

Efek Antioksidan Taurin dalam Menurunkan Kerusakan Ginjal Mencit Jantan (*Mus musculus*) Akibat Stres Oksidatif yang Diinduksi Paraquat

Taurine Antioxidant Effect in Decreasing Kidney Damage in Male Mice (*Mus Musculus*) due to Oxidative Stress Induced by Paraquat

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ABSTRACT

Paraquat toxicity occurs through the high production of reactive oxygen species (ROS) which cause damage due to oxidative stress. Antioxidants can reduce damage through prevention of oxidative stress. Taurine has shown the ability to act as an antioxidant. The aim of this research is to find a effect of antioxidant taurine to decrease kidney damage caused by oxidative stress due to paraquat by looking at the histopathology changes. 25 male adult mice from strain DDY were used and divided into five treatment groups; C(-) (Aquadest IP), C(+) (Paraquat 30mg/kg), P1 (Paraquat 30mg/kg + Taurine 250mg/kg), P2 (Paraquat 30mg/kg + Taurine 500mg/kg) and P3 (Paraquat 30mg/kg + Taurine 1000mg/kg). All groups were given treatment intraperitoneal for twenty one days. The mice were sacrificed where kidney were collected for histopathology preparation. The parameters measured were renal histopathological changes in form of degeneration and necrosis. The results show that taurine administration had an effect on decreased degrees of damage to kidney tubular cells, with a decrease in the mean degree of renal tubular degeneration and necrosis. Degeneration of renal tubular cells in groups (P2) reduced compared to the group (C+) there were significant differences ($P < 0.05$). Necrosis of renal tubular cells in groups (P1, P2) reduced compared to the group (C+) there were significant differences ($P < 0.05$). In conclusion, this research proves that administration of paraquat causes renal histopathological changes which are characterized by degeneration and necrosis. It also proves that taurine dose of 500 mg/kgBB could provided optimal effect.

Keywords: paraquat, taurine, renal tubular cells, degeneration, necrosis

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INTRODUCTION

Herbicide poisoning is one of the problems experienced by developing countries including Indonesia, the use of herbicides in agriculture and plantations showed an increase in the quality and quantity of agricultural products in line with the use of herbicides. Herbicides themselves are not harmful to humans and animals if in low doses, but when low doses accumulate in the body will cause

damage at body organs, id est eyes, skin, respiratory system, liver, heart, kidneys, and gastrointestinal tract (Windarti et al., 2015).

Paraquat is a toxic substance from the group Bipiridulum is one of the commonly used herbicides in Indonesia (Rutherford et al., 2011). Poisoning case in Indonesia shows about 0.3% caused by exposure to herbicides. One of type herbicide ever reported cause poisoning is paraquat group (Sembodo, 2010). The

use of it often does not pay attention to the warning signs, so the risk of exposure to hazardous materials becomes high and increase the residue in plants that are forage feed for livestock. Entrance route paraquat into the body can be through various exposure routes among them are contacted with skin, mucosa, inhalation, and swallowed. Paraquat that enters the body will form the Reactive Oxygen species (ROS), ROS are chemical compounds that belong to free radicals (Wang et al., 2014).

Kidney as the main excretory organ has the function to filter every filtrate circulating in the body includes toxic substances, drugs, and residual metabolic material circulating will be excreted through the kidney (Aryana et al., 2016). The kidney is the main organ responsible for paraquat excretion, the resultant kidney injury may reduce the elimination of paraquat and increase its toxicity in other organs. Known this compound can accrue within renal tubular cells, leading to cycles of reduction and oxidation, generating reactive oxygen species, and ultimately damaging the tubules (Weng et al., 2017). Accumulation of paraquat in the body will certainly change the normal kidney structure toward pathological conditions. This abnormal state will cause impaired renal function, this will lead to various complications of kidney disease (Williams et al., 2016).

Taurine has been reported play an important role as antioxidant (Jong et al., 2012). Administration of taurine supplementation only shows in low to high doses does not cause any side effects of this compound (Waldron et al., 2018).). This substance plays a lot in the physiological process since taurine plays an important role in cell membrane stabilization, modulation of calcium intracellular levels, osmoregulation and detoxification pathways (Maysa et al., 2016).

