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The Potential of Ciplukan Leaf Extract (*Physalis Angulata* L.) to Improve Kidney Function

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ABSTRAK

Gagal ginjal merupakan penyakit tidak menular namun dapat mengancam nyawa manusia. Penyakit ini dapat dialami dari berbagai macam usia mulai dari anak-anak hingga lansia. Salah satu tanaman yang biasanya dijadikan sebagai obat herbal ialah tanaman ciplukan (*Physalis angulata* L.). Gagal ginjal merupakan penyakit tidak menular namun dapat mengancam nyawa manusia. Penyakit ini dapat dialami dari berbagai macam usia mulai dari anak-anak hingga lansia. Salah satu tanaman yang biasanya dijadikan sebagai obat herbal ialah tanaman ciplukan (*Physalis angulata* L.). Penelitian ini bertujuan untuk mengetahui potensi dari daun tanaman ciplukan dalam memperbaiki fungsi ginjal. Penelitian ini dilakukan di Kampus IPB Dramaga, IPB University, Bogor. Tikus putih digunakan sebanyak 24 ekor dan dibagi menjadi 8 kelompok dengan perlakuan dosis 150mg/kg BB dan 300mg/kg BB. Perlakuan dilakukan selama 14 hari dan 28 hari.Etilen glikol digunakan sebagai agen nefrotoksik. Pemberian kombinasi etilen glikol 1 ml/100g BW secara oral dan ekstrak ciplukan dosis 300mg/kg BB secara bersamaan mampu menormalkan kadar ureum dalam darah. Hasil histologi juga menunjukkan adanya perbaikan pada glomerulus ginjal. Ekstrak daun ciplukan memiliki kandungan flavonoid yang berpotensi memperbaiki fungsi dan morfologi ginjal.

Kata kunci: *Ciplukan (Physalis angulata L.), etilen glikol, ginjal, kreatinin, ureum*

ABSTRACT

Kidney disease is a non-communicable disease, but it can threaten human life. This disease can occur in different age groups from children to elderly people. One of the plants commonly used as herbal medicine is the ciplukan plant (*Physalis angulata* L.). The aim of this study is to determine the potential of the leaves of ciplukan plant to improve kidney function. This research was conducted at the Dramaga Campus of IPB, IPB University, Bogor. Twenty-four white rats were used and divided into 8 groups with treatment doses of 150mg/kg BW and 300mg/kg BW. The treatment was carried out for 14 days and 28 days. Ethylene glycol was used as a nephrotoxic agent. Administration of a combination of ethylene glycol with the dose of 1 ml/100g BW orally and ciplukan extract at a dose of 300 mg/kg BW at the same time in the P8 group was able to normalize blood urea levels. Histological results also showed improvement in the renal glomerulus. Ciplukan leaf extract contains flavonoids that potential to improve renal.

Keywords*: Ciplukan (Physalis angulata L.), creatinine, ethylene glycol, kidney, ureum*

INTRODUCTION

Kidneys are organs that have the main function of removing metabolic waste through the process of filtering blood, maintaining water balance, pH, electrolytes, and secreting the hormone erythropoietin. The kidney consists of several parts, including the nephron which is the smallest functional unit of the kidney. Decreased nephron performance is an indicator of decreased kidney function (Vasudevan *et al*., 2017). The kidneys play an important role in the excretion of waste products and toxins such as urea and creatinine as metabolic wastes.

