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MORINGA OLEIFERA EXTRACT CAN INHIBIT GLOMERULUS DAMAGE OF RATTUS NOVERGICUS INJECTED CYCLOPHOSPHAMIDE

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Abstrak

Cyclophosphamide merupakan sitostatika yang digunakan untuk kemoterapi. Cyclophosphamide memiliki efek toksisitas yang menyebabkan kerusakan pada ginjal. Moringa oleifera merupakan tanaman dengan aktivitas antioksidan tinggi. Kandungan antioksidannya berupa flavonoid dan vitamin C yang dapat menghambat kerusakan glomerulus. Banyak penelitian yang telah membuktikan bahwa Moringa oleifera mengandung aktivitas antioksidan yang tinggi. Namun, belum ada penelitian tentang potensi Moringa oleifera dalam menghambat kerusakan glomerulus yang diinjeksi cyclophosphamide. Tujuan penelitian ini untuk menganalisis potensi Moringa oleifera dalam menghambat kerusakan glomerulus Rattus novergicus akibat injeksi cyclophosphamide. Studi eksperimental laboratorium dengan desain post test only control group design. Penelitian ini menggunakan 18 ekor Rattus norvegicus sebagai sampel yang dibagi menjadi 3 kelompok sampel, yaitu K1 adalah kelompok yang hanya diberikan NaCl, K2 adalah kelompok yang diberi NaCl dan diinjeksi cyclophosphamide, dan kelompok perlakuan adalah kelompok yang diberi ekstrak Moringa oleifera. Preparat histopatologi glomerulus dilakukan pewarnaan Hematoksilin Eosin. Data dianalisis secara statistik dengan uji Kruskall Wallis dan Mann-Whitney.Uji Mann-Whitney menunjukkan perbedaan yang signifikan antara kelompok K1 dan K2 dan Kelompok P dan K2 ($p<0.05$). Tidak terdapat perbedaan yang signifikan diantara K1 dan P ($p>0.05$) Pemberian ekstrak Moringa oleifera dapat menghambat kerusakan glomerulus Rattus novergicus yang diinjeksi cyclophosphamide.

Kata Kunci: Cyclophosphamide, kerusakan glomerulus, Moringa oleifera

Abstract

Cyclophosphamide is a cytostatic used in chemotherapy. Cyclophosphamide has a toxic effect that causes damage to the kidneys. Moringa oleifera is a plant with high antioxidant activity. Its antioxidant content in the form of flavonoids and vitamin C which can inhibit glomerulus damage. Many studies have proven that Moringa oleifera contains high antioxidant activity. However, there has been no research about the potential of Moringa oleifera in inhibiting glomerulus damage that was injected with cyclophosphamide. The purpose of this study was to determine the potential of Moringa oleifera in inhibiting glomerulus damage in Rattus novergicus due to cyclophosphamide injection. This was a laboratory experiment with a post-test only control group design. This study used 18 Rattus norvegicus as samples which was divided into 3 sample groups namely K1 is the group that was only given NaCl, K2 was the group that was given NaCl and injected by cyclophosphamide, and the treatment group was given Moringa oleifera extract. Glomerulus histopathological preparations were stained with Hematoxylin Eosin. Data were analyzed statistically by the Kruskall Wallis and Mann-Whitney tests. The Mann-Whitney test showed significant difference between groups K1 and K2 and groups of P and K2 ($p<0.05$). There were no significant differences between K1 and P ($p>0.05$). Giving Moringa oleifera extract can inhibit the glomerular damage of Rattus novergicus, which is injected with cyclophosphamide.

Keywords: Cyclophosphamide, glomerulus damage, Moringa oleifera

1. INTRODUCTION

Cancer is still a serious health problem in Indonesia. The Ministry of Health (2022) states that the prevalence of cancer in Indonesia is expected to continue to increase. According to the 2018 Basic Health Research (Riskesdas), the prevalence of cancer in Indonesia reached 1.79 per 1000 population, up from 2013's figure of 1.4 per 1000 population. Chemotherapy is one way to treat cancer in general. Chemotherapy can be used as an adjuvant or neoadjuvant treatment to kill cancer cells with anti-cancer drugs called cytostatics. The cytostatic drug that is often used is cyclophosphamide (Aruan et al., 2015).

