ACUTE TOXICITY TEST OF RED ONION (*Allium Cepa L*) SKIN EXTRACT ON KIDNEY HISTOPATHOLOGY OF RATS (*Rattus Norvegicus*)

[1] Denny Christian Brahmantya Putra, [2]Dina Helianti, [3]Septa Surya Wahyudi, [2]Rosita Dewi.

[1] Faculty of Medicine/University of Jember, Jember, Indonesia.
[2] Department of Histology, Faculty of Medicine/University of Jember,Jember, Indonesia.
[3] Department of Anatomy, Faculty of Medicine/University of Jember,Jember, Indonesia.

ABSTRACT

Introduction: *Shallots (Allium cepa L.) are known to contain quercetin, which is helpful as an antioxidant, but its accumulation in the body can be toxic.* ***Objective:*** *This study aims to determine the effect of administering red onion skin extract on histopathology and weight of the kidney, body weight, and clinical symptoms, as well as determining the LD50.* ***Material &Methods:*** *This research was conducted according to OECD guideline No. 420 Fixed Dose Procedures.Rats were allocated to a control group that was given 3% DMSO and a treatment group that was given red onion peel extract at a dose of 5000 mg/kgBW.Kidney histopathology slides with Hematoxylin-eosin staining were observed by two observers in five fields of view using Fiji ImageJ software and assessed based on the Kocoglu scoring.Kidney histopathology data was analyzed using the Mann-Whitney test, while kidney weight and body weight gain used the Independent Sample T-test.* ***Results:*** *The treatment group experienced significantly higher body weight gain than the control group. On the other hand, observations of the histopathological appearance and weight of the kidney did not show any significant differences. Observation of clinical symptoms showed normal results with no deaths.* ***Conclusion:*** *Administration of 5000 mg/kgBW red onion skin extract in the acute toxicity test had no effect on the histopathology and weight of kidney rats, but had a significant effect on body weight gain. The LD50 of red onion skin extract was found to be >5000 mg/kgBW.*

Keywords: *Red onion skin extract, acute toxicity test, LD50, kidney histopathology.*

ABSTRAK

Pendahuluan: *Bawang merah (Allium cepa L.) diketahui mengandung kuersetin yang bermanfaat sebagai antioksidan, namun akumulasinya di dalam tubuh dapat bersifat toksik.* ***Tujuan:*** *Penelitian ini bertujuan untuk mengetahui efek pemberian ekstrak kulit bawang merah terhadap histopatologi dan berat ginjal, pertambahan berat badan, gejala klinis, dan menentukan LD50.* ***Bahan & Cara:*** *Penelitian ini dilakukan berdasarkan pedoman OECD No. 420 Fixed Dose Procedure. Tikus dialokasikan pada kelompok kontrol yang diberi DMSO 3% dan kelompok perlakuan yang diberi ekstrak kulit bawang merah dengan dosis 5000 mg/kgBB. Preparat histopatologi ginjal dengan pewarnaan Hematoxylin-eosin diamati oleh dua orang pengamat pada lima lapang pandang menggunakan software Fiji ImageJ dan dinilai berdasarkan skoring Kocoglu. Data histopatologi ginjal dianalisis menggunakan uji Mann-Whitney, sedangkan berat ginjal dan pertambahan berat badan dianalisis menggunakan uji Independent Sample T-test.* ***Hasil:****Kelompok perlakuan mengalami pertambahan berat badan yang lebih tinggi secara signifikan dibandingkan dengan kelompok kontrol. Pengamatan terhadap gambaran histopatologi dan berat ginjal tidak menunjukkan adanya perbedaan signifikan. Pengamatan gejala klinis menunjukkan hasil normal dan tidak ada kematian.* ***Simpulan:*** *Pemberian ekstrak kulit bawang merah 5000 mg/kgBB pada uji toksisitas akut tidak memberikan efek terhadap histopatologi dan berat ginjal tikus, namun memberikan efek terhadap pertambahan berat badan. LD50 ekstrak kulit bawang merah didapatkan >5000 mg/kgBB.*

Kata kunci: *Ekstrak kulit bawang merah, uji toksisitas akut, LD50, histopatologiginjal.*

Correspondence: Dina Helianti; c/o: Department of Histology, Faculty of Medicine/University of Jember. Jl. Kalimantan No.37, Krajan Timur, Sumbersari, Kec. Sumbersari, Jember68121, Indonesia, dinahelianti.fk@yahoo.co.id.