Creatinine is by product of creatine phosphate breakdown in the muscles, and is produced at a constant rate. Creatinine is completely cleared from the blood by filtration process of the kidneys. Each nephron has a glomerulus, which is where the blood is filtered. Assessment of glomerular function is also characterized by changes in excreted creatinine as creatinine clearance levels. In other words, an increase in blood creatinine is a decrease in kidney function (Gounden *et al*., 2020). Urea in the blood or BUN (Blood Urea Nitrogen) is the result of normal protein metabolism. The level of urea depends on the catabolism (breakdown) of proteins in the liver which are excreted in the urine through the kidneys. High level of urea in the body has potency as toxicant

Factors that affect the decline in kidney performance are age and diseases such as diabetes, hypertension, and an unhealthy lifestyle (Vadya and Aeddula, 2020). The Data of RIskesdas on 2018 showed the prevalence of chronic kidney failure in Indonesia increases with age, age group 15-24 (0.1%), 25-34 years (0.2%), 35-44 years (0 .3%), 45-54 years (0.5%), 55-64 years (0.7%). The increasing number of chronic kidney failure was showed at interval age group 65-74 years (0.8%) and in the age group 75 years (0.7%). Chronic kidney failure is prone to occur in the elderly, this is due to decreased body function (degenerative). Increasing age shows a progressive decrease in GFR (Glomerular Filtration Rate) and RBF (Renal Blood flow).

Herbal plants are currently widely used by the public in various types of kidney treatment such as temulawak (Shakti *et al*., 2019) and cat's whiskers (Madyastuti *et al*., 2020). Ciplukan plant (*Physalis angulata* L.) is a plant that has been proven to be efficacious including as an anti-inflammatory (Junior *et al*., 2017), antimetastatic (Hseu *et al*., 2011), as well as an antioxidant (Susanti *et al*., 2015, Nuranda *et al*., 2016). Previous research (Tampie, 2018) reported that all parts of the ciplukan plant have the potential to overcome urolithiasis. The use of ciplukan leaf extract to improve kidney function has never been done. The purpose of this study was to analyze the effect of ciplukan leaf extract (*Physalis angulata* L.) on kidney function in general and specifically in treating kidney damage exposed to nephrotoxic ethylene glycol. Parameters measured were rat blood chemistry levels in the form of urea, creatinine, and rat kidney histology.

MATERIAL AND METHODS

Time and Location

The research was carried out in several places. The rearing of the test animals was carried out at the Laboratory Animal Management Unit and preparation of the ciplukan extraction was carried out at the Physiology Laboratory of the Department of Anatomy, Physiology and Pharmacology, SKHB IPB. Phytochemical analysis of ciplukan was carried out at the Tropical Biopharmaceutical Study Center (TropBRC) IPB and histology of the kidneys was carried out at the Histology Laboratory, Department of Anatomy, Physiology and Pharmacology, SKHB IPB. This research starts in October 2021 to April 2022.

Animal Model

The use of animal models in this study has obtained permission from the Animal Ethics Commission of SKHB IPB University No: 004/KEH/SKE/I/2022.

Twenty-four white rats male (*Rattus novergicus*) with body weights ranging from 200-300 g at 2-3 months of age were divided into 8 treatment groups asseen at table below:

Table 1 Treatment of Animal Model

Groups	Treatment	Dose of Ciplukan Leaf Extract (mg/kg BW)	Days of treatment
P1	Normal	-	14 d
P2	Ciplukan leaf	150	14 d
P3	Ciplukan leaf	300	14 d
P4	EG	-	14 d
P5	EG 14 days and ciplukan leaf 14 days	150	28 d
P6	EG 14 days and ciplukan leaf 14 days	300	28 d
P7	EG and ciplukan leaf 14 days	150	14 d
P8	EG and ciplukan leaf 14 days	300	14 d

*Preparation of Ciplukan Leaf Simplicia (*Physalis angulata L.*)*

The ciplukan plant used was ciplukan leaves from Cimanggu, West Bogor District, Bogor City, West Java Province. The ciplukan leaves used are the young leaves (young leaves are leaves 1-3 from the shoot (Gultom *et al*., 2020)). Furthermore, the leaves were dried at 50 °C for 3 days to facilitate the process of making simplicia. After that, in a blender to get ciplukan leaf powder. Simplicia is stored in a clean, tightly closed container.

