The Effect Of Ethanol Extract Of Batak Onion (*Allium Chinense G. Don.*) On The Kidney Histopathology Of White Rats Induced By Ethylene Glycol

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Abstract.

Ethylene glycol is a chemical compound that when entered into the body can cause damage to kidney cells/tissue. Batak onion is an ethnomedical plant that acts as a natural antioxidant that is very necessary for the body. The purpose of this study was to determine the effect of Batak onion ethanol extract on the score of kidney histopathology damage and glomerular diameter of white rats induced by ethylene glycol. This study used a completely randomized design (CRD) with 25 white rats consisting of 5 treatment groups, namely the negative control group given food and water, the positive control group 0.75% ethylene glycol, treatment group 1 0.75% ethylene glycol and ethanol extract of Batak onion 250 mg/kg BW, treatment group 2 0.75% ethylene glycol and ethanol extract of Batak onion 500 mg/kg BW, treatment group 3 0.75% ethylene glycol and ethanol extract of Batak onion 750 mg/kg BW. The research data were analyzed statistically using one-way ANOVA and Duncan's further test. The results of the study showed that the administration of Batak onion ethanol extract had an effect on the histopathological damage score of the kidneys of white rats ($p<0.05$) with the most optimal extract dose of 500 mg/kg BW and 750 mg/kg BW and had an effect on the glomerular diameter of the kidneys of white rats ($p<0.05$) with the most optimal extract dose of 500 mg/kg BW and 750 mg/kg BW. These results indicate that the administration of Batak onion ethanol extract had an effect on the histopathological damage score of the kidneys and the glomerular diameter of white rats induced by ethylene glycol.

Keywords: *Batak Onion; Histopathology and Ethylene Glycol..*

I. INTRODUCTION

Kidney disease is a disorder of the kidneys characterized by abnormalities in the structure and function of the kidneys such as abnormalities in urine sediment, electrolytes, filtration rate, kidney structure and kidney histology.1 In September 2018, the WHO reported findings of kidney failure in children caused by ethylene glycol contamination in children's syrup. In Indonesia, the Food and Drug Monitoring Agency of the Republic of Indonesia (BPOM RI) has issued the latest information that the presence of ethylene glycol contamination in several children's syrup products is suspected to be the cause of kidney failure in children. Ethylene glycol is a colorless, odorless, and sweet-tasting liquid widely used in various household and industrial products. Ethylene glycol ingested in the body is rapidly absorbed through the digestive tract within 1-4 hours, then more than 80% is converted into toxic compounds. In the kidneys, ethylene glycol will form kidney stones that can cause inflammation and result in oxidative stress in kidney cells/tissues.

Cell/tissue damage can be minimized with antioxidants from outside the body that can be obtained from plants.Batak onion (Allium chinense G. Don) is a plant commonly consumed by people in North Sumatra and often used as a traditional medicine. The Batak ethnic group believes that the compounds in Batak onion extract have medicinal value. Among the compounds found in Batak onion extract are flavonoids, alkaloids, steroids, terpenoids, and saponins.The secondary metabolite compounds contained in Batak onion extract act as natural antioxidants, essential for the body. Antioxidants are compounds that can neutralize free radicals, thus preventing several degenerative diseases such as cardiovascular disease, carcinogenesis, and others. Therefore, given the potential medical value of Batak onion extract and the potential toxic effects of ethylene glycol, research is needed to investigate the effect of Batak onion ethanol extract on the kidneys of white rats induced by ethylene glycol.

II. METHODS

This research is an experimental study using a completely randomized design (CRD) and has received research ethics approval under number 0315/KEPH-FMIPA/2023 from the USU Faculty of Mathematics and Natural Sciences Animal Research Ethics Commission. The experimental animals used in this study were 25 male Wistar rats, calculated using the Federer formula. The rats used weighed 150-200 grams and were 8 to 12 weeks old. The tools and materials used in this study were:Test tubes, beaker glass, aluminum oil, measuring cup, spatula, petri dish, watch glass, measuring flask, dropper, binocular microscope, trinocular microscope, micrometer slide, cover glass, object glass, surgical tool set, organ bottle, safety pin, cotton, surgical tray, gastric sonde, microtome, microtome knife, water bath, paraffin block tool, tissue processor, tissue embedding, timer watch, slide warmer, paraffin dispenser, tissue cassette, batak onion, ethylene glycol, ethanol 96%, FeCl 1% reagent, H2SO4, magnesium, HCl, bouchardat, maeyer, salkowsky, lieberman-bourchard, distilled water, alcohol 96%, DPPH powder, methanol, CMC 0.5%, NBF 10%, hematoxylin and eosin dye, xylol, alcohol 70%, 80%, 90%, paraffin, ethylene, chloroform.

