

Renoprotective Effect Of Ekstrak Etanol Goat Weed (*Ageratum Conyzoides L*) Against Paraquat- Induced Wistar Rats

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Abstract

Pesticide poisoning is a public health problem in Asia-Pacific with 300,000 deaths. Paraquat is the active ingredient in long-used pesticides and is known to form ROS that damage vital organs such as the kidneys and liver. However, the negative impact of exposure to these pesticides can be reduced through the administration of endogenous antioxidants, which can neutralize ROS and protect body cells from ROS damage. Goat Weed plants contain flavonoids, alkaloids, essential oils, phenols, and coumarins, which have antioxidant effects. This study aims to determine the impact of goat weed plant extract (*Ageratum conyzoides L.*) on changes in rat kidney histology exposed to paraquat dichloride. The study involved 24 male Wistar rats with four treatments: K (distilled water), KN (paraquat), P1 (paraquat and goat weed extract 200 mg/kg BW), and P2 (paraquat and goat weed extract 400 mg/kg BW). Extracts and paraquat exposure were administered on days 1 to 7, with extracts administered at one-hour intervals after paraquat exposure. On the 8th day, rat blood samples were taken for MDA and urea testing, and kidneys were taken for histology preparations using the paraffin method and hematoxylin-eosin staining. The main parameters analyzed included kidney index ratio, kidney weight, glomerular area, inflammatory area, necrosis cells, MDA, and urea levels. Analysis was performed using ANOVA test and Duncan's further test ($p < 0.05$). The results showed significant improvements in liver index ratio, liver weight, hepatocyte cell area, inflammatory area, necrotizing hepatocyte cells, MDA, and urea in the 400 mg/kg BW dose treatment ($P < 0.05$). The conclusion shows that a dose of 400 mg/kg BW of goat grass ethanol extract can protect the kidneys and maintain MDA and urea levels of rats exposed to paraquat dichloride.

Keywords: Goat Weed; Histopathology; Kidney; Paraquat; Urea

Introduction

Farmers are the largest working group in many countries, including Indonesia. Pesticides are chemical compounds that have been used to eradicate pests and plant diseases in the agricultural and plantation sectors (Anggarani *et al.*, 2019). Pesticides commonly used in agriculture and plantations are paraquat. Paraquat is one of the active ingredients of *gramoxone-type* pesticides that have long been and are still widely used in crop cultivation throughout the world, including in Indonesia (Muhartono *et al.*, 2017). Paraquat dichloride has the general formula $C_{12}H_{14}Cl_2N_2$. Paraquat

dichloride has a molecular weight of 257.16 g/mol. The chemical name of *paraquat dichloride* based on IUPAC is *1,1-dymethyl-4,4bypyridium dichloride* (Cartaxo *et al.*, 2015).

Pesticide poisoning is a public health problem in developing countries with an estimated 300.000 deaths in the Asia-Pacific region. Paraquat affects the redox cycle and forms Reactive Oxygen Species (ROS) which are highly reactive free radicals (Subbiah & Tiwari, 2021). The formation of reactive oxygen causes toxicity to the organs (Muhartono *et al.*, 2017). The use of paraquat can damage various organs such as the heart, muscles, lymph, suprarenal

glands, and excretory organs such as the kidneys and liver (Muhartono *et al.*, 2017). Research results show that paraquat contamination can cause damage to the excretory system and blood (Alizadeh *et al.*, 2022). In the animal body paraquat forms superoxide (peroxide) which damages tissues, lungs, brain, heart, liver, and kidneys, causing death (Damayanti & Widiyanti, 2016). Blood accumulated with paraquat will activate the enzymes Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) so that their levels in the blood increase and indicate damage to the liver (Tsani *et al.*, 2017).

Indonesia's biodiversity as a tropical country is very high, and various plants that can be potential as natural herbal medicines are easily found (Matatula *et al.*, 2020). The goat weed plant (*Ageratum conyzoides* L.) is one of the disturbing weeds for cultivated plants, but previous research has proven that goat weed plants have allelochemical properties that can increase phenolic content which is an antioxidant (Chahal *et al.*, 2021). Goat weed leaves contain flavonoids, alkaloids, essential oils, phenols, and coumarins. The antioxidant effect of goat weed plants is due to the content of phenol compounds. Phenol compounds are the main class of antioxidants found in plants. Phenol compounds can reduce free radicals by donating their electrons through hydroxyl group hydrogen atoms (Halimatussa'diah *et al.*, 2014). Goat weed plants have an LC₅₀ value of 16.33 µg/ml against myeloma cells (Olowofolahan & Olorunsogo, 2021). The goat weed plant in Indonesia is still rarely utilized, so the purpose of this research is to determine the effect of goat weed extract on changes in kidney histopathology and reduce Malondialdehyde (MDA), and urea levels in the blood in rats exposed to paraquat.