Preparation of Ciplukan Leaf Ethanol Extract

The extraction of ciplukan leaf was using maceration methode and alcohol 70% ethanol as solvent. The ratio of simplisia and solvent was 5:1 then soaked for 3 x 24 hours. Liquid ethanol extract from maceration process then evaporated using a rotary evaporator (40 °C, 50 rpm) to obtained a thick extract from the ciplukan plant. Ciplukan leaf extract then carried out phytochemical screening.

Preparation of Ethylene Glycol

Ethylene glycol is used as much as 0.75% (Wientarsih *et al*., 2012). A total of 0.75 ml of ethylene glycol was added to 100 ml of distilled water. Then the solution was shaken to mix well. Ethylene glycol is mixed in drinking water and given as much as 1 ml/100g BW. The dose of EG given to rats was 1 ml/100g BW.

Making Preparation of Ciplukan Leaf Extract

The ciplukan extract had been prepared as a based solution by diluting 15g of ciplukan thick extract in 500 ml distilled water and 30g of ciplukan thick extract in 500 ml distilled water. A dose of 150mg/kg BW contained 30mg/ml and a dose of 300mg/kg BW contained 60mg/ml. The dose given to the rats was converted according to their respective body weights. The dose of ciplukan leaf extract was used in two doses, 150 mg/kg BW and 300 mg/kg BW. The use of this dose refers to a study (Putra, 2019) that used white rats as experimental animals.

Analysis of Urea and Creatinine

A total of 3 ml of blood was taken directly from the left ventricle. The blood was then centrifuged at 10000 rpm for 10 minutes to obtain blood serum for analysis of urea and creatinine. Urea and creatinine tests were carried out using the Spectrofotometer Hitachi UV/Vis® mouse instrument.

Histological Analysis of Rat Kidney

Rats were necropsied to take out the kidney, then kidney organs were cut with a thickness of ± 5 mm. The samples were transferred into 70% alcohol as a stopping point. Samples were dehydrated in graded alcohol (80%, 90%, 95%, and absolute I, II, III) and cleared (xylol I, II, III) in an incubator at 56 °C. The samples were infiltrated into paraffin I, II, III at 56 °C then were embedded in paraffin and sectioned at 5 µm, and stained with hematoxylin-eosin to be photographed under a microscope.

Data analysis

The data were analyzed by analysis of variance (ANOVA) using SPSS release 16 software. If it had a significant effect, the Duncan test continued with a 95% confidence interval. Histological data microscopic observation of kidney structure was carried out in a comparative descriptive manner.

RESULTS

Secondary Metabolite Content of Ciplukan Extract (Physalis angulata L.)

The results of the phytochemical analysis of ciplukan leaf extract qualitatively showed that the ciplukan leaves used in this study contained alkaloids, tannins, saponins, steroids, and flavonoids (Table 1). The flavonoid content in ciplukan leaves is 2.29% (w/w) which is expected to be an antioxidant. (Phance *et al.,* 2016) reported that flavonoids have a positive effect on human and animal health.

Urea and creatinine levels

BUN (Blood Urea Nitrogen) and serum creatinine was analyzed to assess kidney function. The blood test for BUN is a measurement of the end product of nitrogen from protein and amino acid catabolism, measures the amount of urea nitrogen in the blood, and is directly related to the excretory function of the kidneys (Pandya *et al*., 2016). (Dinar *et al*., 2019) explained that BUN describes the number of nitrogen atoms in the blood combined with urea. Urea is filtered at the glomerulus and reabsorbed in the renal tubules. This increase in BUN is associated with a decrease in the glomerular filtration rate (GFR). Therefore, an increase in urea can be an indicator of injury to the kidneys. The average results of the rats urea and creatinine results can be seen in Table 3.

