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The Antioxidant Capacity of *Peristrophe Bivalvis* (L.) Merr. as Natural-Based Nephroprotection

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ABSTRACT

The kidneys as one of the important body organs have a very important role in maintaining a healthy body. The kidneys function to regulate fluid balance in the body the concentration of salt in the blood, acid-base balance in the blood, and excretion of waste materials such as urea and other nitrogenous waste in the blood. Magenta plants (*Peristrophe bivalvis* (L.) Merr.) contain secondary metabolites, namely alkaloids, saponins, triterpenoids, tannins, and flavonoids as antioxidants. The abundance of antioxidants sourced from natural sources for various diseases is often used as a complementary therapy and is one of the current therapeutic choices. However, the development of natural sources must also consider kidney function during an intervention. The incidence of kidney failure can be caused either by the occurrence of oxidative stress or exposure to drugs and other chemical compounds must also consider the physiological functions of important organs in the body such as the liver and kidneys. This study was conducted to determine the protective role of magenta leaves extract (*Peristrophe bivalvis* (L.) Merr.) on the kidneys after being given an acetaminophen hepatotoxic dose. In this study, the effectiveness of magenta leaves antioxidants and the safety of use was analyzed by looking at the kidney function in the experimental model of Wistar strain male white rats, using a Randomized Post Test Only Control Group Design. Four treatment groups showed that magenta leaves extract (*Peristrophe bivalvis* (L.) Merr.) at 125 and 250 mg/kg BW can protect the kidneys with average creatinine levels of 0.63 and 0.75 and with a normal range (0.7 - 1.2). It means that these two groups could protect the kidney function although in the histopathology test only the group administering extracts of 250 mg/Kg BW showed good results. It can be concluded that administration of the magenta leaves extracts at 250 mg/kg BW can protect renal function as seen from serum creatinine levels. Besides, histopathological features can provide a protective effect on the kidneys with the incidence of necrosis in the kidneys of less than 60% of the toxic dose of acetaminophen.
Keywords: flavonoids; magenta leaves (*Peristrophe bivalvis* (L.) Merr.); nephroprotectiontable

INTRODUCTION

The use of traditional medicine in Indonesia has been going on for a long time before modern medicine was discovered and marketed. Free radicals are atoms that have one unpaired electron and are reactive, and thus they tend to react continuously to form new radicals. Free radicals are very dangerous for the human body because they can damage the components of body cells such as lipids, proteins, and DNA (Oktarini *et al*., 2014). Free radicals can result from air pollution or food consumption. Free radicals underlie cellular reactions to oxidative stress and thus play an important role in the pathophysiology of various diseases (Maulina, 2015). Under normal circumstances, free radicals produced in the body can be neutralized by antioxidants in the body. If the levels of free radicals are too high because of external influences such as air pollution, cigarette smoke, and heavy physical activity, the antioxidants in the body can no longer neutralize the antioxidants outside the body (Oktarini, *et al*., 2014).

The kidneys, one of the important body organs, have a very important role in body health. The kidneys function to regulate fluid balance in the body, the salt concentration in the blood, acid-base balance in the blood, and excretion of waste materials such as urea and other nitrogenous waste in the blood (Sherwood, 2011). The kidney is a nut-shaped organ located on both sides of the vertebral column. The right kidney is slightly lower than the left kidney because it is pressed down by the liver. If the kidneys cannot work as they should, kidney diseases such as kidney failure, kidney stones, nephritis, pyelonephritis, polycystic might arise and cause disability of its function properly (Robbins & Kumar, 2007). As the kidneys significantly affect the human body, they must always be protected from exposure to free radicals. Free radical exposure can be found in chemical compounds, drugs, and natural sources which are widely used in alternative treatment.

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Natural sources used in each treatment must be safe, not to mention scientifically proven first.

