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THE EFFECT OF JENGKOL SEED (*Archidendron pauciflorum*) EXTRACT TO PLASMA MDA LEVEL DUE TO DEEP SECOND DEGREE BURN WOUND IN *SPRAGUE DAWLEY* RATS

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ABSTRACT

Background: During inflammatory phase of the burn healing process, the number of plasma MDA levels tend to increase, which may cause damage towards cells and thus delay the resolution of burn wounds. Some research has been done in order to find alternative treatment for burn wound, including the application of antioxidant. Jengkol contains many antioxidant compounds which are expected to reduce plasma MDA levels, so it can accelerate the healing of burn wound. **Aim:** To prove that administration of jengkol extract can reduce plasma MDA level in Sprague dawley rats which were been given burn wound. **Method:** This experiment was using pre and post-test control group design, with 20 Sprague dawley rats as samples. All of the samples were given deep second degree burn wound which were then divided equally into 2 groups, treatment group (given jengkol extract 500 mg/KgBW) and control group (not given anything). Plasma MDA levels were then analyzed by using TBARS method. Data was analyzed using paired T-test, Wilcoxon, Mann-Whitney, and independence T-test. **Result:** There is no significant difference between the mean of plasma MDA levels in pre and post-test of either control group (p=0.771) or in the treatment group (p=1.00). Using Mann-Whitney test, there isn't any significant difference in the pre-test between control and treatment group (p=0.677) or in the post-test between control and treatment group (p=0.916) by using independence T-test. **Conclusion:** Jengkol extract cannot reduce plasma MDA levels of Sprague dawley rats which were given burn wound.

Keyword: *Jengkol extract, plasma MDA, burn wound, antioxidants*

INTRODUCTION

In 2016, there were 486,000 people in the United States who suffered burn wounds, either caused by fire, electricity, hot air, or irritant substances.[1] Burn wounds are injuries that affect skin or other organic tissue underneath.[2] Damage caused by burns varies depending on the degrees of burn wounds. In deep second degree burn wound, it tends to penetrate to the dermis pars reticular layer. The macroscopic appearance is usually yellowish-white and rather rough lesion. The pain will be decreasing and will not be as bad as either the first or superficial second degree burn wound.[3,4]

The healing process of burn wound consists of 3 phases, which are inflammatory phase, proliferation phase, and the remodeling phase.[5] In the inflammatory phase there is an increase of Reactive Oxygen Species (ROS) or free radicals, which mostly originate from Xanthine Oxidase (XO) (which is important for reperfusion of injured and ischemic tissue) and also due to activation of neutrophil along with macrophages during trauma.[6]

When the amount of ROS inside the body far exceeds the antioxidant levels, it will cause a condition of "oxidative stress". This condition contributes to many pathological issues, such as immunosuppression, sepsis, prolong of the burn wound healing, and even death. With a prolong of healing process, it may increase the risk of secondary infection, which will be more complicated to handle.[6] One of

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indicators of increasing ROS in the body is an increasing of malondialdehyde (MDA) levels.[1]

Nowadays, various methods have been developed to optimize the burn wound treatment, one of which is using antioxidants.[6] Antioxidants can be classified into 2 major groups, which are enzymatic and non-enzymatic. Non-enzymatic antioxidant are antioxidants that are not derived from inside human body, but from vegetables and fruits.[1,7] One of vegetables that is often found in Indonesia is jengkol.[8] Jengkol is rich in antioxidants such as phenols, flavonoids, terpenoids, alkaloid, and based on research conducted in 2013, it has similar structure as cysteine, which is precursor in the formation of glutathione.[9,10]

The aim of this study is to prove that administration of jengkol extract can reduce plasma MDA level in Sprague dawley rats which were been given burn wound.