1. Making Batak Onion Ethanol Extract
   Two kilograms of Batak onion were cut into small pieces and then dried using a black cloth under the sun for 3 x 24 hours. The dried Batak onion was then ground using a blender until it produced a powder. The Batak onion was then dissolved in 96% ethanol at a ratio of 1:10 for 3 x 24 hours. The resulting solution was then concentrated using a rotary evaporator.
2. Experimental Animal Treatment
   The negative control group (KN) was given food and drink, the positive control group (KP) was given 0.75% ethylene glycol, treatment group 1 (P1) was given 0.75% ethylene glycol and ethanol extract of Batak onion at a dose of 250 mg/kg BW, treatment group 2 (P2) was given 0.75% ethylene glycol and ethanol extract of Batak onion at a dose of 500 mg/kg BW, treatment group 3 (P3) was given 0.75% ethylene glycol and ethanol extract of Batak onion at a dose of 750 mg/kg BW. All treatments were carried out for 30 days.
3. Preparation of Histopathology Preparations
   The kidneys, after adding fixative solution, were cut into two transverse and longitudinal sections and then placed in tissue cassettes. The tissue cassettes were then placed in a tissue processing unit, paraffin-embedded using a paraffin block, and sectioned using a microtome at a thickness of 3–4 μm. The tissue was then placed in a water bath at 46°C and placed on a glass slide. The tissue was then stained with hematoxylin and eosin and finally mounted on a cover glass using enthelene.
4. Observation of Histopathological Preparations
   Observation of the preparation on the histopathology score value was carried out using a binocular microscope at 40x magnification for 5 fields of view, for measuring the glomerular diameter using a trinocular microscope at 40x magnification for 5 fields of view. Observation of the kidney histology damage score value using the Manjha Roenigk damage criteria with the normal damage score variable = 1, bleeding (hemorrhage) / parenchymatous degeneration = 2, fatty degeneration / hydropic degeneration = 3, necrosis = 4. In observing the glomerular diameter the application used for visualization of histopathology images is Image J.
5. Data analysis
   Data from observations of histopathological preparations of white rat kidneys were collected and then analyzed statistically using SPSS one-way ANOVA. If a p value <0.05 was obtained, the Duncan test was continued.

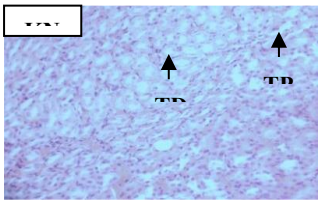
III. RESULTS AND DISCUSSION

The results obtained from observing the histological kidney damage scores for each treatment group showed that the positive control (KP) group had the highest score compared to the other treatment groups. The average histological kidney damage score can be seen in Table 1 below:

Table 1. Mean value of histopathological kidney damage score

Group	Mean Damage Score ± SD	P Value
Negative Control	22.40 ± 3.50a	
Positive Control	42.40 ± 1.81d	
Treatment 1	38.00 ± 2.55c	0,000
Treatment 2	34.40 ± 2.60bc	
Treatment 3	30.80 ± 3.19b	

The results of the one-way ANOVA test on the observation of the score value of kidney histology damage obtained a significance level value (p = 0.000) meaning that the administration of ethylene glycol and ethanol extract of Batak onion for 30 days had an effect on the histological damage to the kidneys of white mice (p <0.05). The histological damage to the kidneys obtained was in the form of bleeding (hemorrhage), hydropic degeneration, fatty degeneration and necrosis and can be seen in Figure 1.



following:

Fig 1 a. Histopathology of the kidneys of white rats in the negative control group, magnification 40x. TP: Proximal Tubule, TD: Distal Tubule