Antioxidants are compounds that can maintain health because of their ability to capture free radical molecules. They possess the ability to inhibit oxidative reactions, the causes of various diseases in the body (Ardawiah, *et al*., 2015). Enzymatic antioxidants or antioxidant enzymes produced by the human body act as antidotes to free radicals generated from endogenous sources such as superoxide dismutase (SOD), glutathione peroxide (GPx), and catalase (CAT). Enzymatic antioxidants also contain primary antioxidants which function to capture free radicals and stop the formation of free radicals (Adrianta, 2018). Antioxidants from natural sources are widely used in complementary therapy for various diseases. However, despite using natural sources in therapy, kidney function needs to be one of the other considerations. The incidence of kidney failure can be caused either by oxidative stress or exposure to drugs and other chemical compounds that might affect the physiological functions of important body organs such as the liver and kidneys.

From the above explanation, plant sources in complementary therapies were examined further in this study by looking at whether they are truly safe for the treatment of various diseases and kidney function. This study examined the use of magenta plants (*Peristrophe bivalvis* (L.) Merr.) as an antioxidant that is safe for kidney function.

METHODOLOGY

Research Design

The research design used was experimental purely with a randomized control-group post-test design. The material used was magenta leaves (*Peristrophe bivalvis* (L.) Merr.) collected from Bitera village, Gianyar-Bali regency. The samples were identified by the Indonesian Institute of Sciences (LIPI), Bedugul Bali for their chemical compounds, such as ethanol, sodium-carboxymethyl cellulose or CMC-Na, standard feed, and aquadest. The tools used were various glass tools, analytical scales, rotary evaporators, Buchner funnels, and a set of tools required for histopathological preparations.

The current research was carried out at the Integrated Laboratory of the Faculty of Pharmacy, Mahasaraswati University, Denpasar in January - April 2019. A total of 600 grams of magenta leaves were collected to obtain simplicia. The simplicia produced was then mashed and macerated with solvents: ethanol with the ratio of 1:7. A Buchner funnel was used to collect the filtrate, and the residue was generated in the same manner. The resulting filtrate was concentrated with a rotary evaporator until a thick extract was obtained.

Kidney Histopathology Analysis

Experimental animals are divided into 4 groups randomly consisting of:

Group 1 (P1); Control Group was given 1ml aquadest orally for 28 days and then on 29th day and 30th given a toxic dose of acetaminophen. On the 31st day, blood was taken through the sinus orbitalis to analyze kidney function after surgery and kidney organs to identify necrosis of kidney cells.

Group 2 (P2); treatment was given by administering 500 mg/kg BW 1ml curcumin emulsion orally for 28 days. On the 29th and 30th days, the group received a toxic dose of acetaminophen, and on the 31st-day blood was taken through the sinus orbitalis to analyze kidney function after that surgery and kidney organs to identify necrosis of kidney cells.

Group 3 (P3); Treatment group was given 125 mg/kg BW magenta leaves extract (*Peristrophe Bivalvis* (L.) Merr.) orally for 28 days. On the 29th and 30th days, a toxic dose of acetaminophen was administered, and blood was taken on the 31st day through the sinus orbitalis to analyze kidney function after surgery and kidney organs to identify necrosis of kidney cells.

Group 4; Treatment group received 250 mg/kg BW magenta leaves extract (*Peristrophe bivalvis* (L.) Merr.) orally for 28 days and then on the 29th and 30th days were given a toxic dose of acetaminophen. On the 31st day, blood was taken through the sinus orbitalis to analyze kidney function after surgery and kidney organs to identify necrosis of kidney cells.

RESULT AND DISCUSSION

Data on kidney function by looking at serum blood creatinine levels (Table I).

A data normality test was conducted, and all p-values were more than 0.05, meaning the data were normally distributed. Then, the homogeneity test was carried out, and the p-value was <0.05. For the next test, nonparametric tests were performed using the Kruskal Wallis test with a p-value of >0.05. Then, the Mann-Whitney U test was utilized to compare differences between groups. Comparison between groups can be seen in Table II.

