



## Effect of Ethanol Extract of Basil Leaves (*Ocimum basilicum*) on the number of M2 Macrophages in the Kidneys of Mice (*Mus musculus*) Using the Unilateral Ureteral Obstruction (UUO) Method

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### Abstract

**Background:** Chronic Kidney Disease (CKD) is a problem in nephrology with a reasonably high incidence rate. The final condition of CKD is the presence of kidney fibrosis in the tubulointerstitial region, depending on the polarization of macrophages. Basil antioxidant efficacious leaves can prevent damage to tubular cells in animals.

**Objective:** To determine the effect of giving basil leaf extract (*Ocimum basilicum* L.) on the amount of M2 macrophages in mice (*Mus musculus*) with the Unilateral Ureteral Obstruction method

**Methods:** This research uses an experimental study using a posttest only with a randomized controlled group design.

**Results:** Data on the administration of basil leaf extract (*Ocimum basilicum* L.) to mice can reduce damage to the kidneys analyzed using LSD test obtained p-value ( $0.000 < 0.005$ ). The amount of M2 macrophages in mice given basil ethanol extract with the most significant dose was 24 mg/20grBW compared to the administration of basil ethanol extract at a dose of 12 mg/20grBW and 48mg/20grBW.

**Conclusion:** Administration of basil leaves extract (*Ocimum basilicum* L.) in mice can reduce damage to the kidneys.

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## INTRODUCTION

*Chronic Kidney Disease (CKD)* is a nephrology condition with a very high incidence and broad and complex etiology. It typically begins without complaints or clinical signs unless it has progressed to the end-stage (terminal kidney failure)<sup>1</sup>. The result of Basic Health Research (Riskesmas) in 2013, the prevalence rate for CKD was 1.5 times, with the highest prevalence in the age group 55 years.<sup>2</sup>

When CKD reaches stage 3, a person is more prone to have renal disease complications such as hypertension and anemia. According to reports, hypertension affects 85 to 95 % of CKD stages 3-5<sup>3</sup>. The presence of renal fibrosis, particularly in the tubulointerstitial area, is the end stage of CKD and is defined by increasing injury to the renal parenchyma tissue and the formation of extracellular matrix, particularly collagen types I and III<sup>4</sup>. This is evidenced by the appearance of macrophages in a pro-fibrotic process or vice versa, particularly in M2 macrophages with phenotypes such as M2a, M2b, M2c, and M2d. M1 macrophages are pro-inflammatory cells because they appear to eliminate various factors that the body considers harmful. After being exposed to an etiology that can harm the kidneys, the inflammatory process begins.<sup>5</sup>

When inflammation is active, M2 macrophages arise, which act as a regulator by releasing IL-10, arginase, TGF-, and HO-1, allowing wound healing and tissue regeneration to begin quickly. The wound healing process depends on the etiologic component's presence; if the etiology of an injury is not addressed, M2 activation will continue.<sup>6</sup>

Basil leaves (*Ocimum basilicum* L) contain antioxidants such as eugenol, flavonoids, and ursolic acid, all of which act as free radical scavengers in the body because it is high in antioxidants<sup>7</sup>. Basil is a member of the genus *Ocimum*, recognized for its high essential oil concentration<sup>8</sup>. Because basil leaf extract had no harmful effect on biochemical and histological examinations of the kidneys and liver, it has the potential to be a nephroprotector.<sup>9</sup>

Unilateral Ureteral Obstruction (UUO) is an experimental model for tubulointerstitial fibrosis that causes hemodynamic and metabolic abnormalities in the kidneys and arthritis and renal inflammation characterized by macrophage infiltration<sup>10</sup>. Tubular epithelial cells and cells in the interstitial space account for approximately 80% of the total kidney volume. There are also a small number of resident mononuclear cells and fibroblasts that are easily measurable during the beginning and progression of renal fibrosis, making UUO an increasingly appealing experimental model for research<sup>11</sup>.