METHOD

Study Design

This study used Pre and Post-Test Control Group Design. The subjects were recruited by simple random sampling. There were 20 *Sprague dawley* rats which met inclusion and exclusion criterias. The inclusion criterias in this study were 12 weeks old male Sprague dawley rats, around 200 grams in weight, healthy, active, and with given deep second degree burn wound. While for the exclusion criteria was rats with anatomical anomaly. Subject was classified as drop out if it died during acclimatization and experiment. Independent variable in this study was 500mg/KgBW jengkol extract, as for the dependent variable was plasma MDA level in *Sprague dawley* rats. This study was conducted for 1 day.

Preparation of Jengkol

Jengkol which was used in this study must have shiny brown, hard-skinned, and distinctive smell, which indicates that the jengkol is ripened enough. Jengkol was obtained from Pontianak, West Kalimantan. The first step of manufacturing jengkol extract was to weigh jengkol seeds (there was 461 grams jengkol which was used in this study). Then jengkol seeds were ground until crushed. Next, added 1000 ml of 96% ethanol solvent into the container which contained samples. After that, the container was covered by aluminum foil and kept at room temperature for 7 days for maceration process. Afterwards, the container was placed on a dark area which was not exposed to the sun (to avoid oxidation). Next step, the extract, which had been filtered, was dried by using steamer. Then, the extract was placed in a container and stored in incubator for 45^{O}C for one night. Furthermore, the thickened extract was diluted using hot water and ready to be used.

Animal Models

In this study, 10 rats would be put into treatment group (given jengkol extract 500 mg/KgBW twice a day), while the other 10 would be put into the control group (not given anything). All of the subjects had their back shaved off, approximately 5 cm in diameter. Before the subjects were given deep second degree burn wound, they were anesthetized using combination of 10% Ketamine and 2% Xylazine. Then, 100^{O}C water was poured into a round border approximately 3.5 cm in diameter, located on the shaved off back. The boiling water was held inside the border for 15 seconds before discarded. This procedure would create a deep second degree burn wound which would have a yellowish-white and rather rough lesion appearance as shown in Fig. 1

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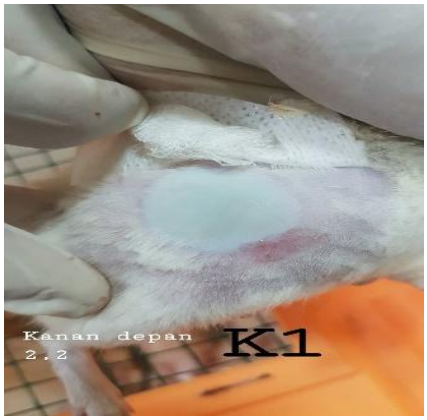

Figure 1. Deep second degree burn wound appearance.

The deep second degree burn wound then was wrapped using sterile gauze, which had been moistened with 0.9% NaCl. Then the administration of jengkol extract for treatment group was carried out.

Laboratory Analysis

Retro-orbital blood sampling was done 6 hours after given burn wound (pre-test) and 18 hours after first blood sampling (post-test). The amount of blood which was taken per subject was approximately 1.5 – 2 cc. Then the blood was centrifuged where plasma samples obtained would be tested further using TBARS (Thiobarbituric Acid Reactive Substances) Assay method.

First of all, put in 200µL of samples into test tube. Then added 2000µL of 15% TCA and 2000µL of 0.37% TBA in 0.25N HCl solution into the test tube. Next, the mixed solution was incubated for 60 minutes at 95^{O}C. The solution was then centrifuged at 3000rpm for 15 minutes. Later, supernatant was taken and put into cuvette, then the absorbance was read using spectrophotometer Stat Fax 3300 at a wavelength of 545nm.

Statistical Analysis

The analysis for numeric data used Saphiro-Wilk for normality test, while paired T-test, independent T-test, Mann-Whitney, and Wilcoxon were used for testing the hypothesis.

Ethics Approval

This study had obtained an ethical clearance from KEPK (Health Research Ethics Commision) with No. 49/EC/H/KEPK/FK-UNDIP/V/2019.

RESULTS

In this study there was no any dropped out subject during experiment.

