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# Effects of Tapak Dara Leaves Extract on Kidney Histology of Aspartame-Induced Mice

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## ABSTRACT

This study aimed to determine the effect of *Catharanthus roseus* leaves extract on kidney histology of mice aspartame-induced. Mice were divided into 4 groups, namely the negative control group (K-) without treatment, the positive control (K+) given aspartame 0.02 mg/day, the dose group 1 (D1) was given aspartame 0.02 mg/day and continued with the *C. roseus* leaves extract at a dose of 0.42 mg/kg. Group 2 (D2) was given aspartame 0.02 mg/day followed by *C. roseus* leaf extract at a dose of 0.84 mg/kg BW. IP administered aspartame for 14 days, and *C. roseus* leaves extract was administered by gavage for 12 days. The data obtained were analyzed by One Way ANOVA and DMRT test with a significance level of 0.05. The results showed that the induction of aspartame at a dose of 0.02 mg/day in mice increased the average number of proximal tubular necrosis cells. *Catharanthus roseus* leaves extract administration at 0.42 mg/kg BW and 0.84 mg/kg BW for 12 days can reduce the number of proximal tubular necrosis cells in aspartame-induced mice. Thus it can be concluded that the administration of *C. roseus* leaves extract can reduce the proximal kidney tubules necrosis but has not been able to reach normal conditions yet.

***Efek Pemberian Ekstrak Daun Tapak Dara terhadap Histologi Ginjal Mencit yang Diinduksi Aspartam***

***ABSTRAK:*** *Tujuan penelitian ini adalah untuk mengetahui pengaruh ekstrak daun Catharanthus roseus terhadap histologi ginjal mencit yang diinduksi aspartam. Hewan uji dibagi menjadi 4 kelompok yaitu kelompok kontrol negatif (K-) tanpa perlakuan, kontrol positif (K +) diberi aspartam 0,02 mg/hari, kelompok dosis 1 (D1) diberi aspartam 0,02 mg/hari dan dilanjutkan pemberian ekstrak daun C. roseus dengan dosis 0,42 mg/kg, dan kelompok 2 (D2) diberi aspartam 0,02 mg/hari dilanjutkan pemberian ekstrak daun C. roseus dengan dosis 0,84 mg/kgBB. Pemberian aspartam dilakukan secara IP selama 14 hari dan pemberian ekstrak daun C. roseus diberikan secara gavage selama 12 hari. Data yang diperoleh dianalisis One Way Anova dan uji DMRT dengan taraf signifikansi 0,05. Hasil penelitian menunjukkan bahwa induksi aspartam dengan dosis 0,02 mg/hari pada mencit meningkatkan rata-rata jumlah sel nekrosis tubulus proksimal. Pemberian ekstrak daun Catharanthus roseus 0,42 mg/kgBB dan 0,84 mg/kg BB selama 12 hari dapat menurunkan jumlah sel nekrosis tubular proksimal pada mencit yang diinduksi aspartam. Dengan demikian dapat disimpulkan bahwa pemberian ekstrak daun C. roseus dapat menurunkan*

*kerusakan tubulus proksimal ginjal namun belum dapat mencapai kondisi normal.*

## INTRODUCTION

Artificial sweetener is an ingredient added to food or drink that can give a sweet taste (Hadju et al., 2013). Aspartame is one of the artificial sweeteners widely used in processed foods and beverages because it is relatively cheap and has a sweetness level of 180-200 times sweeter than sucrose (Dali et al., 2013; Felton, 2006; Wiratmoko & Nurmalasari, 2014). Aspartame is a non-calorie sweetener. Its use is approved by the FDA (*Food and Drug Administration*), which sets an ADI (*Acceptable Daily Intake*) of 50 mg/kg BW/day. At the same time, BPOM sets an ADI at 40 mg/kg BW/day (Abechi et al., 2014; BPOM, 2014; Feehley & Nagler, 2014). The use of aspartame with doses that exceed the limit or be consumed continuously in the long term can endanger the health of the body (Wiratmoko & Nurmalasari, 2014).

