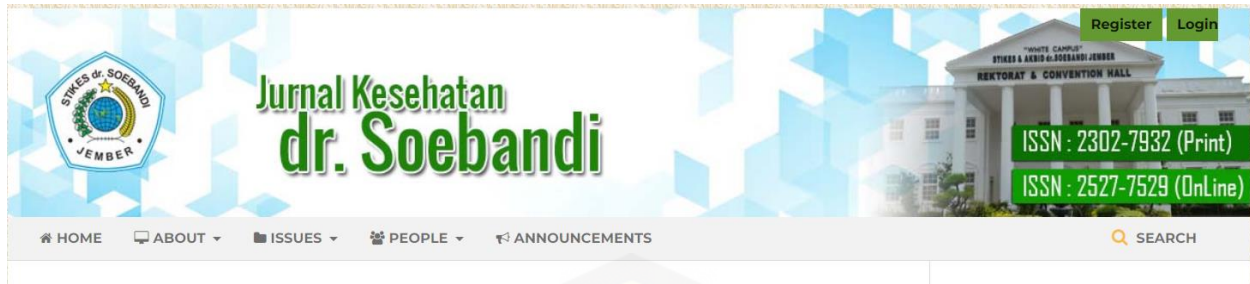


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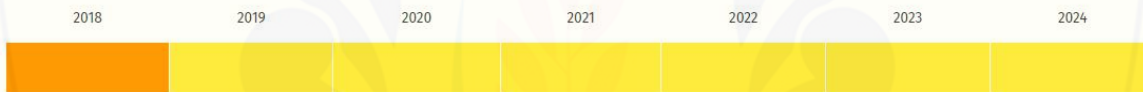
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## Antioxidant Effect of Shallot Peel Infusion on Kidney Oxidative Stress in Diazinon-Induced Wistar Rats

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### ABSTRACT

Diazinon is a pesticide widely used by farmers to control pests. Exposure to the low doses of diazinon can occur continuously through a polluted environment and diazinon residues in agricultural products. It has a nephrotoxic effect through oxidative stress mechanism. Flavonoid as antioxidant can significantly neutralize oxidative stress. Shallot peel is a source of flavonoids. This study was designed to determine the antioxidant effect of shallot skin infusion (SPI) on kidney oxidative stress in diazinon-induced Wistar rats by measuring the flavonoid level of SPI and kidney malondialdehyde (MDA) level. Rats were divided into normal, diazinon, and SPI groups. Diazinon was administered at dose of 40 mg/kgBW for 7 days followed by SPI at doses of 500, 1,000, and 2,000 mg/kgBW for 7 days. The flavonoid level of SPI was measured using spectrophotometry method and the kidney MDA level was measured using ELISA method. The flavonoid level of SPI was 96.8 mg QE/L. The results showed that the normal group had the lowest kidney MDA level of 2.585  $\mu\text{M}/\text{mL}$ , the diazinon group had the highest level of 2.708  $\mu\text{M}/\text{mL}$ , while the SPI group at dose of 500, 1,000, and 2,000 mg/kgBW had renal MDA levels of 2.642  $\mu\text{M}/\text{mL}$ , 2.644  $\mu\text{M}/\text{mL}$ , and 2.593  $\mu\text{M}/\text{mL}$ . Nevertheless, the result of statistical analysis showed that the kidney MDA levels seven days after diazinon administration was not significantly different from the normal group so that SPI administration did not affect the kidney MDA level in diazinon-induced Wistar rats.