Basil leaves contain antioxidants in the form of flavonoid chemicals. When an obstruction occurs, the antioxidants found in basil leaves are hypothesized to protect tubular cells in animals from the non-phenolic chemicals found in essential oils and their phenolic components<sup>12</sup>. This research aimed to determine the effect of Basil leaf (*Ocimum basilicum*) on the quantity of M2 macrophages in the kidneys of mice (*Mus musculus*) using the UUO method.

## METHODS

This experimental study employs a post-test-only research design with a randomized controlled group design. The implementation will occur at the Pharmacology Laboratory, Faculty

of Medicine, Universitas Muhammadiyah Purwokerto, and the Laboratory of Anatomical Pathology, Faculty of Medicine, Gadjah Mada University, Yogyakarta.

The ethics commission has approved this study with the following number: KEPK/FK/II/143/2019. The population of this study consisted of experimental mice (*Mus musculus*). There were at least five male mice in each of the five treatment groups. In this investigation, the groups were divided as follows: K (-): The group that had only surgery and aquadest was the negative control group. K(+): The UUO group is the positive control group given aquadest. P1: 12 mg/20gBW basil leaf ethanol extract and the UUO group. P2: UUO group and a basil leaf ethanol extract of 24 mg/20gBW. P3: UUO group and basil leaf ethanol extract (48 mg/20gBW). M2 macrophages were the dependent variable, while basil leaf ethanol extract was the independent variable. After that, the animals were euthanized for histological evaluation by immunohistochemical staining of anti-Arginase I antibody.

Data analysis. The data from the five groups were then examined using the Shapiro-Wilk method. The data acquired were normally dis-

tributed; thus, the Levene test was used to determine the homogeneity of the data, followed by a different test using the One-way ANOVA test, and finally, the LSD post hoc test.

## RESULTS

The data from the examination were analyzed using the One-way ANOVA test to examine the effect of ethanol extract from basil leaves on the average number of M2 macrophages in the kidneys of mice (*Mus musculus*).

One-Way ANOVA test resulted in a  $p = 0.000$  ( $p < 0.05$ ) value as listed on the Table 1, indicating significant differences in the mean changes in the number of macrophages among multiple groups. The results were then examined with post hoc LSD to check if there were differences among treatment groups or if there was a difference in the average number of macrophages in each treatment group among the five groups in further detail.

The results of the post hoc LSD test showed that the  $p$ -value ( $0.00 < 0.05$ ) as listed on the Table 2 means that there is a significant effect on the administration of basil leaf ethanol extract in mice using the Unilateral Ureteral Obstruction (UUO) method among groups.

Table 1. Effect administration of basil leaves on a number of M2 macrophages in Mice with the Unilateral Ureteral Obstruction method

Groups	n	Mean $\pm$ SD	<i>P</i>
K-	5	11.40 $\pm$ 1.140	
K+	5	139.40 $\pm$ 9.864	
P1	5	84.40 $\pm$ 1.817	*0.000
P2	5	78.00 $\pm$ 5.701	
P3	5	49.80 $\pm$ 6.261	

