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EFFECTIVITY OF KELOR LEAF (Moringa oleifera L.) EXTRACT ON TUMOR NECROSIS FACTOR ALPHA (TNF-α) AND INTERLEUKIN 10 (Il-10) LEVELS IN CYCLOPHOSPHAMIDE-INDUCED CYSTITIS: AN EXPERIMENTAL STUDY

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ABSTRACT

Hemorrhagic cystitis is often associated as an effect of cyclophosphamide (CP) usage in the treatment regimens of malignant and non-malignant diseases. Indonesia has various medicinal plants that can be used as therapeutic developments, one of which is Moringa (*Moringa oleifera L.*). Moringa leaf is part of the plant that is mostly used and contains flavonoids that function as antioxidants. The purpose of this study was to prove the prevention effect of Moringa leaf extract administration on TNF-α and IL-10 levels in hemorrhagic cystitis after CP administration. The research used post-test control only group design. Six-month-old Wistar rats (*Rattus norvegicus*) with a body weight of 150-200 grams, were divided into 3 groups. Control group 1 (K1) was only given 0.9% NaCl orally during the study period, Control group 2 (K2) was given 0.9% NaCl orally and CP, Treatment group (P) was given Moringa leaf extract at a dose of 500 mg/kgBW orally and CP. Each oral doses were given for 7 days before, 1 hour before, and 4 hours after injection of single dose CP at 200 mg/kgBW intraperitoneal. Rat serum was obtained 24 hours after all treatments and followed by examination of TNF-α and IL-10 levels. Based on the data analysis, there was a significant decrease in TNF-α levels and a significant increase in IL-10 levels in rats group given 500 mg/kgBW Moringa leaf extract. Moringa leaf extract at a dose 500 mg/kgBW gives prevention effect by reducing TNF-α levels and increasing IL-10 levels in hemorrhagic cystitis after CP administration.

1. INTRODUCTION

Cystitis is infection of the bladder, in both acute or persistent form, and its severity can variety from slight to excessive, life-threatening bleeding [1]. Hemorrhagic cystitis is a excessive medical manifestation of chemical cystitis, that's regularly related to the chemotherapy drug cyclophosphamide (2- 40%) [2], [3]. Cyclophosphamide (CP) is an oxazaphosphate alkylating agent widely used in the treatment of malignant tumors such as lymphoma, myeloma, chronic lymphocytic leukemia, and non-malignant diseases such as nephrotic syndrome and multiple sclerosis. CP is known to induce cystitis and is commonly observed in patients receiving chemotherapy with these drugs. The usual treatment for CP-induced hemorrhagic cystitis is to administer mesna, a chemical that binds to the CP metabolite acrolein, into the urine. Mesna is also used as an adjunct regimen to the anti-cancer protocol of ifosfamide and cyclophosphamide [4], [5]. In patients with mesna prophylaxis, the incidence of hemorrhagic cystitis is 2025% [6], so alternative therapies are needed to overcome it. A study by [7] Hemorrhagic cystitis in patients taking cyclophosphamide because the factor influencing the development of hemorrhagic cystitis was the dose of CP itself, which is independent of the amount of mesna administered. We did not recommend using Mesna to prevent bleeding.

Acrolein is a CP metabolite that can rapidly invade urothelial cells. Next, acrolein provides intracellular production of reactive oxygen species (ROS) and nitric oxide (NO), either directly or as a nuclear factor kappa light chain enhancer for activated B cells (NFκB) and activated protein 1 (AP1). Activates through.). Activation of NFκB and AP1 increases the expression of tumor necrosis factor α (TNF- α) 2. Mouse models have shown that the end result of this acrolein exposure process is apoptosis and necrosis of urothelial cells, leading to hemorrhagic cystitis.

TNF-α is an inflammatory cytokine. The purpose of the inflammatory process is to remove irritants and promote tissue regeneration. TNF-α may contribute to tissue regeneration by activating anti-inflammatory cytokines such as interleukin 10 (IL10). Interleukin 10 (IL10) is produced by T helper 2 cells (Th2), a subset of CD4 + T cells containing Th1 and Th17, a subset of B cells, macrophages, neutrophils, and some subsets of dendritic cells. It is a cytokine, IL10 can inhibit the ability of bone marrow cells, such as macrophages and dendritic cells, to activate Th1 cells, thereby inhibiting the production of Th1 cytokines. The production of anti-inflammatory cytokines such as IL10 can reduce inflammation and tissue damage by inhibiting an excessive inflammatory response. The role of IL10 as an anti-inflammatory cytokine synergistically with the classical role of TNF as an pro-inflammatory cytokine in maintaining the proper balance of inflammatory responses. The role of these anti-inflammatory cytokines is synergistic with the classical role of TNF- α as an pro-inflammatory cytokine in maintaining the proper balance of inflammatory responses. The balance of pro-inflammatory and anti-inflammatory activates various growth factors that play a role in tissue healing.