Based on this problem it is necessary This research aims to find a effect of antioxidant taurine to decrease kidney damage caused by oxidative stress due to paraquat by looking at the histopathology changes.

MATERIALS AND METHODS

The samples used in the study was 25 male mice (*Mus musculus*) aged 2-3 months with an average weight of 33-37 grams. All of the mice should be in good condition and healthy and were be obtained from Pusat Veteriner Farma (PUSVETMA) Surabaya. The manufacture and observation of mice kidney histopathology slides was conducted in the Veterinary Pathology Department of the Faculty of Veterinary Medicine Universitas Airlangga. The study was conducted in February - March 2021. The 25 DDY strain male mice (*Mus musculus*) were divided into five groups, each group consisting of five mice each. Acclimatation will be carried out for seven days before therapy is applied with the aim of avoiding stress in animals. The study was conducted with five treatments in which each treatment there are five animals that are:

1) Negative Control Treatment (C-): Group of mice that not induced with paraquat and only given aquadest intraperitoneally and given aquadest intraperitoneal as much as 0.4 cc.

2) Positive Control Treatment (C+): Groups of mice were induced by paraquat at a dose of 30 mg/kgBW intraperitoneal and given aquadest intraperitoneal as much as 0.4 cc.

3) Treatment 1 (P1): Groups of mice were induced by paraquat solution at a dose of 30 mg/kgBW intraperitoneal and treated with an taurine dose of 250 mg / kgBW intraperitoneal.

4) Treatment 2 (P2): Groups of mice were be induced by paraquat solution at a dose of 30 mg/kgBW intraperitoneal

and treated with an taurine dose of 500 mg / kgBW intraperitoneal.

5) Treatment 3 (P3): Groups of mice were be induced by paraquat solution at a dose of 30 mg/kgBW intraperitoneal and treated with an taurine dose of 1000 mg/kgBW intraperitoneal.

Paraquat induction was performed intraperitoneally, induction is carried out twice a week, exactly 1 day after the acclimatation period. Administration of taurine was administered once a day intraperitoneally. Mice (*Mus musculus*) after 21 days of therapy was euthanized using cervical dislocation, then performed abdominal surgery for the retrieval of kidney organs. The retrieval of kidney organs is done carefully to avoid damage to the organ tissues. then

fixed with 10% formalin Subsequently, histopathological preparations were made with HE stain.

Kidney histopathology preparation examination was used a light microscope with 400 magnification of five different viewing fields for each slide. The easiest and most commonly observed kidney damage is tubular damage, making kidney damage easier to observe through scores of tubular damage. for tubular damage scores, *i.e.* Degeneration of tubular epithelial cells, and tubular cells necrosis. Lesions and scores of renal histopathological changes can be seen in table 1.

Table 1. Renal Histopathology Scoring (Racusen *et al.*, 1999).

Types of lesions	Degree of damage	Scoring
Degeneration of tubular cells	1. No degeneration	0
	2. <25% degeneration of the tubular epithelium	1
	3. 25%-50% degeneration of the tubular epithelium	2
	4. >50% degeneration of the tubular epithelium	3
Tubular cells necrosis	1. No necrosis	0
	2. <25% necrosis of the tubular epithelium	1
	3. 25%-50% necrosis of the tubular epithelium	2
	4. >50% necrosis of the tubular epithelium	3

RESULT

Kidney histopathological observation scores were analyzed using the Kruskal Wallis test at a confidence level of 5% ($P < 0.05$) for each treatment, followed by the Mann-Whitney test. The results of the antioxidant effect of taurine on the results of the average rank and standard deviation of the rates of degeneration and necrosis of mice renal tubular cells can be seen in the table 2.

Table 2. Mean Rank and Standard Deviation of Degeneration and Necrosis Rate (mean rank \pm SD) of Renal Tubular Cells of Male Mice.