Cyclophosphamide is the most widely used anticancer agent in chemotherapy. Among the 1,000 compounds selected and the antibiotics tested on 33 tumors, cyclophosphamide was the most effective alkylating agent. Cytostatic drugs such as cyclophosphamide are used to kill cancer, but can inhibit normal cell division, which proliferates rapidly so that it can cause toxicity. Cyclophosphamide has a toxic effect in the form of multiorgan damage, which results in severe morbidity and can even be potentially fatal (Faisel, 2012; Widodo et al., 2020; Murti, 2022). Research conducted by Tohamy et al (2021) states that the dose of cyclophosphamide used in chemotherapy can cause kidney damage. Mesna was given as the first injection during cyclophosphamide chemotherapy and again 2-6 hours later in order to reduce the effects of ifosfamide toxicity during chemotherapy, according to Reddy and Winston (2021) Giving cyclophosphamide can induce the formation of free radicals, which cause a decrease in the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), resulting in oxidative stress (El-sheikh dan Rifaai, 2014; Liu et al., 2012). Oxidative stress occurs due to an imbalance between oxidants and antioxidants and antioxidants which play a role in organ damage, so the body needs antioxidants to prevent oxidative stress (Werdhasari, 2014). One of the plants that contain antioxidants is *Moringa oleifera* (Gimenis et al., 2018).

Moringa oleifera is a plant with high antioxidant activity, the most dominant antioxidant contents in its leaves are flavonoids and vitamin C (Fitriana et al., 2016). The content of flavonoids has antioxidant activity, which can inhibit free radicals by capturing ROS, so that ROS in the body is not excessive so as to prevent oxidative stress (Parwata, 2016). The content of vitamin C in *Moringa oleifera* acts by donating electrons, so that it can prevent the formation of other compounds from the oxidation process by releasing a chain of electrons, and vitamin C will be oxidized into a relatively stable ascorbic radical. The reaction which was initially reactive became non-reactive (Razis et al., 2014). Research by Gianosa (2020) has proven that administration of *Moringa oleifera* extract can provide repair of glomerular damage in male Wistar rats with steptozotocin 45 mg/kgbb induced diabetes mellitus.

Many studies have been conducted to determine the antioxidant properties of *Moringa* leaves, but no research has been conducted on the potential of *Moringa* leaf extract against glomeruli damage caused by cyclophosphamide injection. Based on this background, the researchers were interested in conducting research to prove the potential of giving *Moringa oleifera L* extract against damage to the glomerulus of *Rattus novergicus*, which was injected with cyclophosphamide.

2. RESEARCH METHOD

This study is a laboratory experiment with 18 male Wistar strain Wistar rats that meet the inclusion criteria and are in healthy conditions characterized by shiny fur, active movements, and no injuries. They are 2-3 months old, with an average rat body weight of 200 g, and were adapted for 1 week. There were three groups, which were divided into control group 1 (K1), which was given 0.9% NaCl on days 8-14, then on day 15 given 0.9% NaCl, and after 5 hours they were given 0.9% NaCl again. Control group 2 (K2) was given 0.9% NaCl on days 8–14; on day 15, they were given 0.9% NaCl; 1 hour after that, they were injected with cyclophosphamide; and after 4 hours, they were given 0.9% NaCl again. The treatment group (P) was given 0.9% NaCl on days 8–14; on day 15, they were given *Moringa oleifera* leaf extract; 1 hour later, they were injected with cyclophosphamide; and 4 hours after that, they were given *Moringa oleifera* leaf extract again. On the 16th day, each group underwent kidney organ harvesting. Furthermore, the rats were prepared and stained with hematoxylin and eosin (HE). Glomerular histopathological preparations were observed using a Nikon Eclipse E200 light microscope and NIS-Elements software. The data obtained were analyzed by the Shapiro-Wilk test and the Levene test. After that, the Kruskall-Wallis test was followed by the Mann-Whitney test.

3. RESULTS

Damage to the glomerulus of *Rattus novergicus* was analyzed using a Nikon Eclipse E200 light microscope and NIS-Elements D software. The data obtained from the research results were analyzed using the Statistical Package for the Social Sciences (SPSS).

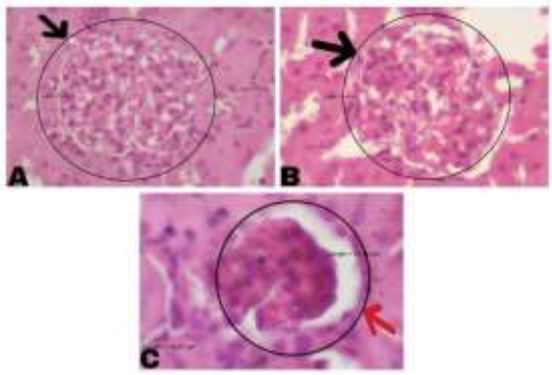

Figure 1. Microscopic view of the glomeruli of the K1, K2, and treatment groups (HE staining, 400x magnification)

Information :
The red color indicates atrophic glomeruli. The black color indicates a normal glomerulus.
A. Normal glomerular appearance (black arrow) in group K1; B. Normal glomerular appearance (black arrow) in the treatment group; and C. Features of glomerular atrophy (red arrow) in groupK2.