INTRODUCTION

Red onions are widely used as a cooking spice mixture and consumed raw as a traditional medicine to relieve fever, dizziness, and influenza. Studies reveal that onions contain a variety of secondary metabolites, including tannins, kaempferol, and flavonoids like quercetin. Red onion skin contains 48 times more quercetin than its bulbs.[1,2] The quercetin content in red onion skin has

the potential as a natural antioxidant that neutralizes free radicals and prevents DNA damage.[2] Red onion skin extract (ROSE) has been proven to repair diazinon-induced rat liver damage.[3] Other studies also show the gastroprotective effect of ROSE in mefenamic acid-induced rats.[4]

Contrary to its benefits, flavonoids in high doses can change their properties to become prooxidants and disrupt the balance of redox reactions that occur in the body.[5] The prooxidant activity of flavonoids is greatly influenced by their structural characteristics. The more hydroxyl groups in flavonoids, the higher their prooxidant activity. Flavonoids from the flavonol group, namely quercetin, have the highest prooxidant activity compared to other types of flavonoids.[5,6]

Quercetin, which is mainly contained in ROSE, will go through the excretion process in the kidneys, so it has the potential to cause damage to this organ.[7] Kidney damage can be assessed from histopathological changes and organ weight.[8] Therefore, it is necessary to carry out acute toxicity tests to determine the effect of ROSE on kidney histopathology.

OBJECTIVE

This study aims to determine the effect of administering ROSE on kidney weight and histopathology, body weight, and clinical symptoms, as well as determining the LD50.

MATERIAL &METHODS

This research was a true experiment with a posttest-only control group design. This research received ethical approval from the Ethics Commission of the Faculty of Medicine, University of Jember, with number 397/UN25.1.10.2/KE/2024.

This research used female Wistar rats (Rattus norvegicus), aged 8-12 weeks, nulliparous, with body weight variations ≤ 20%. After passing an acclimatization period of 7 days, random allocation was carried out based on body weight to determine the control and treatment groups. The toxicity test was carried out based on the OECD acute oral toxicity test guideline number 420 Fixed Dose Procedure9 which is divided into 2 parts, namely the sighting study and the main study. The sighting study aims to determine the initial dose for the main study. The sighting study began by giving ROSE a dose of 2000 mg/kgBW to 1 rat. ROSE is administered orally in a single dose. Next, observations were carried out for 14 days. No clinical symptoms or mortality were found during observation, so the sighting study was carried out again by increasing the dose to 5000 mg/kgBW for another rat. After 14 days of observation, no clinical symptoms or mortality were found, so the main study was carried out at a dose of 5000 mg/kgBW. In the main study, 9 rats were divided into two groups: 5 rats in the control group and 4 rats in the treatment group. The control group was given 3% DMSO, while the treatment group was given 5000 mg/kgBB ROSE. Next, clinical symptoms and mortality were observed for 14 days. Body weight measurements were carried out at the start of the study, once every 3 days and every 7 days. On the 15th day, all experimental units were weighed again and terminated using Ketamine-Xylazine for kidney collection.