Aspartame will undergo metabolism into aspartic acid, phenylalanine and methanol (Sweetman, 2009) in the liver. Methanol is known to have toxic effects on the body because methanol is oxidized to formaldehyde (Hamidah & Yulianti, 2017; Parthasarathy et al., 2006). Formaldehyde can increase the production of *Reactive Oxygen Species* (ROS) compounds to cause oxidative stress (Rohmani et al., 2015; Widayati, 2021). Furthermore, oxidative stress can cause cells, tissues, and organs (Susatiningsih, 2015). One of the body organs that can be affected by oxidative stress due to exposure to aspartame is the kidney (Othman & Bin-Jumah, 2019).

Madboly et al. (2019) said the kidney structure includes capsule, cortex and medulla. In the cortex, there are nephrons which are the structural and functional units of the kidney (Eroschenko, 2008). One of the constituents of the nephron is the proximal tubule which is the part that is most susceptible to damage due to oxidative stress due to an increase in ROS. ROS can react with lipid membranes, resulting in membrane permeability changes that can cause necrosis (Barrera, 2012). Irawati (2007) stated that the administration of aspartame at a dose of 20 mg/20g BW/day by gavage in mice for 28 days resulted in necrosis of the proximal renal tubule. The research of Madboly et al. (2019) also showed that administration of aspartame at a dose of 250 mg/kg/day in rats for 2 months by gavage caused damage to the proximal renal tubule, which was characterized by loss of the *brush border*. However, the role of renal participation in the assimilation of

many substances, including aspartame, have not been considered (Gabr et al., 2011), so it is important to determine the effect of aspartame on renal histology, especially in proximal tubules necrosis.

Kidney damage due to oxidative stress can be inhibited and prevented by administering antioxidants (Wahyuono et al., 2017). One of the antioxidant compounds that play a role in preventing cell damage due to free radicals is flavonoids (Redha, 2010). One medicinal plant containing antioxidant compounds is tapak dara (*Catharanthus roseus*) (Verrananda et al., 2019). The results of the phytochemical test showed that the ethanol extract of tapak dara leaves contained alkaloids, flavonoids, quinones, saponins, polyphenols, and triterpenoids (Fitrianingsih, 2010).

Flavonoids can donate electrons into free radicals to stabilize radical compounds so that they can inhibit oxidation reactions (Akhlaghi & Bandy, 2009; Dewi et al., 2018). Research conducted by (Basith et al., 2016) stated that basil leaves extract (*Ocimum sanctum*) containing flavonoids, which was administered by gavage using the gastric probe method at a dose of 74 mg/20 g for 14 days, could inhibit paracetamol-induced damage to mice's proximal tubular epithelial cells. Likewise, extracts of lakum fruit (*Cayratia trifolia*) at a dose of 230 mg/200g

BW containing flavonoids, given by gavage for 7 days, could prevent damage to the proximal kidney tubules of rats induced by paracetamol (Kurniadi et al., 2018). So far, the study of the effect of aspartame on the kidney has been done; however, the potential effect of Tapak Dara leaves extract to cope with this effect is not yet known. The previous study showed that 5.2 grams of rosy periwinkle (*Catharanthus roseus*, L.) which is boiled in 300 ccs of water until the remaining 100 ccs and given as much as 3.6 cc / oral /day for 7 days in Wistar male rat reduced urea and creatinine levels after treated by gentamycin (Situmeang & Sudharmono, 2019). This indicated that *Catharanthus roseus* potent to the recovery of kidney histological damage. So, the purpose of this study was to determine the effect of administration of *C. roseus* leaves extract on the kidney histology of mice that had been exposed to aspartame.

## METHOD

### Time and Place of Research

This research was conducted from March to August 2020 at the Zoology Laboratory and Botanical Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Jember.

### Research Design

This research employed a completely randomized design (CRD). The test animals were divided into 4 groups, and each treatment group was repeated 6 times. The following is the division of the test groups in this study:

Negative control group: a control group of mice without aspartame or tapak dara leaves extract treatment.

Positive control group: treatment group with aspartame dose of 0.02 mg/day for 14 days.

Dosage group 1: treatment group with aspartame dose of 0.02 mg/day for 14 days, followed by tapak dara leaf extract 0.42 mg/kg BW for 12 days.

Dosage group 2: treatment group with aspartame dose of 0.02 mg/day for 14 days, followed by administration of tapak dara leaf extract 0.84 mg/kg BW for 12 days.