**Keyword:** Shallot Peel, Diazinon, Oxidative Stress, Kidney Malondialdehyde

### ABSTRAK

Diazinon adalah pestisida yang banyak digunakan petani untuk mengendalikan hama. Paparan diazinon dosis rendah dapat terjadi terus menerus melalui lingkungan yang tercemar dan residu diazinon pada produk pertanian. Diazinon terbukti memiliki efek nefrotoksik melalui mekanisme stres oksidatif. Flavonoid sebagai antioksidan diketahui secara signifikan dapat menetralkan stres oksidatif. Kulit bawang merah merupakan sumber flavonoid. Penelitian ini dirancang untuk mengetahui efek antioksidan sediaan infusa kulit bawang merah (IKBM) terhadap stres oksidatif ginjal pada tikus Wistar yang diinduksi diazinon dengan mengukur kadar flavonoid IKBM dan kadar malondialdehid (MDA) ginjal. Tikus dibagi menjadi kelompok normal, diazinon, dan IKBM. Diazinon diberikan dengan dosis 40 mg/kgBB selama 7 hari dilanjutkan dengan IKBM dosis 500, 1.000, dan 2.000 mg/kgBB selama 7 hari. Kadar flavonoid IKBM diukur menggunakan metode spectrophotometry dan kadar malondialdehid (MDA) ginjal diukur pada hari ke-15 menggunakan metode ELISA. Hasil pengukuran kadar flavonoid IKBM 96,8 mg QE/L. Hasil penelitian menunjukkan kelompok normal mempunyai kadar MDA ginjal terendah 2,585  $\mu\text{M}/\text{mL}$ , kelompok diazinon mempunyai kadar MDA ginjal tertinggi 2,708  $\mu\text{M}/\text{mL}$ , sedangkan kelompok IKBM dosis 500, 1.000, dan 2.000 mempunyai kadar MDA ginjal 2,642  $\mu\text{M}/\text{mL}$ , 2,644  $\mu\text{M}/\text{mL}$ , dan 2,593  $\mu\text{M}/\text{mL}$ . Namun, hasil analisis statistik menunjukkan bahwa kadar MDA ginjal 7 hari setelah pemberian diazinon tidak berbeda signifikan dengan kelompok normal sehingga pemberian IKBM tidak mempengaruhi kadar MDA ginjal pada tikus Wistar yang diinduksi diazinon.

**Kata Kunci:** Kulit Bawang Merah, Diazinon, Stress Oksidatif, Malondialdehid Ginjal

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## Introduction:

The usage of pesticides in the agricultural sector in Indonesia has increased rapidly since 1982, even in 1984 Indonesia controlled 20% of the world pesticide market (Mariyono et al., 2018). One of the organophosphate pesticides commonly used in agriculture is diazinon (International Agency for Research on Cancer, 2017). Exposure to the low doses of diazinon is highly risky occur in farmers and communities through polluted soil, water, or air, and consumption of agricultural products containing diazinon residues exceeding the maximum limit of 0.33 mg/day (Ambrus & Yang, 2016). If it happens over a long period of time, diazinon will accumulate in the body and subsequently cause the damage of body tissues. Diazinon has negative effects on various organs, especially kidney which has a vital role in the excretion of diazinon metabolites. It causes an increase in intracellular reactive oxygen species (ROS) resulting in lipid peroxidation in cell membranes with end products including malondialdehyde (MDA). In addition, it causes a decrease in endogenous antioxidant enzymes, damage to mitochondrial membranes, as well as protein and lipid peroxidation leading to oxidative stress. This condition will trigger macrophage activation, pro-inflammatory cytokine production, nitrous oxide system (NOS), and cyclooxygenase-2 (COX-2) expression as the response to inflammatory stimuli (Ogasawara et al., 2017).

Epidemiological data show that the intake of herbal plants with high antioxidant content can neutralize oxidative stress caused by toxic substances. Shallot is one of the horticultural products containing antioxidant compounds, i.e. flavonoid. The shallot peel which is often used as household waste, turns out to contain a high flavonoid compound, a type of quercetin, 3-5 times greater than the tuber. Flavonoid content in shallot peel extract has a capacity to break free radical chain reactions in the body so that it can repair the damaged of body cells (Fuentes et al., 2020; Mobin et al., 2022)

This study was aimed to determine the antioxidant effect of shallot peel infusion (SPI) and its flavonoid content in reducing kidney oxidative stress in diazinon-induced Wistar rats by

measuring the level of kidney MDA and the flavonoid level of IKBM. We designate infusion formula since it only requires easy steps to make, serve, and consume. It also has a fairly high flavonoid content compared to other formulas used in the community.