Indonesia has a variety of natural abundances and could be a medicinal plant that could be used as a source of traditional medicine such as Moringa oleifera L. The leaves are part of the Moringa plant and are most commonly used in traditional medicine because they contain active compounds such as alpha and beta carotene, sterols, saponins, tannins and flavonoids. It removes free radicals and induces the tissue regeneration process. In addition, M. The bioactive ingredients of the oleifera leaf extract resulted in significant induction of elevated levels of IL10 and decreased levels of TNFalpha [1], [10].

2. Methods

A laboratory study using only one post-test control group design. This study was performed on Rattus norvegicus induced by cyclophosphamide and administered with Moringa oleifera L. Monitoring of TNFα and IL10 levels was performed using the ELISA method of the Rattus norvegicus blood assay.

The samples in this study were randomly divided into three groups: control group 1, control group 2, and treatment group. These three groups received different treatments within the given time. After a period of time, the variables in all groups were measured. The observations showed that there were differences in variable values between the control group and the treatment group. This difference is the effect (result) of the treatment [76].

In this study, the samples used have been rats with the Rattus norvegicus species that met the subsequent criteria:

a. Inclusion Criteria

1. Wistar stress male white rats, 6 months old, frame weight 150 to 200 grams
2. Healthy situation characterised through brilliant fur, energetic motion and no accidents

b. Exclusion Criteria

1. Rats with anatomical abnormalities

c. Drop Out Criteria

1. Mice that have been ill due to the fact they didn`t need to consume or died throughout the study
2. Male white rats aren't wholesome or there are anatomical abnormalities (defects).
3. Samples are broken so they're now no longer readable

Determination of the quantity of samples is performed with Federer formula

The survey and data collection took place for a month. Moringa leaf extract was manufactured at the pharmacy of the University of Widia Mandala in Surabaya. The laboratory animals were bred, treated and blood drawn in the biochemistry laboratory of the University of Airanga Veterinary Medicine in Surabaya. Analysis of tumor necrosis factor α (TNFα) and interleukin 10 (IL10) levels by the ELISA method was performed in the laboratory of the Airlangga University Dental Research Center in Surabaya.

3. Results

The study was performed on 24 rats of the Wistar strain of male Rattus norvegicus, 6 months old and weighing 150,200 grams (preparing 6 rats to avoid shedding). Samples were randomly divided into three groups: control group 1 (K1), control group 2 (K2), and treatment group (P), adjusted for 1 week. Group K1 was orally administered 0.9% NaCl, Group K2 was orally and intraperitoneally injected with 0.9% NaCl to administer cyclophosphamide, and Group P was orally and intraperitoneally administered Moringa oleifera L. Cyclophosphamide was administered by internal injection. On day 9, mice were sacrificed and serum was collected to check TNFα and IL-10 levels.

The results of the Shapiro Wilk test show that the TNF-α level of group K1 is p-value = 0.184 (> 0.05), group K2 p-value = 0.849 (> 0.05), and group P-ap value = 0.053 (> 0.05). The results of the data analysis revealed that the data distribution was normal, so the One Way ANOVA test was performed. From the results of the One Way ANOVA test, we found that the p-value = 0.703. Therefore, we can conclude that there is a significant difference in TNF levels based on treatment groups and uniform data variants. Post-hoc LSD testing was continued to find differences between treatment groups. Post-LSD test results showed that it was significant between groups K1 and K2 and P, and between groups K2 and P.

In the results of the Shapiro Wilk test, when examining the IL10 level of group K1, p-value = 0.391 (> 0.05), group K2 p-value = 0.997 (> 0.05), and group p-value = 0.097 (> 0.05). The results of the data analysis revealed that the data distribution was normal, so the One Way ANOVA test was performed. From the results of one-way ANOVA, we found that p = 0.703. Therefore, we can conclude that there is a significant difference in IL10 levels based on data variants similar to those in the treatment group. Post-hoc LSD testing was continued to find differences between treatment groups. The results of the ex-post LSD test showed that P was not significant between groups K1 and K2, P was significant, and that it was significant between the K2 groups.