Materials and Methods

This research was conducted at the Laboratory of Animal Structure and Physiology, Biology Study Program, Faculty of Applied Science and Technology, Universitas Ahmad Dahlan, Yogyakarta. The research was conducted based on procedures approved by the UAD Ethics Committee with the number

022404083. The goat weed plants used were obtained from community land located in Sorosutan, Umbulharjo District, Yogyakarta City. Plant identification was carried out at the Ecology and Systematics Laboratory, Ahmad Dahlan University according to number 341/Lab.Bio/B/VII/2023 with the scientific name *Ageratum conyzoides* L. Animal husbandry and animal testing were conducted at the Universitas Ahmad Dahlan Animal Structure and Physiology Laboratory.

Preparation of goat weed plant extract.

Goat weed plants were taken from the stems and leaves of as much as 15 kg and then made simplistic by drying using a cabinet dryer for 2 days. Simplisia was pulverized and extracted using the maceration method with 96% ethanol solvent for 3 days. The maceration results were then concentrated using a rotary evaporator with a temperature of 40°C until the extract preparation was obtained.

Animal Treatment

The study used Wistar rats (Male, 2-3 months old, weight ± 150 g) acclimatized for 7 days. Rats were grouped into 4 treatments consisting of Control (aquadest), Negative Control (exposed to paraquat), P1 (exposed to paraquat and given 200 mg/kg BW/day extract), and P2 (exposed to paraquat and given 400 mg/kg BW/day extract). Paraquat exposure was given as much as 1 mL/day for 7 days. Paraquat and goat weed extract were administered orally using a sonde for 7 consecutive days.

Preparation and Observation of Histopathology Preparations

On day 8 rats were anesthetized using 10% ether, then euthanized by dislocating the neck. Rats were dissected and kidney organs were taken, then weighed and calculated organ index (%). Organs were washed with physiological saline (10% NaCl) and fixed in 10% BNF solution. Organs were prepared using the paraffin method with hematoxylin-eosin dye. Parameters observed in the kidney organs were the area of inflammation, glomerular diameter, glomerular area, number of glomeruli, number of cells undergoing necrosis in the glomerular area, and

tubules. Observation of histopathology analysis using Image Rester application to see the area of inflammation, necrosis, and glomelurus area.

Data Analysis

All parameters of MDA, urea, and kidney histopathology test results were analyzed using the Analysis of Variance (ANOVA) statistical test. If the results showed a significant difference ($P < 0.05$), further analysis was carried out using Duncan's New Multiple Range Test (DMRT) with a 95% confidence level.

Results and Discussion

Kidney Histopathology

The study showed that the levels of MDA and urea in the blood of rats during treatment showed a change, namely, there was a decrease in MDA and urea levels in P2. The results of the ANOVA test showed that there were significant differences in the MDA levels of rats treated with KN and P2. MDA and urea levels in KN were higher than in other treatments. This is due to the effect of paraquat exposure which can increase MDA and urea levels in the blood. Paraquat increases free radical formation and oxidative stress (Hu *et al.*, 2018). Paraquat causes toxic effects through the production of superoxide anions in living organisms that cause oxidative damage and cell death (Adelakun *et al.*, 2022). Paraquat can reduce superoxide dismutase

(SOD) activity and increase malondialdehyde (MDA) in the organism (Alizadeh *et al.*, 2022). High levels of MDA and urea indicate levels of free radicals in the body. Increased urea levels can indicate the occurrence of kidney function failure (Suryawan *et al.*, 2016).

The administration of the extract for 7 days showed significant differences in MDA and urea levels in P1 and P2 ($P < 0.05$), which were lower than KN (Figures 1 and 2). This is thought to be due to the influence of goat weed leaves such as phenol content which acts as an antioxidant that can protect cells from free radicals (Halimatussa'diah *et al.*, 2014).

Observation of the histopathological structure of the kidney (Figure 3) shows that the kidney is composed of glomerulus and is enveloped by bowman's capsule. The glomerulus can be damaged and shrunk by free radicals caused by paraquat exposure. Exposure to paraquat will cause infiltration of inflammatory cells to the center of the damage, resulting in an inflammatory area (Amin *et al.*, 2020). Tissue areas with small glomerular structures, inflammatory areas, and large numbers of necrosis cells are indicators of microscopic organ damage in the kidney (Hermanto *et al.*, 2022). Figure 4. shows that P1 and P2 have fewer necrotizing glomerular cells compared to KN and show the same glomerular shape and area as the control.