Kidney histopathology preparations were made using the paraffin method and Hematoxylin-eosin (HE) staining. Kidney histopathological observation in 5 fields of view at 400× magnification was carried out by 2 people using a double-blind technique and analyzed with Fiji ImageJ software. Renal histopathological scoring was determined based on Kocoglu[10] scoring (0: no damage, 1: ≤10% damage, 2: 11-25% damage, 3: 26-45% damage, 4: 46-75% damage, 5: 76-100 % damage). Statistical analysis was carried out using IBM SPSS Statistics 25 computer software. Kidney histopathology scoring was analyzed using the Mann-Whitney method, while kidney weight and body weight gain used the Independent Sample T-test method.

RESULTS

Based on the results of the determination of species at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, University of Jember, this research used the red onion variant Allium cepa L. Extraction of red onion skin using the maceration method with 96% ethanol solvent. The extract obtained was 18.2 grams from 200 grams of red onion skin simplicia, so the yield obtained was 9.1%. The total flavonoid content in ROSE obtained was 10.57 ± 0.63% w/w (mg) or equivalent to 105.7 mgQE/g.

This study used random allocation based on body weight to determine the control and treatment groups. The results of the random allocation were then tested for normality and homogeneity and a

significance value of >0.05 was obtained, which means that the results of the random allocation were normally distributed and homogeneous. Next, an independent sample T-test was carried out and a significance value of >0.05 was obtained, which means there was no significant difference in body weight between the two groups.

The lethal dose 50% is determined based on observations of clinical symptoms and mortality in experimental animals. After observing for 14 days in the main test with a dose of 5000 mg/kgBW, there were no clinical symptoms or deaths in the experimental animals. Observations on motor activity, sensory, body posture and gastrointestinal system showed normal results. Based on these results, the LD50 of ROSE is >5000 mg/kgBB and can be included in the Unclassified category in the Globally Harmonized System substance safety classification in accordance with OECD guideline No. 420 Fixed Dose Procedures.

In this study, body weight gain and kidney weight were measured. Weight gain is the difference between body weight before treatment and body weight 14 days after treatment. Kidney weight consists of absolute weight and relative weight. Absolute weight is obtained from direct organ weight measurements, while relative weight is obtained through the following calculations:

$$\frac{\text{Absolute kidney weight (gram)} \times 100\%}{\text{Rat body weight (gram)}}$$

Data on relative and absolute organ weights and body weight gain in Table 1 were then analyzed by homogeneity and normality tests. A p-value of >0.05 was obtained for the three variables, which means that the data on relative and absolute weight gain and organ weights were homogeneous and normally distributed. The results of the independent sample T-test on weight gain data showed a p-value <0.05, which means there was a significant difference between the control and treatment groups. Weight gain in the treatment group was 2.16 times higher than the control group. In the relative and absolute organ weight data, the p-value was >0.05, which means there was no significant difference between the control group and the treatment group.

Kidney histopathological observations included cell necrosis, tubular dilatation, and inflammatory cell infiltration were carried out using Fiji Image J software. The amount of necrosis is obtained by calculating the number of necrosis cells divided by the total number of cells. Tubule diameter measurements were carried out by drawing lines at the longest, shortest and in-between diameters, then measuring the average length of the three lines.[11]

Table 1. Body weight gain and kidney weight.

Groups	Average Body Weight Gain (gram)	Average Absolute Kidney Weight (gram)	Average Relative Kidney Weight (%)
Control	7.6 ± 3.51	0.513 ± 0.028	0.407 ± 0.026
Treatment	16.4 ± 2.51	0.547 ± 0.067	0.400 ± 0.045

