute inflammation contain specific granules like alkaline phosphate, collagenase, lactoferrin, lysozyme, and several non-enzymatic antibacterial basic proteins. Conversely, the azurophilic granules contain compounds such as acid phosphatase, α-Mannosidase, arylsulfatase, β-galactosidase, β-glucuronidase, cathepsin, 5'-Nucleotidase, elastase, collagenase, myeloperoxidase, lysozyme, and defensins. Other types of granulocytes are eosinophil, and basophil [10]. Lymphocytes and monocytes act as inflammatory cells in chronic inflammation and are mononuclear agranulocytes. There are 3 types of lymphocyte which can be classified based on their size, morphology, and function. These are T lymphocyte, B lymphocyte, and NK Cells. T lymphocyte has a role in cell-mediated immunity, B lymphocyte has a role in humoral immunity, and NK Cells kill virus-infected and tumor cells. On the other hand, monocytes transform into macrophages at the site of inflammation, which phagocytes bacteria, other cells, and tissue debris. Furthermore, the monocytes act as the antigen-presenting cell (APC) which has an important role in cellular immunity [8,11]. Table 3 shows the normal range for each type of leucocytes.

Table 3: Normal Range for Each Type of WBC [12]

Type of Leucocytes	**Normal Range**
Basophil	0% -1%
Eosinophil	0% - 5%
Neutrophilic Band	0% - 5%
Neutrophilic segmented	50%-70%
Monocyte	3% - 11%
Lymphocyte	20%-40%

In this study, the positive group had higher levels of neutrophil and lymphocyte than normal; however, the monocyte is within the normal range. Therefore, the positive group had higher levels of chronic active inflammation because they had elevated levels of neutrophils and lymphocyte; nevertheless, normal levels of monocyte indicate that there may be none of the scavenging activity in level tissue. The treatment groups which were given the ethanol extract of *Curcuma zedoria* had a similar number of leucocyte, but the group with increasingly higher dosages had fewer leucocytes. Due to the high level of lymphocyte in positive and treatment groups, this indicates that the ethanol extract of *Curcuma zedoria* has the potential to become toxic in chronic usage. This is consistence with the study conducted by Ongko and his colleagues (2019) who found *that Curcuma zedoria* had a toxic effect on testis of male Wistar rat which shown by the reduction of spermatogenic cell layers and mitosis [13]. There were significant differences observed between the treatment groups and the negative group which indicates that *Curcuma zedoria* did protect the rats against the effect of excess copper exposure. However, it didn't completely protect the rats against the excess of copper exposure as shown by an increased neutrophil level in all groups except the negative group.

5. Conclusion

Ethanol extract of *Curcuma zedoria* had a protective effect against overexposure to copper, but it didn't protect the rats completely from the effects of overexposure.

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