### Tapak Dara Leaves Extract Preparation

Fresh tapak dara leaves were obtained from Blado Kulon village Probolinggo district, cleaned first with running water and then drained. Furthermore, the leaves were dried using an oven at $50^0$C for 4 days, then blended and sieved using a 100 mesh sieve to obtain a powder of tapak dara. The powder was then macerated with 70% ethanol in a ratio of 1:10 for 48 hours. Furthermore, evaporation is carried out using a *rotary evaporator* at a temperature of $80^0$C. The extract is then concentrated at $70^o$C with a water *bath* so that the extract is obtained in the paste form (Aisyah, 2018).

### Animal Test Preparation

The animals used in this study were 24 male mice *(Mus musculus*). The mice were placed in cages with rice husks and sterile wood sawdust covered with iron wire. They were fed by BR-1 pellet as much as 1/10 of their body weight and drank *ad libitum*.

### Animal Treatment

The administration of tapak dara leaves extract with a volume of 1 ml using aquades as a solvent and carried out *orally by gavage*. Aspartame 1 mL was administered intraperitoneally using distilled water. Aspartame exposure was carried out every day for 14 days. The treatment of tapak dara leaves extract was carried out the day after the aspartame treatment and was given every day for 12 days.

### Kidney Histology Preparations

On the $27^{th}$ day, the mice were anesthetized with chloroform and placed on a surgical board in a supine position. Then the test animal was dissected, and an incision was made on the ventral skin. Histological preparations were made using

the paraffin method. The right kidney was removed and washed with 0.9% NaCl.

## Observations

Observations were made by calculating the number of epithelial cells in the proximal tubules that experienced necrosis (pyknosis, karyorrhexis, and karyolysis) in all observations in five fields of view with 400X magnification. Pyknosis is characterized by nucleus shrinking and a dark appearance on Hematoxylin-Eosin staining. Pyknotic nucleus fragments characterize karyorrhexis. While the nucleus characterizes, karyolysis was losing the ability to be stained and not obtained so that the cytoplasm looks empty (Irawati, 2007; Kumar et al., 2007). The percentage of proximal tubular cells damage was made based on the formula from (Deakandi et al., 2017) as follows:

$$\frac{\text{Number of proximal tubular epithelial cells necrosis}}{\text{All visible proximal tubular epithelial cells}} \text{X100\%}$$

Observations were documented by visualizing images in photos of preparations from each treatment using optilab at 400x magnification.

## Data Analysis The data

Histological observations data in the percentage of cells undergoing necrosis were tested for normality using *the Shapiro-Wilk* method. Suppose p<0.05, the data were declared normal. Then the data were analyzed by parametric statistics using *Paired Sample T-Test*. All data analysis processes were carried out using SPSS *Statistics* 25 (Irmawati & Primiani, 2017).

## RESULTS AND DISCUSSION

Research on the effects of free radical control due to aspartame exposure on kidney damage has not been widely used. This study is important to determine the role of antioxidants in dealing with the histology of the kidney.

Observations in the average percentage of necrotic cells in the proximal tubule can be seen in Table 1. Histological observations of the kidney were carried out by counting the number of epithelial cells in the proximal tubule that underwent necrosis. The percentage of cells undergoing necrosis includes cells undergoing pyknosis, karyorrhexis, and karyolysis. The data obtained were then analyzed by using the *Paired Sample T-Test.* The *Paired Sample T-Test* analysis results for the K- and K+ groups can be seen in Table 1.

**Table 1.** The Average Percentage of Proximal Tubular Epithelial Cells that Experienced Necrosis after Aspartame Induced

| Treatment | Necrosis Cells (mean ± sd) (%) |
|---|---|
| K- (without aspartame, without tapak dara leaves extract) | 11.00 ± 3.16 |
| K+ (aspartame, without tapak dara, leaves extract) | 60.33 ± 3.27 |

Based on the results of Paired *Sample T-Test* analysis, there was a known significance value of 0.000 <0.05, so it can be concluded that the average percentage of renal proximal tubular epithelial cells undergo necrosis caused by aspartame treatment. The K+ group showed a higher average percentage of necrosis than the K- group. This is presumably because the effect of aspartame administration can cause proximal tubular epithelial cells to undergo necrosis.