The group receiving cyclophosphamide found that there was shrinkage (atrophy) in the glomerulus so that Bowman's space appeared to have widened, as shown by the red arrow in Figure 1. Meanwhile, the glomeruli in groups K1 (NaCl 0.9%) and P (*Moringa oleifera* + cyclophosphamide) appeared normal.

Table 1. Mean ± SD and p-value of glomerular diameter in the control 1, 2 and treatment groups. The different superscript letters are statistically significant (Mann-Whitney, $p<0.05$)

Group		Mean ± SD	P value
K1	K2 glomerular diameter[a]	41.72 ± 10.68	0.000
	P glomerular diameter		0.467
K2	K1 glomerular diameter[b]	28.1± 7.05	0.000
	P glomerular diameter[C]		0.001
P	K1 glomerular diameter	37.98 ± 6.23	0.467
	K2 glomerular diameter[d]		0.001

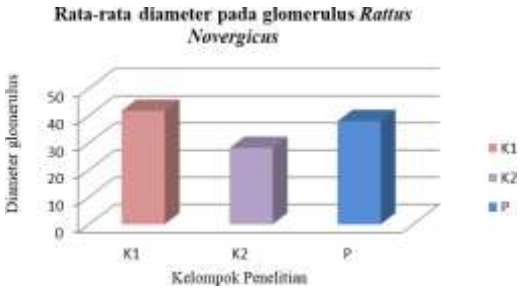

Figure 2. Comparison of the average glomerular diameter in *Rattus Novergicus* for each group

Table 1 shows the average glomerular diameter of groups K1, K2, and P. Based on the results of the study, it was found that the average glomerular diameter in group K1 was 41.72, group K2 had an average diameter of 28.1, and group P was 37.98. Figure 2 shows that group K1 has the highest average diameter and group K2 has the lowest average diameter.

The research data were analyzed using the Shapiro-Wilk test to obtain normally distributed data. The normality requirement is that the probability value be greater than 0.05 ($p > 0.05$). The results of the Shapiro-Wilk test in this study showed a probability of 0.020 in group K1, 0.319 in group K2, and 0.089 in group P, so that it can be concluded that the data in group K1 were not normally distributed because they did not meet the normality requirements ($p< 0.05$). Furthermore, the homogeneity test was carried out with the Levene test. Data is said to be homogeneous if the probability value is greater than 0.05. The results of the Levene test data in this study were 0.029. This means that the data between groups K1, K2, and P has a variant that is not homogeneous.

The hypothesis tests in this study used the Kruskall-Wallis and Mann-Whitney tests. This is because the normality and homogeneity tests contain data that does not meet the assumptions of normality. The hypothesis tested in this study was that the administration of *Moringa oleifera* leaf extract could inhibit damage to the glomerulus of *Rattus novergicus*, which was injected with cyclophosphamide. The basis for decision-making is determined by looking at the probability value (0.05). If the probability value is less than 0.05 ($p<0.05$), then the hypothesis is accepted. The probability value of the Kruskall-Wallis test in this study was 0.000 ($p<0.05$), so it can be concluded that the hypothesis is accepted or that administration of *Moringa oleifera* extract can inhibit damage to theglomerulus of *Rattus novergicus*, which was injected with cyclophosphamide. Then the Mann-Whitney test was carried out to compare whether there was asignificant difference in each group. Testing the data using Mann-Whitney (table 3) shows that between groups K1 and K2, there is a significant difference with a probability value of 0.000, and between group K2 and group P, there is a significant difference with a probability value of 0.001. Whereas in the P and K1 groups, there was no significant difference with a probability value of 0.467.

4. DISCUSSION

Cyclophosphamide is an alkylator in the nitrogen mustard group that causes alkylation in DNA, thereby inhibiting DNA synthesis and function in DNA chains (Ketaren, 2016). Cyclophophamide, which is used to kill cancer cells, not only kills cancer cells but also causes nephrotoxicity, resulting in kidney damage (Brunton et al., 2011; Tohamy et al., 2021). Because cyclophosphamide can reduce the formation of free radicals, causing a decrease in the activity of antioxidant enzymes, oxidative stress occurs (El- sheikh dan Rifaai, 2014; Liu et al., 2012). Oxidative stress caused by free radicals in the form of reactive oxygen

species (ROS) contributes to organ damage (Werdhasari, 2014). Glomerular damage is characterized by shrinkage of the glomerulus, which causes a widening of the distance between the two walls of Bowman's capsule (Saputra, 2019).