Treatment Group	Mean Rank \pm SD	
	Degeneration	Necrosis
C (-)	6.70 ^a \pm 0.729	6.50 ^a \pm 0.521
C (+)	19.60 ^b \pm 0.502	20.40 ^b \pm 0.558
P1	15.70 ^{ab} \pm 0.540	14.10 ^a \pm 0.141
P2	9.30 ^a \pm 0.540	9.50 ^a \pm 0.443
P3	13.70 ^{ab} \pm 0.766	14.50 ^{ab} \pm 0.616

Description: Different superscripts (a,b) in the same column represent a

significant difference between the treatment groups ($p < 0,05$).

The results of the microscopic picture of the kidney tissue of mice in the C (+) experienced massive degeneration as seen from the occurrence of hydropic degeneration (cloudy swelling) with a severity of up to a score of 3, meaning that there were degenerative changes occurring in $>50\%$ of the entire field of view. Control groups C (-) and treatment P2 showed a significantly different picture while, P1 and P3 showed insignificant differences to group C (+).

The results of the microscopic images of the kidney tissue of mice in the K⁺ treatment experienced necrosis with a severity of up to a score of 3, meaning that there were necrosis changes occurring in $>50\%$ of the entire field of view. Control groups C (-), treatment P1, and P2 showed a significantly different picture while P3 showed insignificant differences to group C(+).

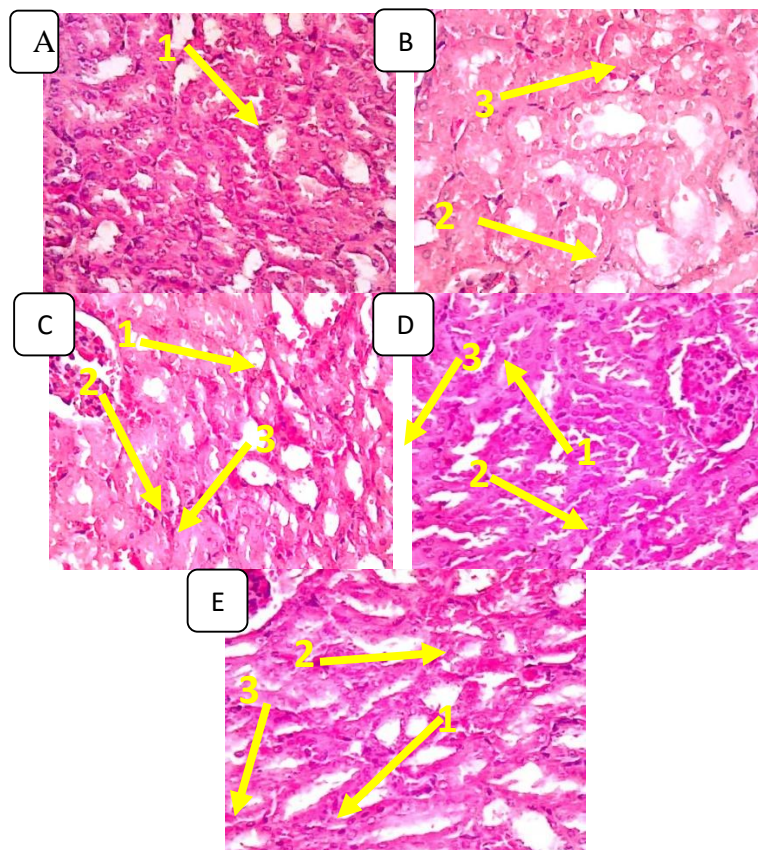


Figure 1. Microscopic image of renal tubular cells of male mice (HE staining and 400x magnification on all group. A. C (-): (1) normal renal tubular cells, B.C (+):(2)

renal tubular cell degeneration (3) renal tubular cell necrosis, C. P1: (1) normal renal tubular cells (2) renal tubular cell degeneration (3) renal tubular cell necrosis, D. P2: (1) normal renal tubular cells (2) renal tubular cell degeneration (3) renal tubular cell necrosis, E. P3: (1) normal renal tubular cells (2) renal tubular cell degeneration (3) renal tubular cell necrosis.