\* One-way ANOVA test, significant if  $p < 0.05$

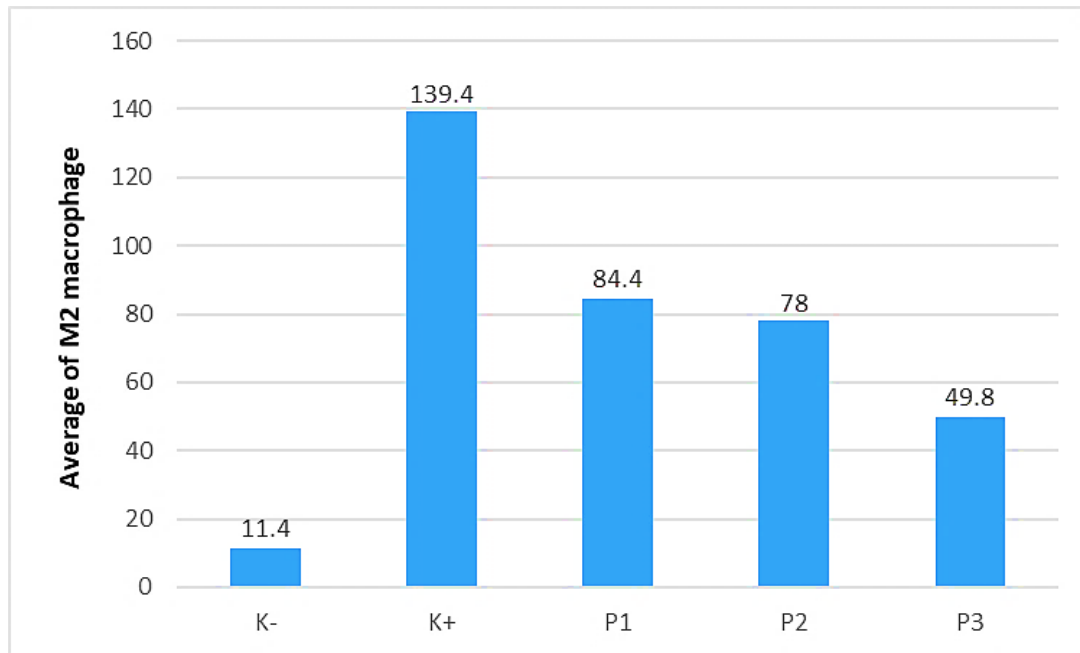
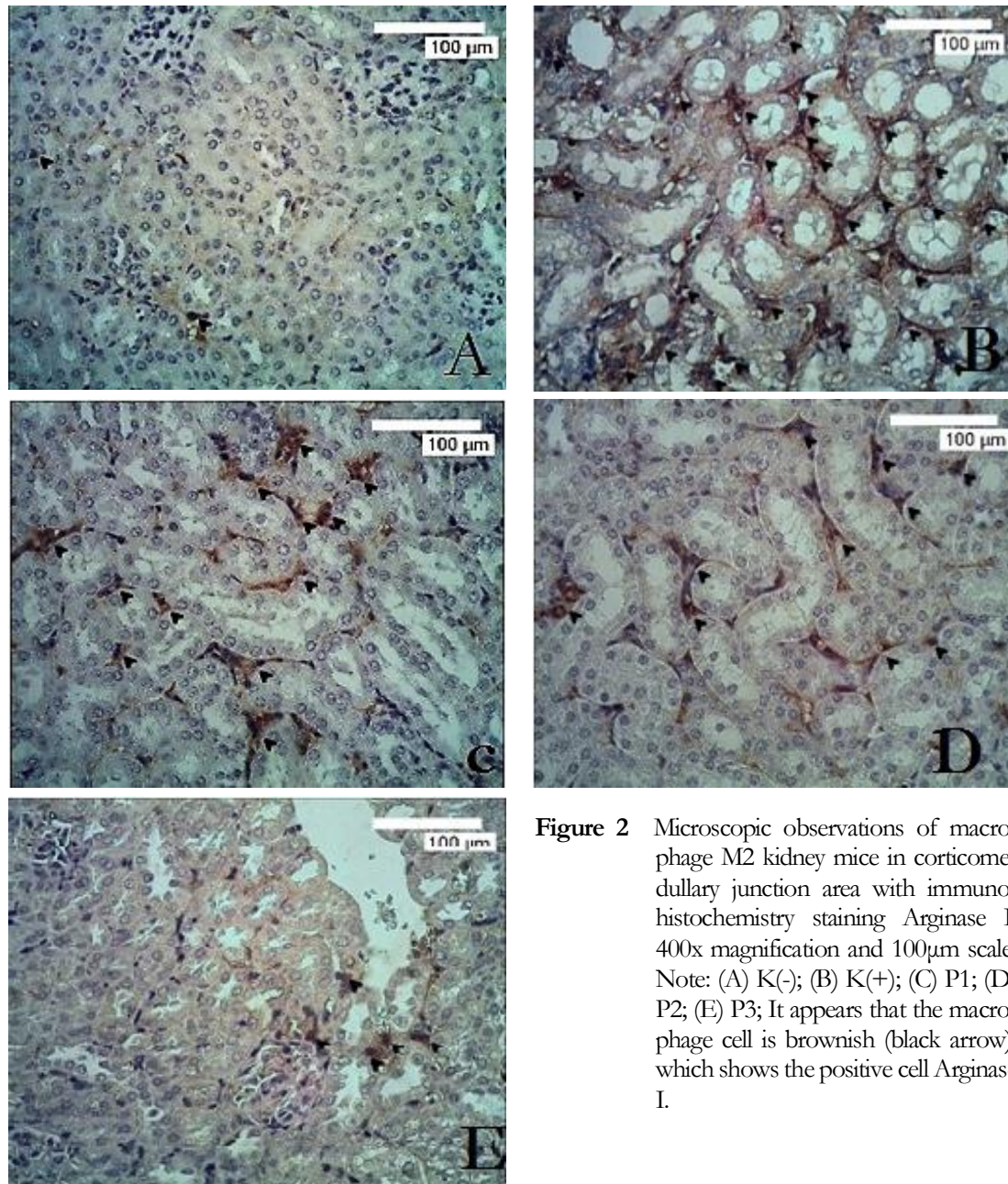