Table 3 showed blood urea levels in rats given

ciplukan leaves 150 (P2) and 300 mg/kg BW (P3) showed the same results as the control rats (P1). The urea levels in blood of P4 (administration EG only) and given Ciplukan afterwards (P5 and P6) increased. The highest increase occurred in P4 namely in the group that only received EG. Urea levels that were given EG together (combination) with ciplukan leaves again decreased like normal rats. EG induction causes acute tubular necrosis which results in reduced kidney performance so that urea levels become higher. As a nephrotoxic agent, ethylene glycol stimulates the formation of calcium oxalate which causes tissue damage. EG toxicity is caused by its acid metabolite, oxalate, which can cause CaOX crystal deposition and metabolic acidosis occurs (Viinamaki *et al*., 2015).

Urea is a waste product of the breakdown of proteins in the body. The buildup of urea can harm the body because it is toxic. The administration of 150 and 300 mg/kg BW of ciplukan extract after ethylene glycol induction for 14 days (P5 and P6) showed a significantly different decrease ($P<0.05$) compared to the treatment only induced by ethylene glycol. However, this treatment did not have an impact on kidney improvement because the urea levels in the P5 and P6 groups were still high. Giving ethylene glycol for 14 days caused toxic effects and kidney damage first and the kidneys only improved after being given ciplukan extract 150 and 300 mg/kg BW.

The combination of ethylene glycol and ciplukan extract at a dose of 150 mg/kg BW (P7) and 300 mg/kg BW (P8) reduced blood urea levels close to normal urea levels. In groups P7 and P8, the damage caused by direct EG induction could be overcome by giving ciplukan extract. The mechanism of secondary metabolites in ciplukan extract in reducing urea levels is thought to be based on antioxidant activity, such as flavonoids, alkaloids, tannins, saponins, and steroids. Flavonoid compounds are strongly suspected to be responsible for the antioxidant activity that can counteract free radicals. Dewi *et al*., (2018) reported that flavonoids are one the polyphenols that have antioxidant properties. Antioxidants can donate electrons to free radicals, so free radicals can be suppressed and not damage body cells.

Creatinine levels in all groups did not appear to change ($P>0.05$). Creatinine is a breakdown product of creatine phosphate in the muscle which is excreted by the kidneys. As a byproduct of muscle metabolism, creatinine is freely filtered by the glomerulus and is not reabsorbed by the renal tubules (Dinar *et al*., 2019). These data are supported by (Diago *et al*., 2020) which states that creatinine permeates through cell membranes, diffuses freely from cells into the blood, is not bound to plasma proteins, is not metabolized in any tissue, and is rapidly excreted by the kidneys through glomerular filtration. Excretion in urine increases as glomerular filtration deteriorates. There are several factors that are suspected of decreasing creatinine. First, it is suspected that there is a disturbance in muscle mass, because almost all the metabolites of creatine phosphate are found in skeletal muscle. (JiHye *et al*., 2015) reported that serum creatinine can represent a marker of muscle mass. In addition, the decrease in creatinine is also a marker of cellular aging and oxidative stress.

Histological Analysis of Rat Kidney Organs

The micromorphological analysis of the rat kidney is presented in Figure 1. Figure P1 is an image of a normal rat kidney. The glomerulus, cell nucleus, and tubules are still clearly visible and there is no edema. The administration of ciplukan extract at a dose of 150mg/kg BW did not show any visible damage, but there was a slight increase in glomerular diameter. The P3 picture shows a shrinkage of the glomerular capillary network so that a larger space appears in Bowman's capsule. Giving ciplukan extract at a dose of 300 mg/kg BW also caused an increase in the diameter of the glomerulus.