DISCUSSION

The excretion of absorbed paraquat (PQ) is carried out by the renal system, through the formation of urine to eliminate almost completely paraquat in an unchanged form, performed by glomerular filtration and active tubular secretion (Oliveira et al., 2008). In the kidney, paraquat shows a lesion that are degeneration and necrosis of the proximal and distal tubular epithelium (WHO, 1984). The renal tubules are the parts that are usually damaged by exposure to toxins. The tendency of the renal tubules to be easily damaged is a result of the renal tubules reabsorb 60% to 80% of the glomerular filtration products. Another issue is expounded to the massive surface area reabsorbed by the renal tubules, the transport system of ions and organic acids, and therefore the ability of the renal tubules to concentrate ions, organic acids, proteins, peptides and heavy metals, that results in accumulation and toxicity within the renal tubular cell (Schnellman and Goldstein, 2001).

Paraquat cause toxicity occurs through the process of oxidative stress due to the exceeds production of Reactive Oxygen Species (ROS) (Ujowundu et al., 2018). Reactive oxygen species can cause damage to the structural membranes of renal tubular cells through the mechanism of lipid peroxidation (Tomsa et al., 2019). Lipid peroxidation causes an increase in membrane permeability and disrupts ion gradient in cells which can cause cell damage (Gaschler and Stockwell, 2017). Cell damage due to exposure to Reactive Oxygen Species (ROS) causes reversible damage i.e. acute cell swelling or hydropic degeneration, and if exposure is continued, acute cell swelling may progress to the point where it does not

recur irreversible damage (necrosis) will occur. Reversible acute cell swelling can emerge as irreversible and development to cell death. In severe, persistent or repeated exposure, acute cell swelling can development beyond the "point of no return" and end up an early degree in the necrosis process (Miller and Zachary, 2017). Necrosis is shown by microscopic images in the form of pyknosis, cariorexis, karyolysis, and cytoplasmic eosinophilic staining (Sotres et al., 2016).

Lipid peroxidation leads to a decrease in oxidative metabolism, a decrease in ATP production due to mitochondrial damage, and the entry of calcium (Ca^{2+}) into the mitochondria, which changes the activity of the Na^+K^+ pump and changes the regulation of cell volume, which leads to it an increase in intracellular calcium. Direct damage to the plasma membrane by lipid peroxidation can have the same effect, namely a dysfunction of the cell volume regulation and an influx of Ca^{2+} , the inability to restore mitochondrial function and cell membrane damage will result in irreversible damage (Miller and Zachary, 2017).

Taurine has been shown to protect cells from lipid peroxidation. Shows that taurine acts as a radical scavenger, a scavenger of reactive oxygen and nitrogen species that cause oxidative damage (Oliveira et al., 2010). Taurine also act as indirect antioxidant show ability to suppress alterations in membrane caused by oxidative stress by stabilize membrane permeability, act as osmoregulator, regulate ion calcium, and enhances the activity of antioxidant enzymes in cellular system. This causes the extracellular water to be unable to penetrate the cell membrane so as to

prevent cell swelling (Baliou et al., 2021). The ability of taurine to increase the activity of the enzyme superoxide dismutase, glutathione peroxidase and catalase which are endogenous antioxidants, endogenous antioxidants can prevent injury due to oxidative stress. (Yu and Kim, 2009)

The treatment group that received the highest dose of taurine in this study, it was expected to get better results than the other treatment groups, but instead showed degeneration that was not significantly different from group C (+). This shows that the administration of taurine 1000 mg/kgBW/day is not good enough to overcome oxidative stress that causes degeneration. It is possible that lower levels of taurine supplementation will increase protection from injury (Dawson et al., 2002). In the group given the highest dose of taurine, the antioxidant activity of taurine decreased, there was a possibility that the administration of taurine at high concentrations could cause some side effects (Dawson et al., 2002). According to Sotler et al (2019) antioxidants can act as prooxidants, chemicals that induce oxidative stress. Because the effect of the concentration of added antioxidants can affect the rate of oxidation. The concentration of antioxidants in the matrix environment can affect the function of antioxidants turning them into prooxidants at high concentrations, antioxidant activity often disappears, even antioxidants become prooxidants.

CONCLUSIONS

In this research, it can be concluded that the dose of 500mg/kgBW/day is the optimal dose of taurine that has a positive effect in reducing kidney damage in form of degeneration and necrosis in male mice (*Mus musculus*) due to oxidative stress induced by paraquat.

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