Figure 1. Average of M2 macrophages in each group

Table 2. Post hoc LSD of basil leaves extract dose

Group	*p	
	K (+)	0.000
K (-)	P1	0.000
	P2	0.000
	P3	0.000
	K (-)	0.000
K (+)	P1	0.000
	P2	0.000
	P3	0.000
	K (-)	0.000
Dose 12 mg/20 gBW (P1)	K (+)	0.000
	P2	0.101
	P3	0.000
	K (-)	0.000
Dose 24 mg/20 gBW (P2)	K (+)	0.000
	P1	0.101
	P3	0.000
	K (-)	0.000
Dose 48 mg/20 gBW (P3)	K (+)	0.000
	P1	0.000
	P2	0.000
	K (-)	0.000

\* *Post hoc* LSD, significant if  $p < 0.05$



**Figure 2** Microscopic observations of macrophage M2 kidney mice in corticomedullary junction area with immunohistochemistry staining Arginase I. 400x magnification and 100µm scale. Note: (A) K(-); (B) K(+); (C) P1; (D) P2; (E) P3; It appears that the macrophage cell is brownish (black arrow), which shows the positive cell Arginase I.

## DISCUSSION

### The effect of administering basil leaves to mice with the Unilateral Ureteral Obstruction method

The One-Way ANOVA test resulted in a  $p=0.000$  ( $p<0.05$ ) value, indicating significant differences in the mean changes in the number of macrophages among several groups. The post hoc LSD test results revealed that the  $p$ -

value ( $0.000 < 0.005$ ) indicated a difference between groups when basil leaves were given to mice using the Unilateral Ureteral Obstruction (UUO) method.

According to the chemical components contained in basil leaves, namely flavonoid compounds, which are a category of flavonoids known to operate as immunomodulators, basil

leaf extract (*Ocimum basilicum. L*) has an immunomodulatory action with an optimum dose of 800 mg/kgBW.<sup>13</sup> Macrophages are one of three cell kinds. Phagocytes in the immune system are widely dispersed in human tissues and vital in innate and adaptive immunity. Monocytes in their mature form Monocytes circulate in the bloodstream and constantly develop into macrophages. These macrophage cells will stay in the tissue (histiocytes) and engage in more active phagocytosis than monocytes and have more granules containing hydrolytic enzymes.<sup>14</sup>

Flavonoid molecules can prevent and repair free radical damage to cells and tissues.<sup>15</sup> Flavonoid chemicals can boost cell regeneration by destroying free radicals, providing a competitive substrate for unsaturated lipids in the membrane, and/or speeding up cell membrane repair mechanisms.<sup>16</sup> Flavonoids are natural antioxidants in basil leaves (*Ocimum basilicum L.*)<sup>17</sup>. Basil leaves (*Ocimum basilicum L.*) contain active chemicals in them, including essential oils, carbohydrates, phytosterols, alkaloids, phenolic compounds, tannins, lignin, starch, saponins, flavonoids, terpenoids, anthraquinones, apigenin flavones, luteolin, flavones o-glucoside apigenin 7-O glucuronide, luteolin 7-O glucuronide, flavone C-glucoside orientin, molludistin and ursolic acid which function as antibacterial<sup>18,19</sup>.