Aspartame will undergo metabolism into three compounds, aspartic acid, phenylalanine, and methanol (Sweetman, 2009). Methanol from aspartame metabolism is oxidized to formaldehyde (Parthasarathy et al., 2006). Formaldehyde has oxygen atoms with negative electrons that can easily bond with other compounds, causing ROS formation (Dhalila et al., 2017). Excessively increases in ROS levels in the

body result in oxidative stress that can damage lipid membranes through a series of lipid peroxidation reactions and result in cell death (Berawi & Agverianti, 2017; Maiese et al., 2010). As an excretory organ, kidneys are susceptible to lipid peroxidation by ROS increase (Othman & Bin-Jumah, 2019).

Kidneys are organs that play a role in removing the remains of the body's metabolism toxic substances, so the kidneys become one of the main target organs of toxic effects (Mayori et al., 2013). The part of the kidney that is most susceptible to toxic substances in the proximal tubule (Susianti et al., 2010). The level of toxic substances in the proximal tubule is higher due to an active process of reabsorption and secretion. Also, the level of cytochrome P450 in the proximal tubule is higher to detoxify or activate the toxicant. Cells in the proximal tubule continuously exposed to toxic substances will experience damage that can lead to necrosis or cell death (Maisaroh, 2021). Necrosis begins with the morphology change of the nucleus (pyknosis), then the nucleus breaks (karyorrhexis) and the nucleus being disappeared (karyolysis) (Worotikan et al., 2017).

An imbalance of antioxidants causes the proximal tubular cells disruption from the body due to increased ROS. This condition can be overcome by giving antioxidants from external, one of which is from tapak dara (*C. roseus*) leaves extracts. The results of observations of proximal tubule cell necrosis after being given by *C. roseus* leaves extract in mice exposed to aspartame can be seen in Table 2.

Based on the *Paired Sample T-Test*, the percentage average of proximal tubular necrosis cells in the K+ group was significantly different from the group D1 and group D2. The percentage of necrotic cells in the D1 and D2 groups decreased compared to the positive control group. This indicates that tapak dara leaf extract's administration can improve the kidney histology of aspartame-induced mice, especially in proximal tubular cells. However, it has not been able to approach normal conditions.

**Table 2**. The Average Percentage of Proximal Tubular Epithelial Cells that Underwent Necrosis after Aspartame Induced and Continued with the Administration of Tapak Dara Leaves Extract

| Treatment | Necrosis Cells (mean ± sd) (%) |
|---|---|
| K+ (aspartame, without tapak dara, leaves extract) | 60.33 ± 3.27 |
| D1 (aspartame and tapak dara leaves extract 0.42 mg/kg bw) | 45.00 ± 2 ,83 |
| D2 (aspartame and tapak dara leaves extract 0.42 mg/kg bw) | 43.17 ± 2.48 |

Dose of aspartame: 0.02 mg

The decrease in the percentage of necrotic cells was thought to be due to the role of flavonoids in tapak dara leaves. Kusuma (2010) showed that basil leaf extract (*Ocimum sanctum* L.) that also containing flavonoids that were administered for 14 days to mice at a dose of 16.8 mg/20 g/BW has not been able to prevent liver cell damage to normal nearly due to the administration of palm oil with repeated heating. Khrestyawan, (2010) stated that flavonoids in cocoa bean extract (*Theobroma cacao*) at a dose of 0.2 ml/20 g BW and 0.4 ml/20 g BW given to mice for 17 days were able to reduce the amount of proximal renal tubular epithelial cells damage induced by paracetamol, but has not been able to restore the renal proximal tubular epithelial cells to be normal.

Tapak dara leaves contain alkaloids, flavonoids, quinones, saponins, polyphenols, and triterpenoids compounds (Fitrianingsih, 2010). Flavonoids are known as antioxidants to protect the body against ROS (Arifin & Ibrahim, 2018; Kapoor & Rani, 2019). Antioxidant mechanism—flavonoid compounds directly by donating hydrogen ions so that they can neutralize the toxic effects of free radicals, and indirectly by