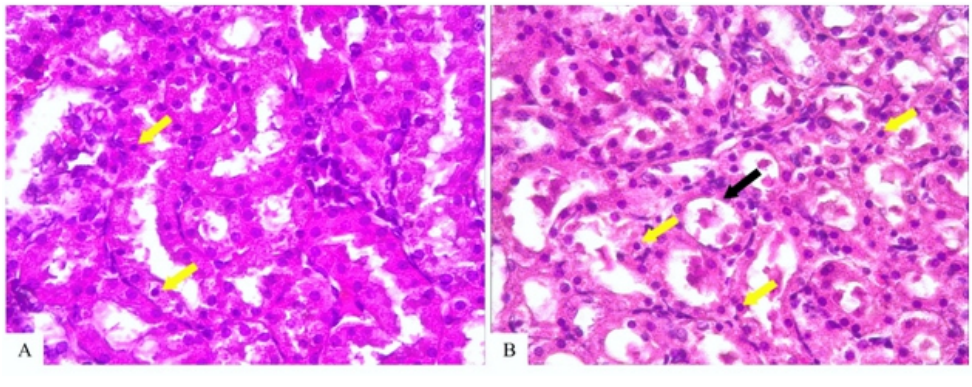

Figure 1. Kidney Histopathology with Hematoxylin-Eosin (HE) staining (400x microscope magnification); A: Control group; B: Treatment group; Yellow arrow: Necrosis cell; Black arrow: Tubular dilatation.

Table 2. Kidney histopathology observation result.

Groups	Necrosis (%)	Tubular Dilatation (%)	Neutrophil Infiltration (%)	Average Scoring ± SD
Control	1.14 ± 0.94	0	0	0.72 ± 0.46
Treatment	1.22 ± 0.79	0.43 ± 0.79	0	0.92 ± 0.28

Inflammatory cell infiltration was observed by counting the number of neutrophil infiltrates.

The histopathological appearance of the kidney is shown in Figure 1. Necrosis cells which are characterized by condensed cell nuclei and a darker color are visible in both the control and treatment groups. Tubular dilatation was only seen in the treatment group. No neutrophil infiltration was seen in either group.Table 2 shows a comparison of kidney histopathology scoring between the control and treatment group. To test the reliability of the data, the results of these observations were carried out with a Cronbach's Alpha test and a value of >0.7 was obtained, which means the data is reliable.The Mann-Whitney test was carried out, and the result was Sig >0.05, which means there was no significant difference in the histopathological appearance of the kidneys between the treatment group and the control group in terms of necrosis, tubular dilatation, and neutrophil infiltration.

DISCUSSION

In this study, a toxicity test was carried out on red onion skin in the form of an extract preparation using ethanol solvent. Red onion skin is used because it contains 48 times more flavonoids than the tubers, the extract preparation was chosen. After all,it contains more flavonoids than the infusion preparation,and ethanol was chosen because it is a good solvent for flavonoids.[1,12] The concentration of ethanol used in making ROSE in this study was 96% and the total flavonoid content was found to be 105.7 mgQE/g. ROSE research by Duan et al[13] with 70% ethanol solvent obtained lower total flavonoid levels, namely 49.63 mgQE/g. This difference in the results of flavonoid levels shows that the solvent concentration influences the flavonoid levels of the extract preparation. This is supported by research by Wahyuni & Yusuf,[14] which proves that 96% ethanol produces more flavonoids than 80% and 60% ethanol. Apart from concentration, Wayan et al[15] proved that differences in temperature and duration of extraction also affect the flavonoid content of the extract. Research on red onion skin flavonoid levels in infusion preparations by Helianti et al[16] obtained a flavonoid content of 96.8 mgQE/L. The higher flavonoid content in the extract preparation is because flavonoids are more soluble in ethanol than water and there is a possibility that the flavonoids will be damaged due to the heating process in the infusion preparation.[12,17]

Red onion skin is a natural ingredient and with a high flavonoid content, it has the potential to be used as a functional food or drink. Flavonoids, which are antioxidants, in high amounts will change their properties to become prooxidants and cause a chain reaction that has negative effects on the body.[5] Therefore, an acute toxicity test of red onion skin is needed to determine its safety classification. The type of flavonoid contained in red onion skin is quercetin. The accumulation of quercetin in the body can trigger auto-oxidation in mitochondria, whichcauses an increase in ROS.[5] Apart from that, quercetin can also reduce Cu2+ ions to Cu+ and Fe3+ to Fe2+, which initiates the formation of ROS through the Fenton reaction.[5] Quercetin can also bind radicals. free and produces the semiquinone quercetin, which is then oxidized to quinone. Quercetin quinone is a reactive form, so it needs to be neutralized by the endogenous antioxidant, namely GSH. As a result, there is a decrease in the amount of GSH, which functions to neutralize ROS.[18]