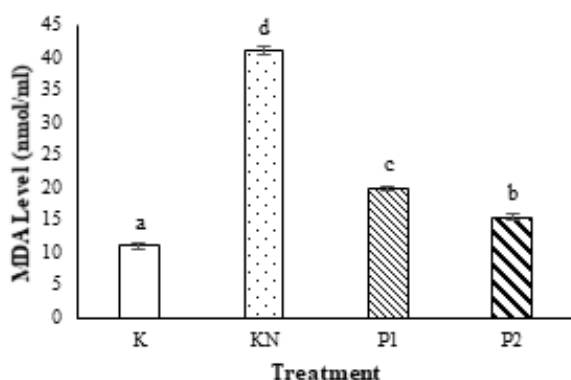


Figure 1. MDA levels of rat kidney in the treatments. K : Control (0 mg/kg BW extract and not exposed to paraquat chloride), Negative Control/KN (exposure to paraquat chloride without extract administration), P1 (200 mg/kg BW extract and exposure to paraquat chloride), and P2 (400 mg/kg BW extract and exposure to paraquat chloride); Mean $\pm$ SD; a-d Different superscripts in the same column indicate significant differences ($p < 0.05$).

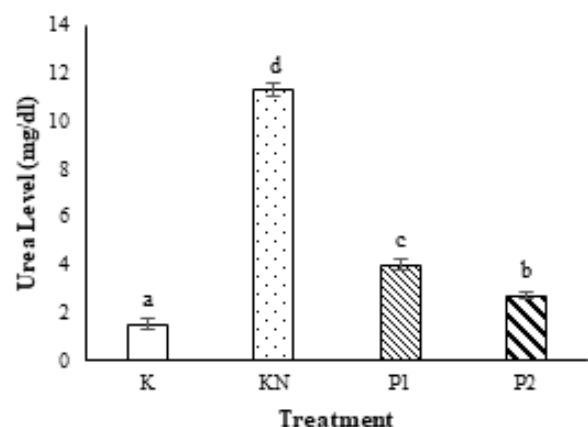


Figure 2. Urea levels of rat kidney in the treatments. K : Control (0 mg/kg BW extract and not exposed to paraquat chloride), Negative Control/KN (exposure to paraquat chloride without extract administration), P1 (200 mg/kg BW extract and exposure to paraquat chloride), and P2 (400 mg/kg BW extract and exposure to paraquat chloride); Mean $\pm$ SD; a-d Different superscripts in the same column indicate significant differences ($p < 0.05$).

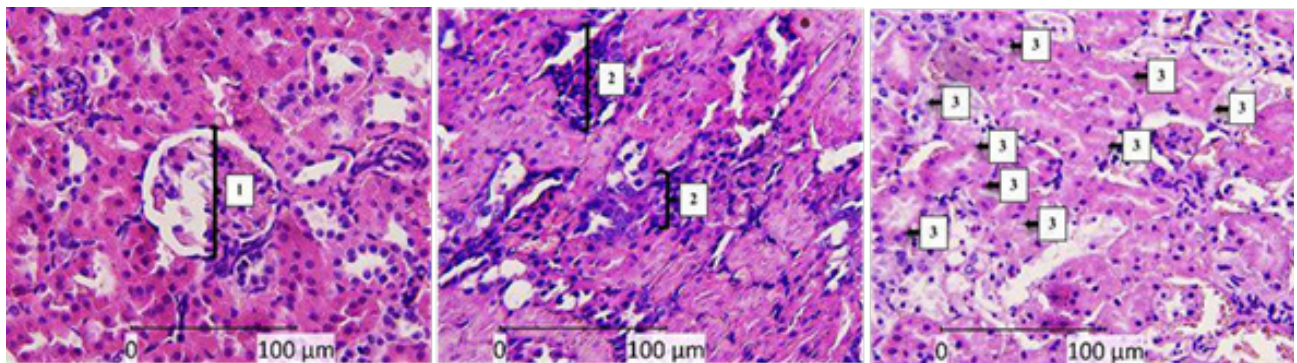


Figure 3. Histopathologic structure of rat kidney found in the treatment. Notes: 1. Glomerular diameter; 2. Inflammatory area; 3. Necrosis cells; Hematoxylin eosin staining; Scale bar 100 μ m.

