Activity of red dragon fruit (*Hylocereus polyrhizus*) juices on doxorubicin-induced nephropathy in rats

B F Prasetyo¹, H Shabrina², V Juniantito¹ and I Wientarsih¹

¹Departement of Clinic, Reproduction and Pathology, Faculty of Veterinary Medicine, Bogor Agricultural University, Agatis Street, Bogor, 16680, Indonesia
²Veterinary Internship, Bogor Agricultural University, Agatis Street, Bogor 16680, Indonesia

Email:bayupr@apps.ipb.ac.id

Abstract. Nephropathy is a common side effects following treatment by an antineoplastic drug, doxorubicin. Dragon fruit is rich in antioxidants which are also beneficial for supporting effective tissue regeneration and reduce detrimental sequelae of chemotherapy. The aim of this study was to determine attenuation capacities of red dragon fruit juice in doxorubicin (DOX)-induced renal damages, based on histopathological findings. Male *Sprague-dawley* rats, consist of 18 rats were divided into three groups. Group I was given NaCl 0.9% (saline) at the dose of 0.4 ml/rat intraperitoneally (IP) weekly for 4 weeks. Group II was given DOX to induce nephropathy at the dose 4 mg/kg body weight (BW) IP weekly for 4 weeks. Group III was given DOX at the dose 4 mg.kg BW IP weekly for 4 weeks and red pitaya juice at dose 4 ml/kg perorally (PO) three times daily for 5 weeks. Nephrotoxicity was assessed by counting total glomerular that have protein deposisition and tubular cells necrosis. Treatment in group III significantly (p<0.05) decreased the numbers of glomerular that have protein deposition and tubular necrotic cells as compared with group II. These results suggest that red dragon fruit juice has the potential nephroprotective activities in doxorubicin-induced nephropathy.

1. Introduction

Doxorubicin (DOX) is an anti-cancer drug from anthracycline class that have side effect on heart, liver, and kidney. Therefore, the use of this drug is restricted [1]. DOX can induced nephotoxicity in kidney. A correct mechanism for nephrotoxicity induced with DOX is remained unknown, but there is a possibility it was formed through free radicals, free iron, and oxidative destruction from biological macromolecule [2]. One way to prevent and reduce the toxical effects is to use a substance that has anti-inflammation and antioxidant characteristics.

Kidney induced with DOX shows histological changes in the form of congestion in renal blood vessels and glomerulus, degeneration of tubules up to necrosis, and infiltration on interstitial tissue by inflammatory cells dominated by macrophages and lymphocytes [3]. An excessive usage of DOX results in accumulation of free radicals that lead to oxidative stress, thus an antioxidant is needed to prevent the free radicals. One of the materials with rich antioxidants needed to resist damage by free radicals in the body is dragon fruit.

Dragon fruit, or pitaya, is a tropical fruit belongs to a cactus family of *Cactaceae*. An example of dragon fruits widely cultivated in Indonesia is red dragon fruit (*Hylocereus polyrhizus*). Research shows that *Hylocereus polyrhizus* could be used as anti-inflammatory agent [4], antimicrobials agent [5] and antiproliferation agent [6]. Furthermore, the flesh of red dragon fruit contains higher...
antioxidant levels than white dragon fruit [7]. The substances that have antioxidant activity in the dragon fruit is to be expected can help regenerate tissues and reducing nephrotoxicity effect on cancer patients who use DOX.

2. Materials

Tools used in the experiment were cage, digital weighing scale, 1 ml and 3 ml syringes, gastric tube, necropsy kit, specimen bottles, label paper, microscope, object glass, cover glass, tissue cassette, tissue basket, automatic tissue processor, paraffin embedding console, waterbath, and microtome.

This research also used materials that consisted of 18 male rats strain Sprague-Dawley aged 8–12 weeks with average weights of 150 grams, NaCl 0.9%, 10% buffered Neutral Formalin (BNF), Pyrantelpamoate (Combantrin® 125 milligrams, Pfizer, Jakarta, Indonesia), amoxicillin (Hufanoxil® 125 mg / 5 ml, HUFA, Indonesia), benzyl metronidazole (Flagyl®, Aventis Pharma, Indonesia), doxorubicin injection (doxorubicin hydroxide 50 mg, Actavis, Indonesia), and dragon fruits. The making of histological slides, staining of Hematoxylin-Eosin (HE) and Masson’s Trichrome (MT) used xylol, 95%, 85%, 70% absolute alcohol, paraffin, Mayer’s Haematoxylin, eosin, Carazzi’s Haematoxylin, 0.75% orange G, 1% acetic acid, ponceauxylidinefuchsin, 2.5% phosphotungstic acid, aniline blue, and purified water.

3. Methods

3.1. Making red dragon fruit juice

Red dragon fruit’s skin was peeled and the flesh was taken for about 100 grams. The dragon fruit’s flesh was cut and pulverized with added 100 milligrams of water. The extract from the pulverizing process was filtered with a sieve.