When there is an increase in ROS, the kidneys have a natural mechanism to maintain homeostasis by forming endogenous antioxidants.[19] Production of these endogenous antioxidants can reduce the damage caused by ROS in conditions of oxidative stress. However, if the increase in free radicals is not compensated, ROS can react with nitrogen bases and deoxyribose, resulting in DNA oxidation.[5,20] ROS can also react with phospholipid membranes, causing lipid peroxidation and cell membrane damage. This condition can lead to damage and necrosis of tubular cells. Next, cells that experience necrosis will detach from the basement membrane into the lumen of the tubule. If the damage that occurs cannot be repaired, the

accumulation of necrotic cells in the tubules will cause obstruction and increase intratubular pressure so that the tubule lumen will dilate.[21] ROS can also trigger inflammation by activating nuclear factor κB (NF-κB).[22,23] Histological features of necrosis cells is characterized by the nucleus shrinking and darker color, while inflammation is characterized by infiltration of inflammatory cells, especially neutrophils.[24,25]

In this study, the LD50 of ROSE was found to be >5000 mg/kgBW and was classified as "Unclassified" based on the GHS. Research on the LD50 of ROSE in rats by Ojuade et al[26] and Builders et al[27] obtained an LD50 value of >2000 mg/kgBW. Similar research was also carried out by Dibal et al[28] on mice and obtained LD50 ROSE results of 3807 mg/kgBW. This shows that ROSE is safe to consume at a dose of 5000 mg/kgBB in a single dose. It is necessary to carry out further toxicity test studies such as sub-chronic and chronic to determine long-term effects.

Analysis of body weight gain in this study showed a significant difference. The body weight gain in the treatment group was greater than the control group. Research on rats by Lee et al[29] stated that administering ROSE could increase food intake in rats. Quercetin, which is contained in ROSE, is known to increase protein absorption in the small intestine.[30] Quercetin can also maintain the balance of normal flora in the small intestine, thereby increasing food absorption.[31] On the other hand, in rats induced by a high-fat diet, quercetin has the opposite effect as anti-obesity and has the potential to reduce body weight.[32,33] The effect of ROSE administration on relative and absolute kidney weight in this study did not cause a significant difference. These results are supported by research by Ojuade et al[26] which showed that administration of ROSE at a dose of 500 mg/kgBW did not cause significant changes on kidney weight.

The dose of 5000 mg/kgBW ROSE in this study was proven not to cause significant changes in the histology of the kidneys of Wistar rats. These results are in line with research on mice by Phukan et al[34] which stated that there were no histological abnormalities in the kidneys given 2000 mg/kgBW of onion peel-derived gold nano-bioconjugate. On the other hand, research on mice by Dibal et al[28] showed that administration of onion skin extract at a dose of 1600 mg/kgBW caused tissue bleeding and lymphocyte aggregation in the kidneys. These differences in results could be due to differences in the types of experimental animals, extract preparation solvents, and the acute toxicity test methods used. The toxicity test in this study was carried out on rats using 96% ethanol and the OECD Fixed Dose Procedure No. 420, while research by Dibal et al[28] was carried out on mice using ethyl acetate solvent and a toxicity test method developed by Lorke (1983).

CONCLUSION

The acute toxicity test of ROSE in this study resulted in an LD50 of >5000 mg/kgBW. Administration of ROSE at a dose of 5000 mg/kgBW had no effect on histopathology and kidney weight, but did have an effect on body weight gain. Further research with a longer duration needs to be carried out to evaluate the long-term effects of ROSE.

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