DISCUSSION

The glomerulus is a special collection of capillaries as the filtering unit of the kidney which is surrounded by Bowman's capsule. The development of kidney disease can lead to increased pressure in the glomerulus and this pressure can result in capillary injury (Embry *et al*., 2016). Photomicrographs showed that ethylene glycol-induced rats had damage to Bowman's capsule and tubular edema was seen (P4 Figure 2). Ethylene glycol metabolism will cause nephrosis. Nephrosis is a degenerative change in the kidneys caused by the impaired exchange of substances that causes the glomerular capillaries to not function properly, resulting in glomerular edema. The glomerular diameter in the P4 group was enlarged compared to normal size. Chronic deterioration of glomerular pressure is characterized by the expansion of glomerular capillaries and glomerular diameter (Sasaki *et al*., 2018). The renal capsule space also appears to be atrophic.

Table 4 shows the expansion of the glomerular area and diameter caused by damage to the glomerulus due to ethylene glycol induction. The toxic effect of ethylene glycol will increase glomerular permeability so that substances are not filtered properly. The substances will collect in Capsule Bowman space.

Capsule Bowman which is pushed by the filtrate fluid will expand and there will be a widening in the size of the glomerular area and diameter glomerulus.

Giving ciplukan extract at a dose of 150 mg/kg BW and 300 mg/kg BW for 14 days (P5 and P6) after ethylene glycol-induced rats showed no effect,

Table 2 Phytochemical Test Results and Total Flavonoid Ciplukan Leaf Extract

Sample	Phytochemical	Result	Unit
Physalis angulata L. leaf	Alkaloid	Positive	-
	Flavonoid	Positive	-
	Total Flavonoid	2.29	% (b/b)
	Tanin	Positive	-
	Saponin	Positive	-
	Quinon	Negative	-
	Steroid	Positif	-
	Triterpenoid	Negative	-

Description: The results were tested at the Tropical Biopharmaca Research Center (TROP-BRC) IPB University

Table 3 Urea and Creatinine in rats level in mg/dL

Groups	Ureum (mg/dL)	Creatinine (mg/dL)
P1	22.67 ± 2.68	0.10 ± 0.00
P2	20.00 ± 2.64	0.16 ± 0.06
P3	22.00 ± 0.00	0.13 ± 0.06
P4	28.00 ± 1.73	0.13 ± 0.06
P5	25.33 ± 2.08	0.16 ± 0.06
P6	27.67 ± 1.52	0.10 ± 0.10
P7	22.33 ± 2.08	0.13 ± 0.06
P8	20.33 ± 1.15	0.13 ± 0.16

Description: Results of the Research and Diagnostic Laboratory of the Teaching Animal Hospital (RSHP) SKHB IPB. Results of rat kidney urea and creatinine level in mg/dL (mean±SD) on the administration of ethylene glycol and ciplukan extract. P1: normal, P2: ciplukan 150mg/kg BW 14 days, P3: ciplukan 300mg/kg BW 14 days, P4: EG 14 days, P5: EG 14 days + ciplukan 14 days at a dose of 150mg/kg BW, P6: EG 14 days + ciplukan 14 days at a dose of 300 mg/kg BW, P7: EG + ciplukan 14 days at a dose of 150 mg/kg BW, P8: EG + ciplukan 14 days at a dose of 300mg/kg BW.

Table 4 Area and Length Glomerulus of Kidney

Groups	Area (µm)	Length (µm)
P1	6039.76	86.39
P2	6918.63	86.24
P3	7001.32	89.24
P4	9310.77	10.86
P5	9503.11	11.,02
P6	9143.50	104.57
P7	8881.40	98.27
P8	5856.24	86.71

Description: Results of the Research and Diagnostic Laboratory of the Teaching Animal Hospital (RSHP) SKHB IPB. Results of rat kidney urea and creatinine on administration of ethylene glycol and ciplukan extract. P1: normal, P2: ciplukan 150mg/kg BW 14 days, P3: ciplukan 300mg/kg BW 14 days, P4: EG 14 days, P5: EG 14 days + ciplukan 14 days at a dose of 150mg/kg BW, P6: EG 14 days + ciplukan 14 days at a dose of 300 mg/kg BW, P7: EG + ciplukan 14 days at a dose of 150 mg/kg BW, P8: EG + ciplukan 14 days at a dose of 300mg/kg BW.