3.2. Animal’s treatment

Each rat was adapted to cage environment for about a week and continued with pre-treatment drugs that consisted of anthelminticspyrantelpamoate 10 mg/kg of body weight (BW) once, amoxicillin antibiotics 20 mg/kg BW for the next 5 days twice everyday, and metronidazole antiprotozoal agent 20 mg / kg of body weight for 3 days every morning at the same time with antibiotics treatment.

The rats were divided into 3 groups, every group consists of 6 rats. The group I: rats were injected using physiological NaCl intraperitoneally (IP) with a dose of 0.4 ml/rat every week for 4 weeks. The group II: rats were injected with DOX 4 mg/kg BW IP every week for 4 weeks. The group III: rats were injected with DOX 4 mg/kg BW IP every week for 4 weeks and combined with the intake of dragon fruit juice with a dose of 4 ml/kg BW orally every day for 5 weeks. The intake of dragon fruit juice started on the same day with the DOX injection. In the fifth week, the rats were euthanized. The kidney was taken and inserted in specimen bottles contained formalin and labeled. Subsequently, the organ samples were blocked with paraffin and then stained with Hematoxylin-Eosin (HE) colours.

3.3. Masson’s Trichrome staining and blind scoring

Masson’s Trichrome staining was started with deparaffinization and flushing using purified water. The object glass was soaked inside a mordant solution, and then immersed in Carrazzi’shematoxylin solution for 40 minutes, rinsed using purified water, immersed in 0.75% Orange G solution for 2 minutes, rinsed with 1% acetic acid solution twice, immersed in ponceauxylidinefuchsin for 15 minutes, rinsed with 1% acetic acid solution twice, immersed in 2.5% phosphotungstic acid solution for 10 minutes, rinsed with 1% acetic acid solution for twice, immersed in aniline blue for 15 minutes, rinsed with 1% acetic acid solution for twice, and then immersed in 95% alcohol solution for 3 minutes. The next step was dehydration. The result of staining showed the nuclei in dark blue colour; muscles and elastin in red; fibrin and calcium in purple; hyaline in light blue; and collagen connective tissues as well as mucus in greenish blue. The slides of formed connective tissues were observed with the blind scoring method.
Scoring was done by a pathologist who unaware of the sample’s group identity to minimize the subjectivity. The scoring criteria of renal fibrosis with Masson’s Trichrome were based on Chen et al. [8] and Gibson-Corley et al. [9]. The scoring criteria of the fibrosis was divided into 4 kinds, which consisted of: 0 (< 5% or very few), +1 (6-25% or mild), +2 (26-50% or moderate), and +3 (> 50% or severe).²

3.4. Observation variables
Necrotic cells and glomeruli that have protein sediment were calculated using Image J software on 5 microscopic field for every single organ. The necrotic cells were calculated on 0.05 mm² microscopic field while glomeruli that have protein sediment was calculated on 0.23 mm² microscopic field.

3.5. Data analysis procedure
The quantitative data were analysed using SPSS software, ANOVA variety analysis method. And then Duncan’s test was used to find the existence of significant difference (p < 0.05) among the treatment groups. Qualitative data were analysed descriptively.

4. Result and discussion
The results of histopathological observation in the form of glomerulus that have protein sediment and necrotic cells calculation are shown in table 1.

| Group | Glomeruli sediment (per 10 glomerulus) | Necrotic cell(s) per 0.05 mm² |
|-------|----------------------------------------|-----------------------------|
| I     | 1.61±0.76a                             | 18.16±2.95a                 |
| II    | 4.30±1.82b                             | 25.10±4.99b                 |
| III   | 2.48±1.07a                             | 19.40±2.78a                 |

a, b, c Difference of superscript in the same column shows a significant difference (p<0.05)

The number of glomerulus that have protein sediment shows that between group I and III reveal no significant difference (p < 0.05). Additionally, there are significant differences between group II (DOX-only treatment) with group III. Red dragon fruit-supplemented rats tend to show lower number of glomerular protein sediments. Hence, the intake of red dragon fruit juice ameliorates the severity of glomerular protein deposition. In addition to glomerular protein deposition, there are marked tubular necrosis. The number of necrotic cells in the group II (DOX-only treatment) significantly higher (p < 0.05) as compared with group III, indicating there is inhibition of tubular necrosis in DOX-treated rats supplemented with red dragon fruit.

The number of glomerulus which had a protein sediment and necrotic cells on group II is the least compared to the DOX-only treated groups. This is because in Group III, supplementation of red dragon fruit juice which contain major antioxidants for effective tissue repair may reduce detrimental effects of DOX in kidney.