The results of the analysis can be explained as follows (1) between the negative control group and the group administered with curcumin tablets as a standard, the p-value was 0.878. In other words, there was no significant difference, meaning that the administration of curcumin

Table I. Serum creatinine results data

Mice No.	Group			
	P1	**P2**	**P3**	**P4**
1	1,3	1,75	0,52	0,74
2	1,76	1,32	0,81	0,8
3	1,82	2,03	0,82	0,91
4	1,83	1,04	0,54	0,71
5	1,35	1,3	0,66	0,77
6	1,3	1,8	0,24	0,61
7	1,35	1,4	0,72	0,8
8	0,96	1,5	0,75	0,7
$\bar{X}$ ± SD	1,49 ± 0,26	1,51±0,32	0,63±0,19	0,75±0,88

Table II. Serum creatinine analysis

	Group	**p-value**
P1 (Control)	P2 (curcumin 500mg/ kg BW)	0.878
	P3 (125 mg/ kg BW)	< 0.001
	P4 (250 mg/ kg BW)	< 0.001
P2 (500mg/ kg BW curcumin)	P3 (125 mg/ kg BW)	< 0.001
	P4 (250 mg/ kg BW)	< 0.001
P3 (125 mg/ kg BW)	P4 (250 mg/ kg BW)	0.328

Table III. Sccoring of necrosis Analysis

	Group comparism	**p-value**
P1 (Kontrol)	P2 (curcumin 500mg/KgBB)	1,00
	P3 (125 mg/KgBB)	1,00
	P4 (250 mg/KgBB)	< 0.001
P2 (curcumin 500mg/KgBB)	P3 (125 mg/KgBB)	1,00
	P4 (250 mg/KgBB)	< 0.001
P3 (125 mg/KgBB)	P4 (250 mg/KgBB)	< 0.001

tablets had the same effect on the kidneys. Kidney function increased above the normal level due to the induction of toxic doses of acetaminophen; (2) Between the negative control group and the group administered with 125mg/ kg BW of ethanol extract from magenta leaves, it shows the p-value was <0.001. There was a significant difference, meaning that the administration of ethanol extract from magenta leaves (*Peristrophe bivalvis* (L.) Merr.) had different conditions in the control group when seen from the results of the analysis (serum creatinine levels). Ethanol extracts of magenta leaves can protect kidney function, as well as P1 and P4; (3) Between P2 and P3, P2 and P4, there were also significant differences (p <0.001); (4) The 125 mg/ kg BW and 250 mg/ kg BW groups showed that the two treatments had the same effect (p=0.328). In this case, the levels of creatinine in groups P3 and P4 were normal, and extracts could be considered for providing a protective effect on the kidneys.

Analysis of Kidney Histopathology

Analysis of the kidneys was performed to identify the role of extracts to protects the kidneys from renal necrosis. Examination of glomerular and tubular necrosis of white rat's kidney tissue in five areas obtained a p-value of <0.001, which means that there were differences in 2 or more groups. Then, the Mann-Whitney U test was performed. The results of the comparison are explained in Table III.

Between the negative control group and the group administered with curcumin tablets as a standard, there was no significant difference (p =1.00), meaning that the groups with 500 mg/kg BW curcumin tablets showed similar results to the control group given a toxic dose of acetaminophen. Value scoring showed more than 60% of necrotic kidney cells and differences between groups P1 and P3, P2 and P3.

Between the negative control group (P1) and P4, there were significant differences

(p<0.001). Value scoring indicated that between the negative control group and the group administered with curcumin tablets as a standard, there was no significant difference (p=1.00) between the groups administered with 500 mg/kg BW curcumin tablets and the control group administered with a toxic dose of acetaminophen. Value scoring showed less than 60% of necrotic kidney cells.

Results of Glomerular Histopathology of White Rat's Tubular Kidney Tissue in Areas
Group P1 (Administration of Toxic placebo and parasetamol)

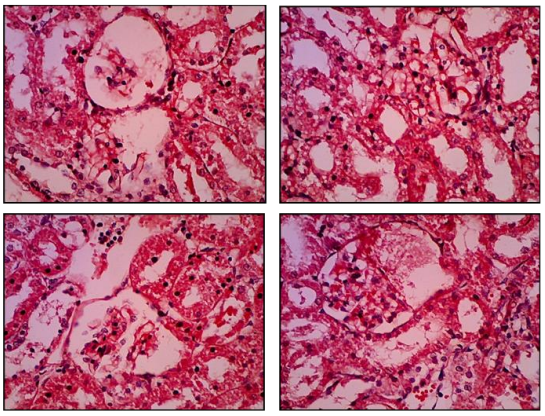

Figure 1. Renal histopathology of the control group

Figure 1 shows several histological findings of the kidneys, exposure to a toxic dose of acetaminophen for kidney function, and necrosis that appears more than 60% in glomerula tissue and tubular kidney tissue