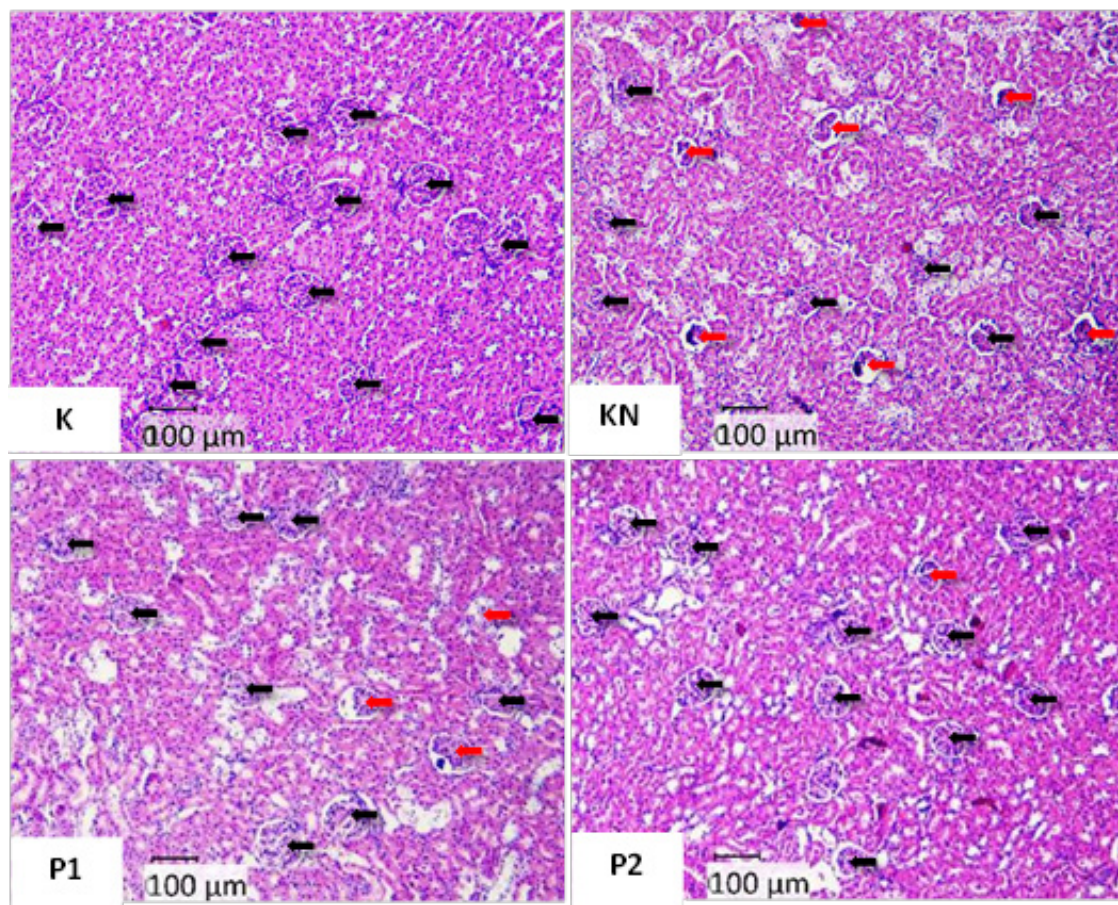


Figure 4. Histopathologic structure of rat kidney in the treatments. K : Control (0 mg/kg BW extract and not exposed to paraquat chloride), Negative Control/KN (exposure to paraquat chloride without extract administration), P1 (200 mg/kg BW extract and exposure to paraquat chloride), and P2 (400 mg/kg BW extract and exposure to paraquat chloride). Black arrows (glomerulus), red arrows (glomerulus necrosis), black arrows (glomerulus necrosis), and red arrows (glomerulus necrosis).

glomerulus showed that there were significant differences in the diameter and area of the glomerulus ($P < 0.05$). Glomerular diameter showed the smallest size in KN, while the highest was in K. Glomerular area showed that P2 was higher than the other treatments, and the smallest glomerular area was in KN, as shown in (Table 1). Reactive oxygen species

(ROS) are free radicals produced during normal cellular metabolism. Continuous administration of paraquat dichloride can increase ROS levels (Kong *et al.*, 2019). ROS are known to damage renal endothelial cells, glomerular mesangial cells, and renal tubular epithelial cells, affecting kidney function and structure (Fakhruddin *et al.*, 2017).