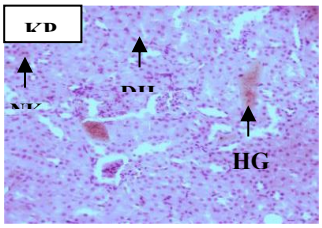


Fig 1 b. Histopathology of the kidneys of white mice in the positive control group, magnification 40x. HG: Hemorrhage, DH: Hydropic Degeneration, NK: Necrosis

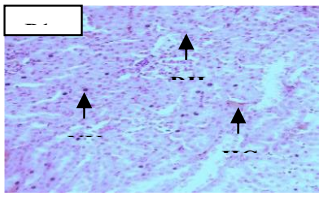


Fig 1 c. Histopathology of the kidneys of white mice in treatment group 1, 40x magnification. HG: Hemorrhage, DH: Hydropic Degeneration, NK: Necrosis

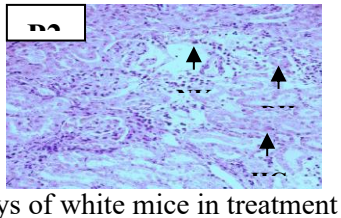


Fig 1 d. Histopathology of the kidneys of white mice in treatment group 2, magnification 40x. HG: Hemorrhage, DH: Hydropic Degeneration, NK: Necrosis

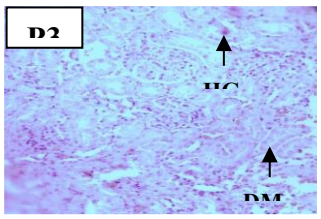


Fig 1 e. Histopathology of the kidneys of white mice in treatment group 3, 40x magnification. HG: Hemorrhage, DM: Fatty Degeneration.

OnGlomerular diameter observations showed that the positive control group (KP) experienced a reduction in glomerular diameter compared to the other treatment groups. The results of the glomerular diameter measurements can be seen in Table 2 below:

Table 2. Glomerular diameter measurement results

Group	Glomerular Diameter ± SD	P Value
Negative Control	125.59 ± 15.72d	
Positive Control	72.37 ± 9.76a	
Treatment 1	87.83 ± 3.99b	0,000
Treatment 2	98.17 ± 2.44bc	
Treatment 3	107.93 ± 3.93c	

The results of the one-way Anova test on the measurement of the glomerular diameter of the kidneys of white rats obtained a significance level value (p = 0.000), meaning that the administration of ethylene glycol and ethanol extract of Batak onion for 30 days had an effect on the glomerular diameter (p <0.05).

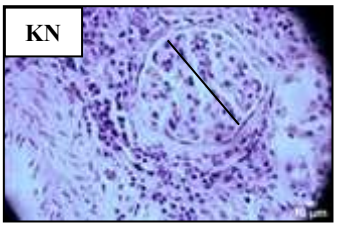


Fig 2 a. Glomerular diameter of white mice in the negative control group, 40x magnification

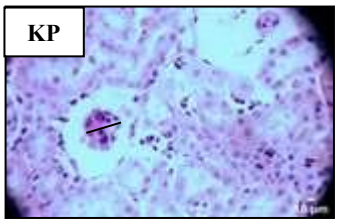


Fig 2 b. Glomerular diameter of white mice in the positive control group, 40x magnification

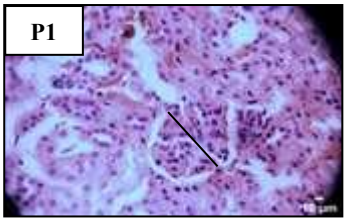


Fig 2 c. Glomerular diameter of white mice in treatment group 1, 40x magnification

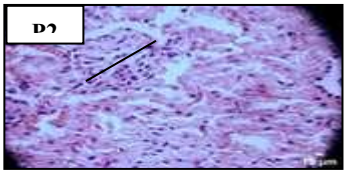


Fig 2 d. Glomerular diameter of white mice in treatment group 2, magnification 40x

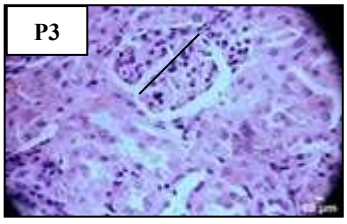


Fig 2 e. Glomerular diameter of white mice in treatment group 3, magnification 40x

Based on observations of the histopathological damage scores for the kidneys in the negative control group, the average kidney structure remained within the normal range. The nephron, such as the distal tubule, exhibited normal lumen structure and tubular cells. In the proximal tubule, an elongated lumen with a brush border and granular cytoplasm are seen, which are characteristics of normal proximal tubules.