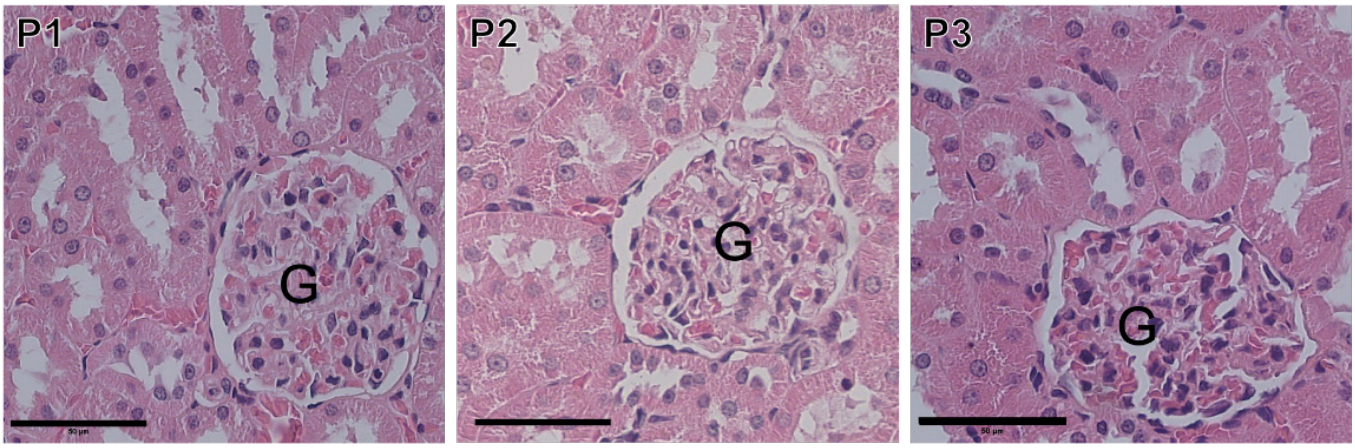

Figure 1 Photomicrograph of rat kidney on induction of ciplukan extract with hematoxylin-eosin staining. Bars = 50µm. In P1 normal group; the glomerular are still normal condition. In P2 ciplukan leaf extract at a dose 150 mg/kg BW group and P3 ciplukan leaf extract at a dose 300 mg/kg BW group; the glomerular and tubular injury was not found. (G: glomerular).