## Methods:

### The preparation of SPI

The shallot peels are soaked in 2% salt water and washed using running water. The washed shallot peels are dried in the sun in the morning and evening. The dried shallot peels are mashed using a blender. The SPI is made using two stacked pans. The first pan was filled with water and subsequently heated, the second pan was placed on top of the first pan and used to place 20% infusion solution consisting of 10 grams of shallot peels *simplicia* and 50 mL of aquadest. The solution was heated at 90°C for 15 minutes. The hot solution resulted is filtered using flannel. Hot water is added to reach a volume of 50 mL (Direktorat Obat Asli Indonesia, 2012). The serial dilution was made to obtain the treatment dose of SPI.

### The total flavonoid level measurement

The total flavonoid level of SPI was qualitatively and quantitatively measured using aluminium chloride colorimetric according to the method of (Puspitasari et al., 2017).

### Animals and treatment

This study constitutes experimental laboratory research with posttest-only control group design. We used *Rattus norvegicus* Wistar strain, male, aged 3 months, and weighing 150-200 grams. Rats were acclimatized for seven days with ad libitum feeding. They were randomly divided into 5 groups consisting of a normal control group, a diazinon group, and three SPI groups. The normal control group was administered corn oil at dose of 5 mL/kgBW/day orally on day 1 to 7, followed by aquadest at dose of 10 mL/kgBW/day orally on day 8 to 14. Diazinon and SPI groups were induced by diazinon at dose of 40 mg/kgBW/day orally on day 1 to 7. On day 8 to 14, diazinon group was administered aquadest at dose of 10 mL/kgBW/day orally, whereas each of SPI group

was administered SPI at dose of 500, 1,000, and 2,000 mg/kgBW/day. This research has received ethical approval from the Research Ethics Committee, Faculty of Medicine, University of Jember with reference number 1470/H.25.1.11/KE/2021.

### The kidney MDA level measurement

On the day 15, the experimental animals were executed and the kidney MDA levels were measured using the enzyme-linked immunosorbent assay (ELISA) (rat kit for MDA Elabscience, United States ELISA) based on the manufacturer's protocol.

### Statistical analysis

The average of kidney MDA levels were analyzed using Kruskal-Wallis test (Sujarweni & Endrayanto, 2012).

### Results:

The total flavonoid level of SPI in unit of mg QE/L, i.e. quercetin equivalent is shown in Table 1.

**Table 1. The total flavonoid level of SPI**

Tested For	Sample Code	Test Result (mg QE/L)
Total flavonoid level	SPI	96.80

The average of kidney MDA level of each group is displayed in Table 2.

**Table 2. The average of kidney MDA level ( $\mu\text{M}/\text{mL}$ )**

Group	Mean	SD
K0 Normal	2.585	0.102
K1 Diazinon	2.708	0.084
P1 SPI 500	2.642	0.110
P2 SPI 1,000	2.644	0.009
P3 SPI 2,000	2.593	0.113

Based on the data, it descriptively showed that the lowest kidney MDA level was in the

normal group with a mean value of 2,585  $\mu\text{M}/\text{mL}$  while the highest kidney MDA levels was found in the diazinon group with a mean value of 2,708  $\mu\text{M}/\text{mL}$ . This indicates that the diazinon group which was only administered sterile water for seven days had the highest renal MDA levels, whereas in the SPI group at dose of 500, 1000, and 2000 mg/kgBW had lower kidney MDA levels, 2,642  $\mu\text{M}/\text{mL}$ , 2,644  $\mu\text{M}/\text{mL}$ , and 2,593  $\mu\text{M}/\text{mL}$ , consecutively.

The higher the dose of SPI, the lower the kidney MDA level. Kidney MDA levels in the SPI group at dose of 2,000 mg/kgBW was closest to the normal kidney MDA level. The normality test for kidney MDA levels using Shapiro Wilk showed that the data for each group were normally distributed ( $p > 0.05$ ). The homogeneity test using Levene test showed that the data is not homogeneous ( $p < 0.05$ ). Hence, the data analysis was performed using Kruskal-Wallis as presented in Table 3.