Table 1. Histopathology Structure of Kidney in Various Treatments

Variable	C	CN	P1	P2
Kidney Weight (g)	0,97 ± 0,09 ^b	0,80 ± 0,10 ^a	0,91 ± 0,08 ^{ab}	0,97 ± 0,08 ^b
Kidney Index (%)	0,53 ± 0,01 ^a	0,96 ± 0,13 ^c	0,75 ± 0,10 ^b	0,62 ± 0,03 ^{ab}
Inflammatory area (mm ²)	1,28 ± 0,21 ^a	2,91 ± 0,21 ^b	1,41 ± 0,22 ^a	1,22 ± 0,26 ^a
Number of Glomerular necrosis Cells	2,20 ± 0,44 ^a	9,20 ± 0,44 ^c	3,80 ± 0,83 ^b	2,00 ± 0,70 ^a
Number of Tubular necrosis Cells	13,00 ± 1,58 ^a	31,20 ± 0,83 ^d	22,80 ± 1,30 ^c	15,60 ± 1,14 ^b
Glomerular Diameter (µm)	80,44 ± 3,73 ^c	57,74 ± 2,03 ^a	72,89 ± 3,69 ^b	79,04 ± 2,62 ^c
Glomerular area (mm ²)	3,98 ± 0,31 ^c	2,34 ± 0,19 ^a	3,05 ± 0,55 ^b	4,12 ± 0,61 ^c
Number of Glomerulus	23,40 ± 1,82 ^c	11,00 ± 1,22 ^a	21,20 ± 1,48 ^b	25,00 ± 1,00 ^c

Description: Control (0 mg/kg BW extract and not exposed to *paraquat chloride*), Negative Control/CN (*paraquat chloride* exposure without extract administration), P1 (200 mg/kg BW extract and *paraquat chloride* exposure), and P2 (400 mg/kg BW extract and *paraquat chloride* exposure). Mean ± SD: a-d Different superscripts in the same column indicate significant differences (p<0.05).

Reactive Oxygen Species (ROS) as a key regulator of cell signaling is also involved in regulating cell growth, development, inflammation, and apoptosis (Zhang *et al.*, 2020). Paraquat can cause severe damage to vascular endothelial cells (Hu *et al.*, 2018). The volume of distribution of paraquat is 1.2-1.6 L/Kg, excreted mainly through urine, with a rate of 82.9% in 48 hours (Shahzad *et al.*, 2017). Giving goat weed ethanol extract gave the highest improvement in the treatment (P2). The results showed the best results were close to (K). This is because goat weed extract contains several phytochemical compounds such as flavonoids, terpenoids, and alkaloids (Thorat *et al.*, 2018). These phytochemical compounds act as antioxidants in capturing free radicals (Setiawan *et al.*, 2020). Phytochemicals are natural compounds in plants that function as protective agents against disease and damage (Forni *et al.*, 2019).

Cells that are damaged will regenerate themselves, and ethanol extract from goat weed can significantly reduce oxidative stress and inflammation (Bahtiar *et al.*, 2017). This is due to antioxidant activity (Nasrin *et al.*, 2013; Jasvidianto *et al.*, 2020). Antioxidants protect the body by counteracting incoming free radicals (Hilaliyah, 2021). In addition, antioxidants in goat weed can also cause anti-inflammatory effects, this is due to the presence of phenol content (Trinh *et al.*, 2020). The anti-

inflammatory mechanism is by inhibiting the activity of the enzyme cyclooxygenase (COX) so that prostaglandins are not formed which causes inflammation (Saputri *et al.*, 2020). Our study showed a decrease in inflammation in rat kidney tissue after administering goat weed extract. Phenol, alkaloid, and flavonoid compounds can act as antioxidants to fight free radicals (ROS) and reduce oxidative stress that causes necrosis (Pay *et al.*, 2022). Flavonoids also play a role in inhibiting cells that cause inflammatory reactions (macrophages and neutrophils). So MDA and urea levels in rats exposed to paraquat dichloride and given goat weed plant extract levels are close to K treatment rats, although the decrease is greater in P2 compared to P1.

Conclusion

The results concluded that the administration of ethanol extract of goat weed plants at a dose of 400 mg/kg BW for 7 days was able to repair damage to the kidney organs of Wistar rats given exposure to paraquat dichloride. The suggestion of this study is to test the levels of SGOT and SGPT in the blood of wistar rats after being given goat weed plant extract to determine antioxidant activity in the body.

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