Group K1 is the group with 0.9% NaCl administration. This group had the highest average diameter measurement compared to the other groups. This illustrates that the glomerulus is in normal condition. 0.9% NaCl solution is a liquid used to replace lost body fluids and is a sanction for electrolyte poisoning. The kidneys are vital organs for the process of excretion in the filtration of substances originating from the bloodstream such as potassium, sodium, amino acids, glucose, and air that the body needs. Based on the results of these studies, it appears that the function of kidney filtration in the glomerulus is still running normally.

The group that received 0.9% NaCl and cyclophosphamide injection had the smallest average glomerular diameter.This illustrates that there is damage to the glomerulus. In the histopathological picture, there is inflammation (edema) in the glomerulus. Inflammation of the glomerulus can occur due to an increase in capillarypermeability, so that the glomerular capillaries become permeable to protein. This increase in glomerular permeability then causes the glomerular filtration load to increase and eventually becomes inflammation (edema). One sign of glomerular edema is atrophy or shrinkage of the glomerular capillaries, so thatBowman's spaces appear enlarged (Yuniarti et al., 2015).

Damage to the glomerulus is caused by the activity of cyclophosphamide, which has a toxiceffect and causes damage to the kidneys (Tohamy et al., 2021). The toxic effect of cyclophosphamide is caused by the ingredients contained therein, such as phosphoramide and acrolein metabolites, which can induce the formation of free radicals in the form of reactive oxygen species (ROS). The continuous production of ROS in the body causes the activity of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione S-transferase to decrease due to efforts to neutralize excess free radicals (El-sheikh dan Rifaai, 2014). ROS production will exceed antioxidants in the body, resulting in an imbalance. The relationship between the production of free radicals and antioxidants in the body is called "oxidative stress". Excess reactive oxygen species (ROS) in the body will attack the lipid, protein, and DNA components, causing damage. Oxidative stress will stimulate an increase in lipid peroxidation, whichwill decompose the end product in the form of malondialdehyde (MDA). Increased MDA levels indicate that there is damage to the organ. Murti research (2022) states that cyclophosphamide has a toxic effect in the form of multiorgan damage, andone of the toxicities that can be triggered is nephrotoxicity.

The group that received Moringa oleifera extract (MO) and cyclophosphamide injection had a larger mean glomerular diameter than the group that received only cyclophosphamide injection.This illustrates that the damage to the glomerulus is minimal. Administration of Moringa oleifera extract 1 hour before cyclophosphamide injection and 4 hours afterward assumes that administration of Moringa oleifera as an antioxidant is effective in protecting and repairing damage to the glomerulus. supported by Widowati research (2014), which said that MO can repair and protect cells from damage. It is suspected that giving MO can be used as a prophylactic drug to reduce the occurrence of ifosfamide (high doses of

cyclophosphamide). This is supported by Reddy and Winston research(2021) on the prevention of ifosfamide by giving Mesna 1 hour before and 2-6 hours after cyclophosphamide chemotherapy. Administration of mesna is useful as a prophylactic drug in patients receiving chemotherapy with cyclophosphamide (Reddy dan Winston, 2021).

It is assumed that giving MO has a similar role to giving Mesna, but giving Mesna has side effects such as nausea, vomiting, abdominal pain, and hypersensitivity reactions such as rashes. *Moringa oleifera*, as an antioxidant, is able to counteract free radicals. The dominant antioxidants in MO leaves are flavonoids and vitamin C. The content of flavonoids has antioxidant activity that can inhibit free radicals by capturing ROS, thereby preventing oxidative stress, regenerating damaged tissue, andinhibiting inflammatory processes (Parwata, 2016; Syahrin et al., 2016).

The content of vitamin C in *Moringa oleifera* acts as an antioxidant by donating electrons, considering that free radicals have unpaired and reactive electrons, so that vitamin C can protect important biomolecules (proteins, lipids, proteins, and DNA) from damage by oxidants originating from exposure. chemicals that trigger toxic effects (Murti, 2022). Gianosa (2020) demonstrated that administration of *Moringa oleifera* could improve glomerular damage in male Wistar rats induced by streptozotocin.

5. CONCLUSIONS AND SUGGESTIONS

The conclusion of the research results is that the administration of *Moringa oleifera* extract can inhibit damage to the glomerulus of *Rattus novergicus*, which is injected with cyclophosphamide. It is hoped that more research will be conducted on the development of *Moringa oleifera* to serve as a prophylactic drug in cyclophosphamide injection, similar to Mesna.

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