**Table 3. The Kruskal-Wallis Test<sup>a,b</sup>**

	MDA
Kruskal-Wallis H	5.196
df	4
Asymp. Sig.	.268

a. Kruskal Wallis Test

b. Grouping Variable: KODE

The result of data analysis showed that there was no significant difference of kidney MDA levels among the normal group, diazinon group, and SPI group, so that the high and low levels of kidney MDA were not statistically significant.

### Discussion :

The measurement of flavonoid level of SPI showed that 1 L of SPI contains 96.8 mg of QE. It was higher than *Guazuma ulmifolia* leaf infusion containing 46.2 mg QE/L flavonoid (Nisak, 2020) and not different from cherry leaf extract containing 93.21 mg QE/g flavonoid (Puspitasari et al., 2017) yet it is lower compared to turmeric leaf stew containing 224.61 mg QE/L flavonoid (Labban, 2014). Nonetheless, it is easier to obtain shallot peel than turmeric leaves.



The flavonoid level of SPI is lower than shallot peel in extract formula. (Setiani et al., 2017) stated that 1 gram of shallot peel extract (SPE) contains 149.2 mg flavonoid QE, even (Rahima et al., 2022) studied that 1 gram of SPE contains 228.1 mg QE. The flavonoid content of SPE was 1.5-2.5 times higher than SPI. However, infusion preparation is more applicable for the society than extract preparations.

In case this result is applied as a functional drink, especially in the agro-industrial society, a cup of SPI about 200 ml contains 19.36 mg QE flavonoid, while SPE usually consumed in the form of capsule/tablet about 1 gram contains 149.2-228.1 mg QE flavonoid. Hence, it can be appraised that in one consumption, the flavonoid content in SPI is 8.5-13% of SPE. Further study is still needed to analyze the effectivity of SPI and SPE in the process of structural and functional tissue repair and the underlying mechanism. Whether the difference of flavonoid level which is quite high between SPI and SPE is in line with the tissue repair, or even not significantly different. (Labban, 2014) revealed that the difference of flavonoid content in curcuma between the infusion formula and extract formula is not in line with tissue function improvement and the biochemical status in the body. A threefold difference in flavonoid level does not mean that there is a threefold improvement of tissue function, although there is a significant improvement between the two formulas. The higher dose of flavonoid administration is not always followed by the repair of structural, functional or biochemical status of the body. Therefore, this study constitutes the initial step to establish the amount of flavonoid intake needed to prevent the process of structural and functional tissue damage induced by diazinon exposure.

Many studies demonstrated that diazinon, an organophosphate pesticide, damage body tissues through oxidative stress pathway. The most widely used indicator of oxidative stress is MDA, a metabolite of lipid peroxidation. It causes the increase of MDA dan the damage of kidney. (Ajibade et al., 2016) studied that diazinon at dose of 3 mg/kgBW for 20 days resulted in the increase of serum MDA level and the damage of kidney

histopathology (Wisudanti et al., 2019) revealed that diazinon at dose of 40 mg/kgBW for five days caused the increase of serum MDA level and kidney damage (Noori et al., 2016) also stated that diazinon 100 mg/kgBW for four days induced the increase of serum MDA level and the damage of kidney histopathology. In addition, (Sargazi & Heidarpour, 2018) specifically showed that diazinon at dose of 10 mg/kgBW for seven weeks caused the increase of kidney MDA level and the damage of kidney. Those studies have established that diazinon at low or high doses, in acute or chronic administration, could increase both serum and kidney MDA levels.

(Aramjoo et al., 2021) stated that diazinon decreases endogenous antioxidant level especially glutathion and increases ROS production which is in line to the damage of kidney leading to stress oxidative condition and kidney toxicity. It is resulted from oxygen molecules oxidation producing ROS such as *superoxide radical* ( $O_2^{\bullet-}$ ) dan *hydrogen peroxide* ( $H_2O_2$ ) triggering the peroxidation of unsaturated fatty acid in the cell membrane so that it increases MDA production (Sargazi & Heidarpour, 2018).