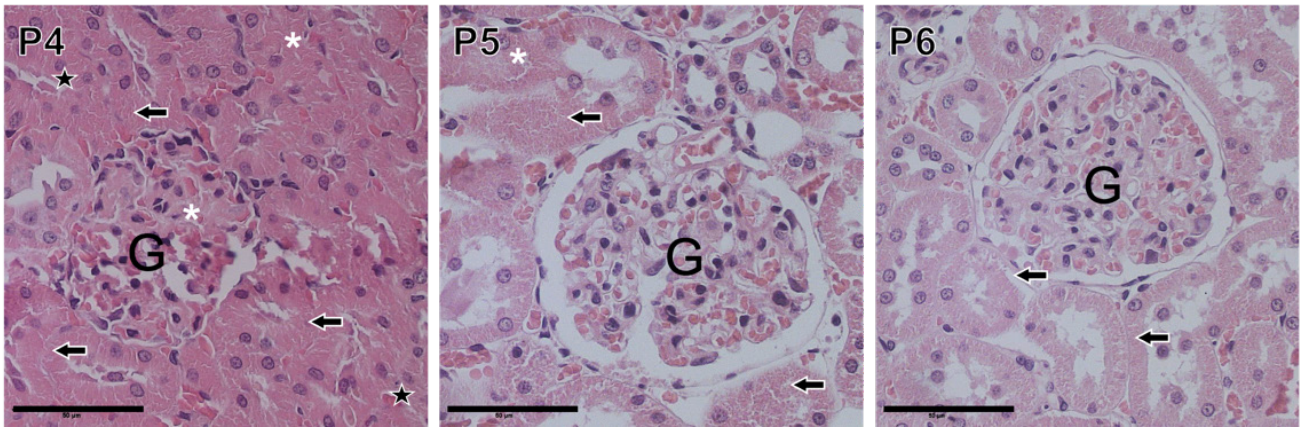

Figure 2 Photomicrograph of rat kidney on induction of EG and ciplukan extract with hematoxylin-eosin staining. Bars = 50µm. In P4 EG group; there are tubular and glomerular edema (asterisk), protein deposits (star), and cell nucleus reduced (arrows). In P5 EG 14 days and ciplukan leaf extract 14 days at a dose 150 mg/kg BW group; there was still tubular edema (asterisk) and, cell nucleus appear reduced (arrows). In P6 EG 14 days and ciplukan leaf extract 14 days at a dose 300 mg/kg BW group; cell nucleus appear reduced (arrows). (G: glomerular).

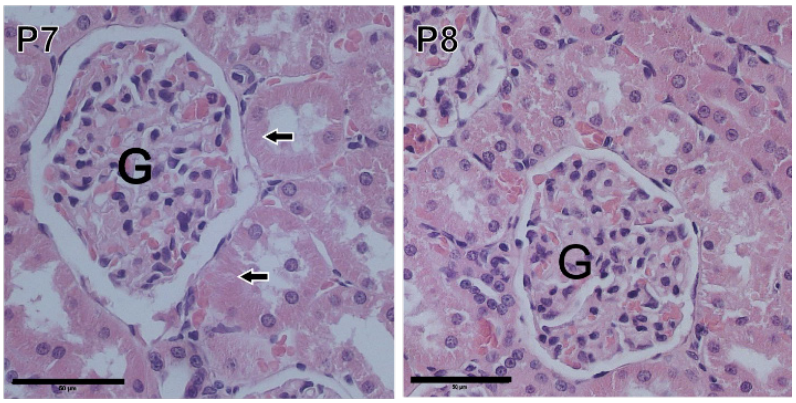

Figure 3 Photomicrograph of rat kidney on induction of EG and ciplukan extract with hematoxylin-eosin staining. Bars = 50µm. In P7 EG and ciplukan leaf extract at a dose 150 mg/kg BW group; cell nucleus appear reduced (arrows). In P8 EG and ciplukan leaf extract at a dose 300 mg/kg BW group); the glomerular are normal condition. (G: glomerular)..

namely the size of the glomerulus in this group experienced an increase in diameter size and there was a reduction in the nucleus in the kidney tubules image P5. Glomerular edema was also seen in both groups. The combination of EG and ciplukan dose of 150 mg/kg BW (P7) still did not give real results. The picture of the glomerulus looks normal but the size of the glomerulus still shows an enlargement compared to the normal size. The effect of the results occurred in the administration of EG and ciplukan at a dose of 300mg/kg BW (P8). Glomerular size in this group was close to normal size, and Bowman's capsule damage due to ethylene glycol administration seemed to have improved. This is possible because of the effect of giving ciplukan extract.

The content of flavonoid secondary metabolites in ciplukan acts as an antioxidant and can protect the body's epithelial cell structure including the kidneys (Wientarsih *et al*., 2014). Antioxidants contained in ciplukan leaf extract can fight toxins and inhibit cell oxidation so that the damage caused by ethylene glycol can be reduced. However, based on the results of statistical analysis level (P>0,05) which shows the results are not significantly different.

From the level of blood urea and diameter of glomerular, it can be discussed that the administration of ethylene glycol and ciplukan extract simultaneously can prevent damage. But giving ciplukan extract after being given ethylene glycol could not restore kidney function.

"All authors declare that there are no conflicts of interest".

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