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Efek Ekstrak Etanol Kulit Bawang Merah dalam Menurunkan Kadar Malondialdehid Ginjal Tikus Wistar yang Diinduksi Diazinon

Effect of Shallot Peel Ethanol Extract in Reducing Kidney MDA Levels in Diazinon-induced Wistar Rats

Mukhammad Arif Hadi Khoiruddin[1], Dina Helianti[2*)], Muhammad Hasan[3], Rosita Dewi[2], Sheilla Rachmania[2]

[1]*Faculty of Medicine, University of Jember, Jember, Indoensia*
[2]*Department of Histology, Faculty of Medicine, University of Jember, Jember, Indonesia*
[3]*Department of Anatomy, Faculty of Medicine, University of Jember, Jember, Indonesia*

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*)*Corresponding author:*
E-mail: dina_helianti@yahoo.co.id

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Abstract

Diazinon is an organophosphate pesticide often used by farmer. The residue of diazinon may contaminate agricultural products and if accidentally consumed in a long term, may potentially led to health problems such as kidney disorders. Shallot (Allium cepa) peel was recently proposed to help recover the kidney damage due to its high flavonoid antioxidant content. This study aims to determine the antioxidant effect of shallot peel extract in reducing oxidative stress caused by diazinon with malondialdehyde (MDA) level indicator on the kidney of Wistar rats (Rattus norvegicus). This research was a true experimental with an in-vivo posttest-only control group design. The 28 rats were divided using a simple random sampling technique. The groups were the control group (K0), the diazinon group (K1), SPEE (Shallot Peel Ethanol Extract) groups which are P1 (300 mg/kg BW), P2 (600 mg/kg BW), P3 (900 mg/kg BW), P4 (1200 mg/kg BW) and P5 (2400 mg/kg BW). At the end of the study, the MDA kidney levels was analysed using the TBARS method. Effective doses were found in 600 mg/kg BW/day, 900 mg/kg BW/day, 1200 mg/kg BW/day, and 2400 mg/kg BW/day. The optimal dose of shallot peel extract in this study was 600 mg/kg BW/day. This study showed the effectiveness of shallot peel extract in reducing kidney MDA levels in diazinon-induced Wistar rats.

Keywords*: shallot peel, diazinon, kidney MDA level*

Introduction

Agriculture is one of the most important sectors in Indonesia. Numerous farmers are using pesticides to do pest control to increase production due to their effectiveness, low cost, and convenience (Balai Penelitian Lingkungan Pertanian, 2013). One of the most used pesticides worldwide—organophosphate—is effective in eradicating pest, but improper and excessive usage of this pesticide may leave residue in agricultural products (Odukkathil & Vasudevan, 2013). Serious acute damage in kidney cell could occur as a result of continuous consumption of such contaminated products by either farmers or the general community (Lee et al., 2015). One type of organophosphate that is widely used is diazinon (Akan et al., 2013).

Previous research done by Mufliha showed diazinon residue found in mustard plant (Muflihah et al., 2015). Metabolism of diazinon becomes diazoxon resulting in reactive oxygen species (ROS) accumulation which in turn can lead into health problem if the diazinon-contaminated agricultural product is accidentally consumed in the long term (Ozbek, 2012). Increased ROS lead to oxidant and antioxidant imbalance called oxidative stress which in turn causes lipid peroxidase (Edem et al., 2012). This condition can be detected in the body through the level of MDA.

The state of oxidative stress in the body caused by diazinon will lead to further damage in body tissue, particularly kidney as the main path of diaxozon excretion (Abdel-Daim, et al., 2018). This was revealed by Wisudanti et al. (2019)

through histopathological changes in Wistar rat kidney cells following diazinon administration orally.

To counter the progressive kidney damage caused by diazinon, an antioxidant compound is often advised since its expected to neutralize free radicals hence reducing the cellular damage (Parwata, 2016). Shallot peel is currently uprising as a natural product which is containing antioxidant with flavonoid, saponin, and tannin as the active compounds (Elsyana & Tutik, 2018). It also contains 3-5 times quercetin flavonoid compared to the tuber part. Shallot peels are easily retrieved from household waste due to their being the main ingredients in cooking.

Shallot peel utilization is needed as an antioxidant-rich natural product to reduce kidney cell damage induced by diazinon contamination through oxidative stress reduction marked by kidney MDA. This research was aimed to explore the shallot peel extract in reducing kidney MDA level of diazinon-induced-Wistar Rat.

Methods

This study was a true experimental with in vivo posttest-only control group study design and has been granted an ethical clearance from Faculty of Medicine University of Jember Ethical Committee with certificate number 1521/H25.1.11/KE/2021. The study was done in 3-month time in Pharmacology Laboratory, Biochemistry Laboratory, and Laboratory Animal House in Faculty of Medicine, University of Jember. Twenty-eight 10-12 weeks old Rattus norvegicus with a weight between 150-230 grams were divided into 7 groups as calculated using the Federer formula. The control group (K0) was given corn oil for 7 days before the administration of 3% dimethyl Sulfoxide (DMSO) for another 7 days, and the diazinon control group (K1) was given corn oil for 7 days before the administration of diazinon for another 7 days. The experiment groups were given diazinon for 7 days followed by Shallot Peel Ethanol Extract (SPEE) with doses of 300 mg/kg BW/day, 600 mg/kg BW/day, 1200 mg/kg BW/day, and 2400 mg/kg BW/day. At the end of the experiment, all rats were terminated using ether. Kidneys were isolated, washed, and soaked in 0.9% NaCl before immediately analysed for their kidney MDA level.