According to Putra<sup>20</sup>, basil leaf extract functions as an immunomodulator to the phagocytic activity of macrophage cells at doses of 400 mg/kg BW and 800 mg/kg BW that are not significantly different or have the same effect as the positive control Imboost Force at a dose of 0.975 mg/kg BW in mice. The phago-

cytic capacity of macrophage cells did not differ significantly all over the three-dose variations with Imboost force as the positive control. Active vitamin D reduces the establishment of renal fibrosis by reducing renal tubular damage, the M1/M2 macrophage ratio, and myofibroblast numbers.<sup>21</sup>

### **The number of M2 macrophages in mice induced by ethanol extract of basil leaves (*Ocimum basilicum*)**

The number of M2 macrophages produced in mice by an ethanol extract of basil leaves (*Ocimum basilicum L.*) was from five different treatment groups. The number of M2 macrophages produced by ethanol extract of basil leaves (*Ocimum basilicum L.*) in mice was highest in the K(+) group, reaching  $139.40 \pm 9.864$ . In mice induced by ethanol extract of basil leaves (*Ocimum basilicum L.*), the number of M2 macrophages was the lowest in the P3 group ( $49.80 \pm 6.261$ ). This could be because macrophages' phagocytic capability has reached its limit, and the antioxidant content of basil leaves is optimal in protecting against cell membrane damage when an ethanol extract of basil is administered at a dose of 24 mg/20grBW.

Basil leaf extract (*Ocimum basilicum. L*) exhibits an immunomodulatory effect based on activity and phagocytic capacity criteria, with an optimum dose of 800 mg/kgBW.<sup>13</sup> Basil leaves (*Ocimum basilicum L.*) from Africa, India, and Asia have antioxidant, antibacterial, insecticide, and therapeutic properties such as anti-inflammatory, antipyretic, and analgesic properties. They are used as vegetables, flavor enhancers, and in the treatment of ailments (migraine, stress, fever, diarrhea).<sup>8,22,23</sup> Basil leaves are

widely used to treat fevers, canker sores, internal heat, coughs, nausea, gas laxatives, and laxative menstruation.<sup>24</sup>

The effect of basil leaf extract administration on enhancing phagocytic activity can be seen in the average value of the phagocytic index being  $> 1$  for all dose groups, indicating that basil leaf extract has the ability to act as an immunostimulant. A rise in the carbon clearance index indicates a rise in the phagocytic function of mononuclear macrophages and the non-specific immune system.<sup>25</sup> Macrophages are immune cells that play a critical part in the body's defense against infections. Phagocytosis is a function of macrophages in the innate immune system that tries to remove extracellular particles, damaged or dead cells, and harmful germs.<sup>26</sup>

These cells develop from spinal cord precursors known as promonocytes, which divide into monocytes and enter the circulatory system. These monocytes will undergo a series of modifications before settling in the tissue as macrophages.<sup>27</sup> Basil leaf extract has immunostimulant properties. The B cells in the spleen become active and create enormous numbers of antibodies composed of B cells, T cells, macrophages, dendritic cells, natural killer cells, and red blood cells that grab foreign bodies (antigens) from the blood traveling through the spleen.<sup>28</sup> Tumor-associated macrophages are macrophages located in the extracellular space during tumor formation (TAMs). TAMs are critical cellular components of human cancer. However, it is still difficult to understand how the process and mechanism work<sup>29</sup>. Pathogens, dead cells, and some extracellular matrix components are phagocytosed by macrophages. Macrophages have a role in

the regulation of organ homeostasis and remodeling. Macrophages induce angiogenesis and aid tissue remodeling during tissue regeneration or repair by secreting proteases and growth factors. The expression of various markers, including CD<sub>68</sub> in humans, can be used to identify macrophages in tissue. The majority of macrophages are present in the wound healing perivascular area.<sup>30</sup>

## CONCLUSION

Administration of basil leaf extract (*Ocimum basilicum* L.) on mice can reduce damage to the kidneys and decrease the amount of M2 macrophages in Mice with Unilateral Ureteral Obstruction (UUO) method, with the optimum dose being 24 mg/20 gr BW.

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