Table 1. Mean plasma MDA levels

Group	Mean ± SD (µmol/L)		Sig.	Δ Mean
	Pre	Post		
Control	0.026 ± 0.009	0.0279 ± 0.015	0.771*	0.0019
Treatment	0.0274 ± 0.009	0.0286 ± 0.014	1.00**	0.0012
Sig.	0.677***	0.916****		

*paired T-test (significant if p<0.05)
**Wilcoxon (significant if p<0.05)
***Mann-Whitney Test (p<0.05)
****Independence T-test (p<0.05)

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Based on Table 1, the highest mean of plasma MDA level was in experimental post-test group (0.0286 µmol/L) and the lowest mean of plasma MDA level was in control pre-test group (0.026 µmol/L).

Based on paired T-test difference test, it was known that there was no significant difference between pre and post-test of control (p=0.771). As from Wilcoxon test, there was no significant difference either between pre and post-test of treatment group (p=1.00). By using Mann-Whitney Test, there was no significant difference in pre-test between control and treatment group (p = 0.677). While from Independence T-test, no significant difference was found either in post-test between control and treatment group (p=0.916).

DISCUSSIONS

In this study, there were 20 experimental male rats which were used. Male rats were used because there were no hormonal influences, such as estrogen, that could affect oxidative stress levels in this study.[11,12] In addition, the age of rats in this study was around 10-12 weeks, in which at such age rats were in the young adult phase, making it ideal for this study. The rats' body weight in this experiment varied from 159 until 234 grams.

Based on the data obtained in this study, there was an increase in the mean plasma MDA levels in both groups, control and treatment, even though the increase of plasma MDA levels in the pre and post-test of control group was greater than in the treatment group.

In this study, there was no significant differences between plasma MDA levels in both pre and post-test of the treatment group. In a study conducted by Davood Mehrabani et al in 2015, the result of administering antioxidants in the case of burn wound appeared to be significant on the 14th day.[12] However in this study, the procedure for administering jengkol extract and observation were only carried out for 1 day and thereafter not continued. This procedure was based on the research conducted by Quan Fang et al in 2017, where it was said that the highest increase in MDA was about 6 hours after the subject was given burn wound. After 6 hours, the MDA levels would decrease gradually.[13] However in this study, plasma MDA level for pre-test (6 hours after given burn wound) did not have a significant difference from plasma MDA levels for post-test (18 hours after pre-test blood sampling). This result might be in accordance with research conducted by Al-Jawad in 2009, where the decrease in serum MDA levels only began to have significant difference on the third day and after the seventh day the difference even became more significant.[1] This research was corresponding to the theory of healing process, in which there were 3 phases, which are inflammatory phase, proliferation phase, and remodeling phase.[5] During inflammatory phase, the number of macrophage and PMN cell were abundant. As the result, macrophage produced a lot of pro-inflammatory cytokines, which caused more damage around the wound, while the PMN cell would produce myeloperoxidase enzyme which had a role in development of free radical. This phase would be going on for 1 until 3 days.[16] In this study, plasma MDA level during post-test was higher than plasma MDA level during pre-test, either in control or treatment group. This result could be possible, considering the inflammatory phase was still going on until the third days, which meant that free radicals (such as MDA) were still produced more and more. In that case, the jengkol extract might not perform well enough because of the ongoing process.

The other factor which might affect the insignificance result might be because there was no variation of dose in this study.

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Thus, it was hard to assess whether the dose used in this study was the optimum dose of jengkol extract as antioxidant. In this study the dose of jengkol extract was 500mg/KgBW.[17,18] This dosage selection was based on several research which had plants extract as antioxidant agent in their experiments. Mostly the dose used in antioxidant experiment was around 500 mg/KgBW, which then was also used in this study. However, those research did not use jengkol extract, thereby 500 mg/KgBW might not be the optimum dose for jengkol extract as antioxidant. Other study which used jengkol extract as anti-hyperlipidemia had been carried out, where the optimum dose was 385 mg/KgBW.[19] However, there was none research done to prove whether that dose could function optimally as antioxidant.

CONCLUSION

The administration of jengkol extract cannot reduce the plasma MDA levels in *Sprague dawley* rats which were given burn wound.

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