Histopathological observations of the kidneys of white mice from the positive control, treatment 1, treatment 2, and treatment 3 showed kidney damage such as hydropic degeneration, fatty degeneration, hemorrhage (bleeding), and necrosis.Hemorrhage (bleeding) is a condition in which blood leaks from the blood vessels surrounding the renal tubules, usually caused by several factors, one of which is toxic substances. Hydropic degeneration is damage characterized by swelling in the tubular epithelial cells caused by a decrease in the amount of ATP in the cell membrane, resulting in the failure of the ion pump, disrupting the balance of fluid and ions in the tubular epithelial cells, which then ends in swelling of the cells. Fatty degeneration is caused by the accumulation of excess fat in the cytoplasm, characterized by vacuoles pushing the cell nucleus to the edge.

Induction with 0.75% ethylene glycol can form free radicals, namely Reactive Oxygen Species (ROS). In the renal tubules *Reactive Oxygen Species*(ROS) will react with cell membrane lipids which causes lysis of the cell membrane and endoplasmic reticulum so that fat accumulates in the cytoplasm7. Fatty degeneration is also a sign of severe damage which is the beginning of cell death (necrosis).Necrosis is cell death in tissue characterized by changes in the cell nucleus. Necrosis occurs due to the loss of cell membrane homeostasis, resulting in the entry of water and ions from the extracellular space into the cell. 10 In this study, the cell death (necrosis) phase was still in the pyknotic stage. Pyknotic is the initial stage of cell death, characterized by a shrinking and darker-colored cell nucleus.Observations of the glomerular diameter of the kidneys of white mice in the positive control group (KP) showed a shrinkage in glomerular diameter compared to other treatments. The shrinkage in glomerular diameter occurs due to the induction of ethylene glycol which causes damage to the glomerular capillaries, resulting in increased permeability that can reduce the glomerular filtration rate. Ethylene glycol induction can cause a shrinkage in glomerular diameter and an enlargement of the Bowman's space6. Damage to the glomerular capillaries will cause toxic substances, blood cells, and proteins to escape, accumulating in the space between the glomerulus and Bowman's capsule, causing the glomerulus to become compressed and shrink.

Overall, the treatment group given the Batak onion ethanol extract had lower histological scores and better glomerular diameter compared to the positive control group. This is due to the secondary metabolite compounds contained in the Batak onion ethanol extract, which act as antioxidants.The terpenoid compounds contained in the ethanol extract of Batak onion can prevent the adhesion of calcium oxalate crystals and the development of kidney stones caused by ethylene glycol. 5 The antioxidant compounds contained in the ethanol extract of Batak onion can reduce free radicals by the mechanism of donating one antioxidant electron to free radicals. Free radical electrons that enter due to ethylene glycol induction will react and bind with cell membrane molecules (lipid peroxide). This reaction will cause damage to the structure of the cell membrane. The antioxidant compounds contained in the ethanol extract of Batak onion will then donate one electron to the free radical so that the free radical is non-reactive.This ion stability will reduce oxidative stress in cells and tissues, resulting in an increased glomerular filtration rate. Annisa et.al (2021) also demonstrated that the antioxidant compounds in pineapple peel vinegar can increase the glomerular diameter of the kidneys of white rats with diabetes mellitus.

IV. CONCLUSION

Based on the research results, it can be concluded that administration of Batak onion ethanol extract affected the histopathological appearance of the kidneys of white rats. Doses of 500 mg/kg BW and 750 mg/kg BW were the most optimal doses for the histopathological damage score and glomerular diameter of the white rat kidneys. Further research is needed on toxic doses of ethylene glycol to determine the stages of cell damage (necrosis) in the form of karyoerexis and karyolysis.

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