The shallot peel used in this experiment was taken from the fried shallot industry in Silo, Jember District. The shallot peel was soaked in salted water, washed, and dried under morning and afternoon sunlight. Dried shallot peel was crushed using a blender and macerated using 96% ethanol. The resulting filtrate was condensed using a rotary evaporator (Elsyana & Tutik, 2018). 600 grams of shallot peel powder produced 52.1-gram extract. It was dissolved into 5 mL DMSO with 3%/kg BW dilution (Hajighasemi & Tajik, 2017). The diazinon dose given was 40 mg/kg BW/day (Wisudanti *et al.*, 2019). To obtain 40 mg diazinon in 5 ml solution (which is rat stomach maximum capacity), 1 ml diazinon for each 74 ml corn oil was used.

The kidney MDA level measurement was using Thio-Barbituric Acid Reactive Substance (TBARS) method. Following 0.9% NaCl washes, each 1 g tissue was dissolved into 9 ml of 1.15% KCl to make tissue homogenates, centrifuged, and the supernatant was mixed with 1 ml of 1% trichloro acetate acid (TCA) and 1 ml of 0.67% TBA in boiling water for 90 minutes. The solution was cooled and centrifuged at 1000 rpm for 10 minutes, and the supernatant was read under 532 nm. The kidney MDA level was converted using standard curve with y=0,0045x + 0,0753 and r^2 = 0,9906 (µM/mL). The resulting MDA level was further analysed using SPSS software. The statistical analysis using in this experiment is the Kruskal Wallis Comparison Test followed by the Wilcoxon Mann Whitney post hoc test (Dahlan, 2015).

Results

The average of kidney MDA levels for each group can be seen in Table 1. The highest kidney MDA level was 80.1 µM/mL found in the diazinon (K1) group, while the lowest kidney MDA level was 34.71 µM/mL found in the normal group (K0). In the SPEE group, the lowest kidney MDA level was 38.16 µM/mL at a dose of 1200 mg/kg BW (P4).

Table 1. The average of kidney MDA level

Group	The average of kidney MDA level (µM/mL)	Deviation standard
K0	34.71	5.36
K1	80.1	4.88
P1	67.71	8.83
P2	50.99	1.53
P3	53.21	1.78
P4	38.16	5.76
P5	46.55	2.72

Table 2. The post hoc test of kidney MDA level

Group	Group	Significance (p)
K0	K1	0.021*
	P1	0.043*
	P2	0.083
	P3	0.083
	P4	0.564
	P5	0.386
K1	P1	0.248
	P2	0.021*
	P3	0.021*
	P4	0.021*
	P5	0.021*

*p<0.05: significant difference between control group and diazinon/SPEE group

The results of data analysis using Shapiro Wilk normality test and Levene homogeneity Test showed that the data were not normally distributed (p<0.05) so that the comparison test was performed using Kruskal Wallis with p=0.002 (p<0.05) and subsequently analyzed using post hoc Wilcoxon Mann Whitney test. The results showed that kidney MDA level in normal group (K0) had a significant difference with the diazinon group (K1) and SPEE at a dose of 300 mg/kg BW (P1), but did not have a significant difference with the SPEE group at a dose of 600 mg/kg BW (P2), 900 mg/kg BW (P3), 1200 mg/kg BW (P4), and 2400 mg/kg BW (P5).

In addition, it was found that the diazinon group (K1) did not have a significant difference to the SPEE group at a dose of 300 mg/kg BW (P1), but had a significant difference to the SPEE group at a dose of 600 mg/kg BW (P2), 900 mg/kg BW (P3), 1200 mg/kg BW (P4), and 2400 mg/kg BW (P5). The results of post hoc test analysis can be seen in Table 2.

Discussion

The data analysis of kidney MDA levels demonstrated a significant difference between control and diazinon group (p<0.05). It resembles the study carried out by Karimani *et al.* (2018) which revealed that diazinon at dose of 10 mg/kg BW during 7 weeks significantly increased kidney MDA level. Salehi et al. (2015) stated that single dose diazinon 100 mg/kg BW in 24 hours significantly increased kidney MDA level. This result is also supported by Wisudanti et al., (2019) presenting that diazinon at dose of 40 mg/kg BW during 5 days induced kidney toxicity characterized by kidney histopathological damage

Diazinon toxicity is provoked by diazoxone, a product of diazinon biotransformation, which inhibits AChE (Martin-Reina et al., 2017). Diazoxon is produced through two phases of metabolism. Phase I, oxidative desulfurization, is a diazinon phase reacting with CYP to form a hydrophilic oxono-organophosphate with easy conjugated characteristics (Wang & Shih, 2016; Badr, 2020). Phase II, hydrolysis phase, is a detoxification process mediated by PON and CE which results the final product, diazoxone, which is more water soluble and very reactive due to the presence of diethyl molecules with OH-group (Badr, 2020).