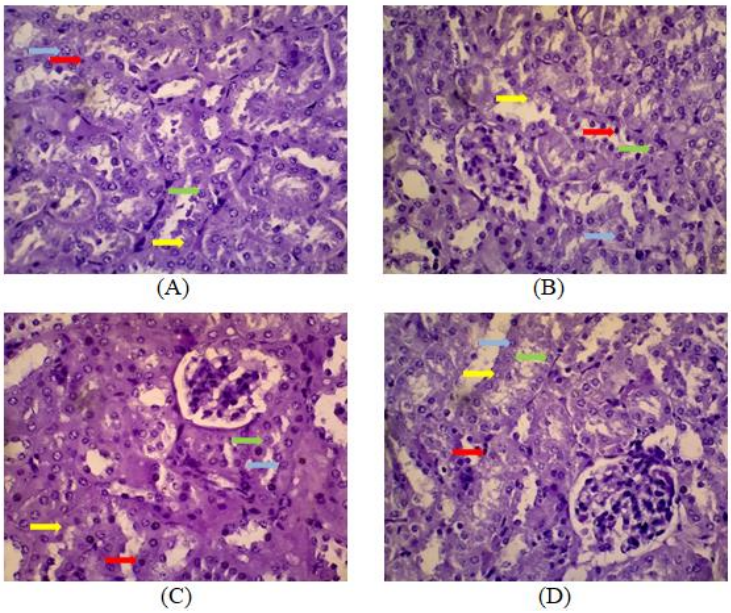
increasing the expression of endogenous antioxidant genes through activation of *nuclear factor erythroid 2 related factors 2* (Nrf2) (Sumardika & Jawi, 2012). According to Procházková & Bousova, (2019), flavonoids can stimulate internal antioxidant enzymes and suppress enzymes related to the formation of free radicals Wirawan, (2018) stated that giving ciplukan leaves (*Physalis minima* L.) containing flavonoids for 21 days to white rats at a dose of 50 mg/kg BW, 100 mg/kg BW, and 150 mg/kg BW can regenerate the proximal tubular epithelium cell damaged by streptozotocin-induced.

Based on the results of the *Paired Sample T-Test*, it is known that the average percentage of renal proximal tubular epithelial cells that underwent necrosis at the dose 0.42 mg/kg BW and 0.84 mg/kg BW groups showed no significant difference (Chinnala, et al., 2014). However, giving a dose of 0.84 mg/kg BW had a greater effect on reducing the percentage of necrotic cells than the 0.42 mg/kg BW dose group. It showed that the higher the dose given, the greater the effect of flavonoids in reducing the percentage of proximal tubular epithelial cell necrosis. Khrestyawan, (2010) showed that the administration of cocoa bean extract at a dose of 0.4 ml/20g BW was more effective in reducing the amount of damage to the proximal renal tubular epithelial cells induced by paracetamol compared to a dose of 0.2 ml/20g BW. It indicated that flavonoids substances in *Catharanthus roseus* extract could reduce the necrotic cells. Phytochemical analysis of *Catharanthus roseus* ethanolic extract by Chinnala et al., {2014) showed the presence of various secondary metabolic constituents such as flavonoids, phenols, alkaloids, and terpene. These substances can be used as hepatoprotection in Wistar rats induced by paracetamol. Treatment of 200 and 400 mg/kg b.w *Chataranthus roseus* ethanolic extract increased GSH level and decreased MDA level.

Histology of the proximal tubules of male mice after being given aspartame and tapak dara leaf extract can be seen in Figure 1. Proximal tubular necrotic cells include pyknotic, karyorrhexis and karyolysis. Pycnotic is the beginning of the damage, characterized by small dark nuclei by HE stained. It is followed by karyorrhexis and karyolysis. Karyolisis was characteristic of nuclei disappearing so that cytoplasmic seen empty.

From this figure, the necrotic cells of proximal tubule epithelial (D) are less than aspartame treatment. The proximal tubule structure seems more compact and tends to be similar to control (A).




(A) Normal; (B) Aspartame 0.02 mg/day; (C) Aspartame 0.02 mg/day + Tapak Dara 0.42 mg/kgBW; (D) Aspartame 0.02 mg/day + Tapak Dara 0.84 mg/kgBW; green arrow: normal cells; blue arrows: karyorexis cells; yellow arrow: karyolytic cells; red arrow: pyknotic cells.

**Figure 1.** Cross-section of the proximal tubule of male mice after being treated with Hematoxylin-Eosin staining and magnification of 400X.

## CONCLUSIONS AND SUGGESTIONS

In conclusion, administration of Catharanthus roseus leaves extracts potent to reduce necrosis of proximal tubular histology in aspartame-induced mice. In this study, the administration of the extract has not shown results that are close to normal, so further research is needed regarding the dose and duration of administration of the extract.

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