4. Discussion

The use of cyclophosphamide (CP) as a medical treatment, especially in malignant tumors, often causes complications in the form of hemorrhagic cystitis [3]. Bladder inflammation is caused by acrolein, a metabolite of CP, which invades the urothelial wall and develops cystitis. Of the pro-inflammatory cytokines of the bladder wall that cause inflammation of the bladder wall. It is also detected in systemic bloodstream [2].

Hemorrhagic cystitis after CP administration is a challenge for urologists and physicians who use CP in their treatment regimens. Continued bladder lavage (CBI) or treatment with mesna is equally effective in preventing hemorrhagic cystitis [68]. However, both options have their drawbacks. CBI is an invasive procedure, while Mesna is a chemical that causes gastrointestinal side effects such as vomiting., pain. Stomach and constipation [69].

Induction of hemorrhagic cystitis in experimental animals can be done using intraperitoneal injection of CP. Research conducted by [11], [48] used a single intraperitoneal dose of 200 mg/kg/BW injection of CP in experimental animals wistar rats, causing bleeding in the bladder wall, severe edema, vascular congestion, and infiltrated inflammatory cells on histopathological examination 24 hours later.

Examination of IL-10 levels in this study showed there was a significant increase in levels in the Treatment group (P: 84.04 ± 22.23) which is a group of rats with bladder inflammation who were given Moringa leaf extract, compared to the Control group 1 (K1: 44.40 ± 14.88) which is a group of healthy mice. IL-10 levels in the treatment group also increased significantly when compared to the control group 2 (K2: 61.84 ± 15.58), which is a group of rats with bladder inflammation. This indicates that administration of ethanol extract of Moringa leaves at a dose of 500 mg/kgBW increased serum IL-10 levels.

In the innate and adaptive immune responses, IL-10 is the main anti-inflammatory cytokine that works by inactivating T cells and macrophages to prevent an exaggerated response to inflammation. The production of IL-10 is predominantly carried out by monocytes, which has a pleiotrophic effect on the immune and inflammatory systems. Both T cells, monocytes, and macrophages have the ability to reduce IL-10 effector activity and function. (Harijanto, 2000). IL-10 can inhibit the synthesis of proinflammatory cytokines produced by monocytes and mononuclear cells in the lamina propria of peripheral blood. Other effects of IL-10 in the immune response are increasing its own production via CD4+ cells, activating B cells by re-regulation of MHC-II expression, and enhancing IgA responses [38], Mora, 2006).

Administration of CP can cause inflammation in the form of necrosis of bladder epithelial cells so that it will release DAMPs (Damage-associated molecular pattern), which is a molecule that is released from the cells experiencing the necrosis.

In this study, the increase in IL-10 was due to the ability of Moringa leaf extract to prevent cell damage by reducing DAMPs, which are molecules released from the necrotic cells, thereby regulating the immune response by activating Th2 cells (T-helper 2). These Th2 cells will secrete IL-10 which works to suppress the inflammatory response.

DAMPs will be recognized by the Toll Like Receptor (TLR) which will then be captured by the Antigen Presenting Cell (APC) so that it will activate the immune response. This immune response is to regulate MHC 2 and will then regulate Th-0 cells, which will then differentiate into Th1 and Th2. Th1 cells activate pro-inflammatory cytokines, while Th2 cells activate anti-inflammatory cytokines

Moringa oleifera with its active flavonoid substance, namely quercetin, can activate excessive NF-B due to inflammation that occurs after CP injection. This is in accordance with the study of Woan et.al (2015) which showed a significant increase in IL-10 production in LPS-induced macrophages given ethanol extract of Moringa flowers. Moringa oleifera has anti-inflammatory properties by inhibiting NO and proinflammatory cytokines on the NF-B signaling pathway.75 This will decrease the secretion of the proinflammatory cytokine TNF- and increase the anti-inflammatory cytokine IL-10.

5. Conclusions

Moringa leaf extract at a dose of 500 mg/kgBW gives prevention effect by reducing TNF-α levels and increasing IL-10 levels in hemorrhagic cystitis after CP administration.

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