The reactive nature of diethyl causes its interaction with lipids, nucleic acids, proteins, and DNA which increases ROS production (Ayala et al., 2014; Casares et al., 2019). The interaction between ROS and lipid triggers lipid peroxidation inducing intramolecular changes at the lipid double bond leading to MDA formation (Ayala et al., 2014; Fritz & Petersen, 2013).

MDA will react with DNA to produce MDA-DNA which cause apoptosis and decrease the activity of intracelullar antioxidants (catalase, glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase, glutathione S-transferase) (Ayala et al., 2014). This process occurs continuously and become distress to the body which decreases the ability to regenerate into its origin (resilience) and the ability to maintain its homeostasis (allostasis) leading to cellular dysregulation and kidney damage demonstrated by the degeneration and glomerular atrophy, cortex hemorrhage, necrosis, pyknosis, hypertrophy of Bowman capsule, and mononuclear infiltration in cortex (Cakici & Akat, 2013; Mansour et al., 2017).

Diazinon-induced lipid peroxidation also disturbs Na-K ATPase pump causing by ATP depletion which emerges problem in passive diffusion and active transport of calcium and magnesium ions in the ascending loop of Henle (Mount, 2014). If it continues, parenchymal and hydropic degeneration will occur, resulting in anaerobic glycolysis and disruption of SERCA in maintaining the function of calcium storage in the cytosol. The increase of calcium storage causes the degradation of nucleic acids, lipids, and proteins, generating necrosis and mitochondrial damage (Illarramendi et al., Karak, 2019; 2014; Phyu, et al., 2020).

Based on the comparative test, there was no significant difference between diazinon group and SPEE group at a dose of 300 mg/kg BW/day and there were significant differences at a dose of 600 mg/kg BW/day, 900 mg/kg BW/day, 1200 mg/kg BW/day, and 2400 mg /kg BB/day. In addition, the comparative test between control group and SPEE group at doses of 600 mg/kg BW/day, 900 mg/kg BW/day, 1200 mg/kg

BW/day, and 2400 mg/kg BW/day showed no significant difference (p>0.05). It indicates that SPEE at doses of 600 mg/kg BW/day, 900 mg/kgBW/day, 1200 mg/kg BW/day, and 2400 mg/kg BW/day decrease kidney MDA level equal to normal. It means that the four doses neutralize diazinon induced-oxidative stress. This is in accordance with research conducted by Wulandari (2019) that administration onion skin ethanol extract at a dose of 600 mg/kg BW could relieve kidney tissue inflammation in mefenamic acid-induced Wistar rats. Bardos et al. (2018) using SPEE at the same dose 600 mg/kg BW showed that SPEE had protective effect to liver damage induced by paracetamol.

Kidney MDA level in SPEE group indicates that the more dose of SPEE, the less kidney MDA level. Nevertheless, SPEE at dose of 2400 mg/kg BW tends to increase of kidney MDA level. Under certain conditions, flavonoids can change their properties from antioxidants to pro-oxidants related to their high concentrations and the presence of redox active metals (Eghbaliferiz & Iranshahi, 2016).

Flavonoid, saponin, and tanin contained in SPEE play roles as antioxidants. Flavonoid regulates the modulation of SERCA to prevent the excessive of calcium storage so that it can induce cell regeneration, reduce necrosis, improve the morphology of mitochondria, and extend cell life (Illarramendi et al., 2014). Flavonoid also has essential roles in SOD and catalase activation catalyzing superoxide anion to hydrogen peroxide and oxygen which is more stable so that no further damage occurs (Birben et al., 2012; Noori, 2012; Suwardi & Noer, 2020; Wahjuni, 2015).

Saponin and tanin boost hydroxyproline production resulting in the increase of collagen growth rate in granulation tissue hence it can accelerate the regeneration and re-epithelialization process (Khunkar et al., 2021). They also avoid hydroperoxide formation consequently inhibiting oxidative stress which can damage cells (Ahmad et al., 2017).

Conclusion

Shallot peel ethanol extract dose of 600 mg/kg BW/day, 900 mg/kg BW/day, 1200 mg/kg BW/day, and 2400 mg/kg BW/day effective reduces the increase of kidney MDA levels in diazinon-induced Wistar rats. The optimal dose of shallot peel extract in this study was 600 mg/kg BW/day.

Conflict of Interest

The authors have no conflict of interest to declare.

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Author Contribution

MA and DH drafted the concept, compiled the research design, collected and analyzed the data, and prepared the manuscript draft. MH drafted the concept, compiled the research design, and interpret the data. RD and SR revised and finalized the manuscript for publication.

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