Group P2 (Administered with 500mg/ kg BW curcumin tablets and a toxic dose of acetaminophen)

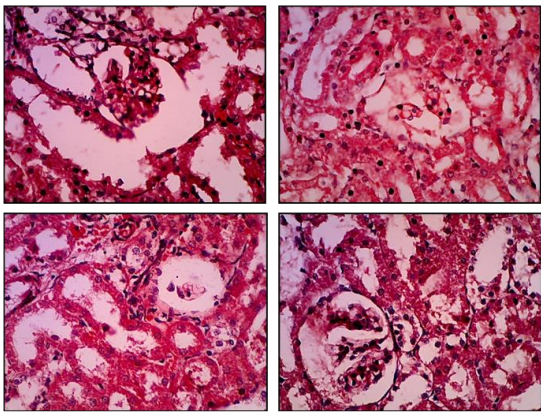

Figure 2. Histopathology of renal group giving curcumin tablets

Figure 2 presents several histological findings of the kidneys, exposure to a toxic dose of acetaminophen for kidney function, and necrosis that appears more than 60% in glomerular tissue tissue and tubular kidney tissue.

Group P3 (Magenta ethanol extract 125 mg / kg BW and toxic dose *acetamenophen*)

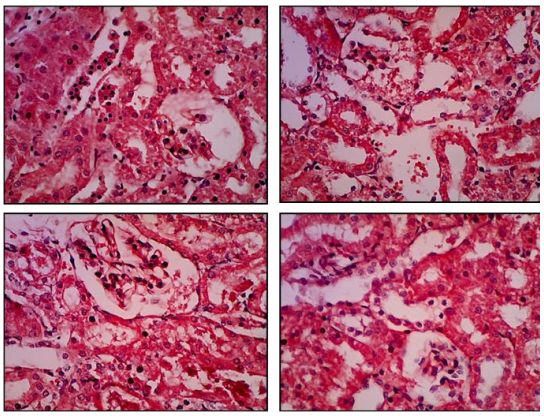

Figure 3. Histopathology of kidney groups giving curcumin tablets

Figure 3 displays several histological findings of the kidneys, exposure to a toxic dose of acetaminophen to kidney function, and necrosis that appear more than 60% in glomerular tissue and tubular kidney tissue.

Group P4 (Administered with 250 mg/ kg BW ethanol extract from magenta leaves and a toxic dose of acetaminophen)

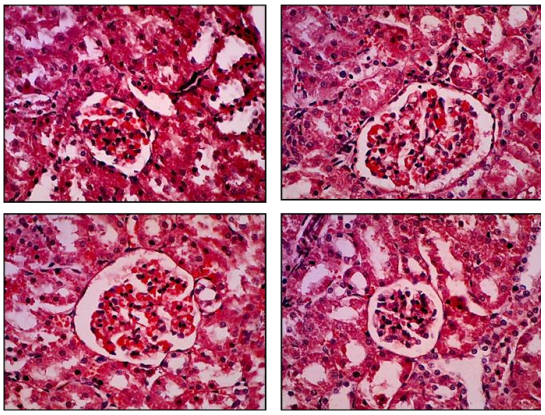

Figure 4. Histopathology of the kidney group of ethanol extract of magenta leaves 250 mg / KgBB

Figure 4 shows several histological findings of the kidney, exposure to a toxic dose of acetaminophen for kidney function. It appears that magenta leaves extract could protect the kidneys