The protein sediment on glomerulus is formed because of an increase in glomerulus’s capillary permeability to plasma proteins such as plasma albumin, therefore causes the protein mass accumulation on mesangium up to Bowman’s space and lumen tubule [10][11]. This corresponds to the previous research showing that the toxic effect on DOX intake in the form of an increase of glomerulus’s capillary permeability[12]. The existence of protein resulting in the histological image of hyaline droplets in light red colour [13] (figure 1).

Necrosis is defined by the death of cell and tissue in an organism. Necrosis is indicated by a cell under going pycnosis with the histopathological image of shrinking nucleus, agglutinating of chromatin, and unstructured mass forming [11] (figure 2). According to [13] necrosis might be caused by ischemia or because of toxic substances. Chemical substances such as chemotherapeutic,
antineoplastic, and antibiotic agent can induce cell destruction. DOX is known to have a side effect that can increase the production of reactive oxygen species (ROS). This radical will react with lipid, protein, and carbohydrate membrane resulting in the induction of cell destruction [11].

![Figure 1. Histopathological observation in kidney HE stain, ↑A: protein deposition in Bowman’s chamber, ↑B: protein deposition in lumen of tubuli.](image1)

![Figure 2. Histopathological observation in kidney HE stain, ↑C: necrotic tubular cells.](image2)

According to [14] there are two mechanisms of ROS forming caused by DOX induction. First, DOX forms a chelate with iron to cause a chain reaction resulting in the forming of new free radicals. Second, the C ring in the anthracycline class has the shape of quinone, therefore if induced it will form semiquinone which is a form of free radical and triggering the formation of other free radicals.

Doxorubicin is excreted through kidney [14]. According to [15] if a chemical substance is actively excreted from the blood to urine, that chemical substance will be previously accumulated in the proximal tubule and causes the destruction of the kidney. Epithelium cells in tubule are easily destroyed because of contacts with toxic substance excreted through kidneys. If this goes on then it will cause an irreversible destruction indicated by a necrosis.

| Sample                  | Parameter | Result |
|-------------------------|-----------|--------|
| Red dragon fruit juice  | Phytochemistry | +      |
|                         | Flavonoid     | Wagner -|
|                         |              | Mayer - |
|                         | Alkaloid      | Dragendorf -|
|                         | Tanin        | -      |
|                         | Saponin      | +      |
|                         | Quinon       | -      |
|                         | Steroid      | -      |
|                         | Triterpenoid | +++    |

A decrease in the quantity of protein sedimentation on glomerulus and necrotic cells of the tubule in a group III can be caused by chemical substance contents in the red dragon fruit. The result of phytochemistry test on Table 2 shows that red dragon fruit juice contains flavonoid, saponin, and triterpenoid. The flavonoid, according to its function, is a primary antioxidant that can stabilize free
radicals. The mechanism of this compound as an antioxidant is with donor hydrogen from its own hydroxyl group and affecting in the formation of a product that is more stable and less reactive [16]. Triterpenoid is a compound used as antioxidant, anticancer, and anti-inflammation [17]. The existence of triterpenoid inside red dragon fruit can reduce the destruction caused by DOX. The result of a research has proven that the extract of red dragon fruit has the effect of nephroprotection from DOX induction.

The high quantity of connective tissues in the interstitium indicates a chronic inflammation because the renal parenchyma which undergoes necrosis is replaced with connective tissues [18]. There is no significant difference between the three treatment groups (table 3). The result of scoring is plus one (+), indicates that connective tissues in the interstitial cortex in the kidney are mild (6-25%) accompanied by thickening of kidney capsule (figure 3).

Table 3. Result of scoring fibrosis.

| Group | Scoring |
|-------|---------|
| I     | +       |
| II    | +       |
| III   | +       |

+: Interstitial fibrosis in cortex of kidney is mild (6-25% accompanied by thickening of kidney capsule

According to [19], fibrosis is a common pathophysiological response in many tissues undergoing destruction. DOX in this research is only given for 4 weeks and not causing a chronic damage, therefore the result of scoring valuation has not a significance difference among the three treatment groups. According to Purwanto’s report [20], the intake of DOX has not a significant difference in type-I collagen DOX group and control in the fourth week. Type-I collagen is a result of TGF-β1 stimulation in receptors of renal fibroblast interstitial cell membrane that causes the occurrence of interstitial fibrosis.

DOX is called a destructive substance to kidney cells (nephrotoxic). That side effect is caused by ROS as the result of DOX reaction in cancer therapy. ROS damaging the kidney cells which will trigger macrophage to excrete cytokine such as TGF-β1. TGF-β1 will stimulate type-I and type-IV collagen that causes interstitial fibrosis and glomerulosclerosis [20].

5. Conclusion
Administration of red dragon fruit juice may prevent or reduce the effects of DOX-induced nephrotoxicity in rats in the form of a decrease in the number of glomerular that have protein sediment and necrotic cells of tubular epithelium.

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