In this study, the doses and period administration of diazinon was determined based of the study carried out by (Qodar et al., 2019) who demonstrated the damage of kidney structural histopathology in diazinon-induced rats. The period of SPI administration was determined based on (Wisudanti et al., 2019) who studied the steps of tissue repair after diazinon induction. They revealed that the cell improvement to the normal condition needed ten days since diazinon was administered. It was related to the stress oxidative (Gonçalves et al., 2021) Hence, this study used diazinon at dose of 40 mg/kgBW for seven days followed by aquadest, SPI at dose of 500 mg/kgBW, 1,000 mg/kgBW, and 2,000 mg/kgBW for seven days. On day 15, we measured kidney MDA level.

The descriptive observation showed that the diazinon group only administered aquadest for seven days had the highest kidney MDA levels, while the SPI group at doses of 500, 1,000, and 2,000 mg/kgBW showed lower kidney MDA

level. Administration of SPI at dose 500 mg/kgBW decreased 0.066  $\mu\text{M}/\text{mL}$  of kidney MDA caused by diazinon but quadruple SPI dose at 2000 mg/kgBW only reduce it by 0.115  $\mu\text{M}/\text{mL}$ . The data showed the higher the dose of SPI, the lower the kidney MDA level, but the SPI dose increases is not proportional to the kidney MDA level decreases. The statistical analysis also showed insignificant results.

The data analysis showed that although the highest kidney MDA level was found in the diazinon group, it was not significantly different from the normal and SPI groups. It was probably related to the body capacity to carry out the cell repair within seven days after diazinon administration. It was initiated from the body's mechanism to neutralize the oxidative stress condition. (Abdel-Daim et al., 2019) stated that hepatocyte structural repair occurred five days after diazinon administration. Cell undergoing 100% congestion decreased to 50% and 10% pyknosis, while within seven days, congestion decreased to 30% and 5% pyknosis. The structural cell improvement indicated the decrease of stress oxidative condition was potentially related to the balance between ROS production and antioxidant defense which really affected the cell repair. Conversely, stress oxidative condition will block the activation of cell repair (Gonçalves et al., 2021).

The SPI administration for seven days did not significantly decrease kidney MDA level in diazinon-induced Wistar rats. It was going from the high level of kidney MDA in the diazinon group which was not significantly different from the normal group. As explained, it was appropriate to the body capacity to execute the cell repair so that SPI administration as natural ingredient containing high antioxidant compounds did not significantly reduce the lipid peroxidation having decreased. Based on this result, aside from the antioxidant effect of SPI on lipid peroxidation, it is necessary to further investigate the effect of SPI on the kidney antioxidant defense by measuring the antioxidant capacity of the kidney such as the thiol group and ferric reducing antioxidant power (FRAP).

Oxidative stress condition is strongly influenced by the balance between ROS production and antioxidant defense. The cessation of diazinon administration indicates the absence of ROS trigger, so that the body will responsively neutralize the existing oxidative stress condition by increasing endogenous antioxidants such as glutathione, superoxide dismutase (SOD) and catalase. This condition needs to be associated with the improvement of the histopathological structure of kidney cells which takes a longer time; whether the process of cell repair always be followed by continuous oxidative stress conditions or not. (Abdel-Daim et al., 2019) explained that the administration of another natural ingredient, i.e. curcumin after the administration of diazinon could improve tissue structure to normal on day 4 compared to the group without curcumin which needed more than 10 days. Because the effect of SPI in improving kidney structure is not observed in this study, further observation on the histopathological structure of kidney tissue related to stress oxidative condition is needed whether the decrease of MDA level seven days after diazinon administration in line with the improvement of kidney tissue.

### Conclusions:

Based on the result, it can be concluded that the kidney MDA levels seven days after diazinon administration was not significantly different from the normal group so that SPI administration did not affect the kidney MDA level in diazinon-induced Wistar rats.

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