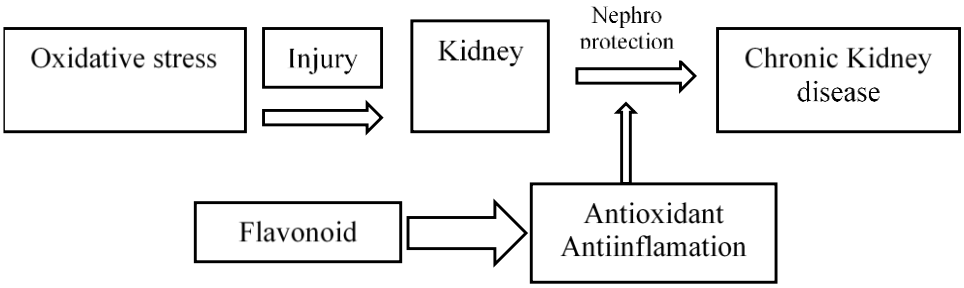

Figure 5. Flavonoid as a nefroprotection (*Modification from Vargas, et al., 2018)*

as necrosis in glomerular tissue nd tubular kidney tissue occurred less than 60%.

DISCUSSION

The protective effect was best found in the group given 250 mg/kg BW ethanol extract from magenta leaves. The analysis of tubular kidney tissue showed less than 60% necrosis was found in tubular cells, but the kidneys could still function as shown in the group administered with ethanol extract. The serum creatinine test showed both groups administered with 125mg/ kg BW ethanol extract and 0.63 acetaminophens, and 250 mg/kg BW ethanol extract and 0.75 acetaminophens, respectively (Dose range of acetaminophen from 0.7 - 1.2) could protect kidney function although, in the histopathology test, only the group was given 250 mg/kg BW ethanol extract had good results. It can be concluded that 250 mg/kg BW ethanol extract administered could protect kidney function seen from the serum creatinine levels and histopathologic image (necrosis occurred less than 60%) against acetaminophen toxic dose exposure.

Huxtable (1988) states that the kidneys can work normally and be protected from exposure to free radicals although the nephron still functions around 30-35%. However, a long-term disabled nephron can cause excessive solute (dissolved ingredients), and thus the kidneys will have difficulty in regulating the urine concentration. Excessive dissolved ingredients in animals experiencing oliguria become the initial signs of acute kidney failure (Di Bartola, 1981).

The antioxidant capacity of flavonoids on magenta leaves extract is the result of phenolic hydroxy groups in the molecular structure. It can also occur because of free radicals and their activities as metal pullers. Antioxidants generally have several functions, one of which is to yield hydrogen atoms and slow down the rate of oxidation which inhibits the formation of lipid radicals. Yielding hydrogen atoms can change lipid radicals to more stable ones that do not cause further damage. (Owolabi, 2014). According to Vargas *et al*. (2017) flavonoids protect the kidneys through a decrease in apoptosis, a marker of caspase-3. The inhibition of cisplatin-induced apoptosis will support the survival of kidney cells.

This present study explains that flavonoids were also found in Magenta leaves (*Peristhrope Bivalvis* (L.) Merr.). Flavonoids in magenta leaves can protect the kidneys as seen from serum creatinine levels produced (within normal limits) at a dose of 250 mg/kg BW as well as from the occurrence of necrosis in the kidney compared to groups administered with a toxic dose of acetaminophen without extract. Flavonoids in magenta leaves as antioxidants are considered to provide direct protection by donating electrons to free radicals. Therefore, they will not cause oxidative stress which can result in kidney injuries. Indirectly, flavonoids in magenta leaves can trigger activation of endogenous antioxidants through activation of transcription factors namely Nrf-2. Nrf 2 entering the cell nucleus can bond with the antioxidant response element (ARE) and activate endogenous antioxidants. The antioxidants formed will reduce oxidative stress to prevent kidney injury. Due to the induction of excessive chemical compounds, increases regulation of p65 NF-κB protein expression involving several pro-inflammatory cytokines (e.g., iNOS and TNF-α). Flavonoids also have the anti-inflammatory capacity that inhibits activation of NF-κB, and thus inflammatory mediators such as (TNF-alpha, IL-10) are not expressed. Flavonoids can reduce phosphate-NF-κB p65 and phospho-P38 MAPK activation resulting in a decrease in apoptosis.

This study proves that the administration of ethanol extract from magenta leaves to the kidneys can also provide preventive and protective effects from further damage to kidney cells induced with a toxic dose of acetaminophen.

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