DIPONEGORO MEDICAL JOURNAL
(Jurnal Kedokteran Diponegoro)
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
Volume 9, Nomor 2, Maret 2020

LONGAN PEEL EXTRACT (DIMOCARPUS LONGAN L.) AS AN ANTIOXIDANT AGAINST OXIDATIVE STRESS IN WISTAR RATS INDUCED BY TOXIC DOSES OF PARACETAMOL

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ABSTRACT

Background: Oxidative stress is an imbalance between free radicals and antioxidants, characterized by increase in malodialdehyde (MDA) levels. Stress oxidative caused by paracetamol overdose consumption and can be a serious problem for the structure and liver cell function. Oxidative stress prevented and controlled by antioxidant. Longan peel has phenolic and flavonoid which can potentially be sources of natural antioxidants. This study aimed to prove the longan peel extract had an antioxidant effect that could affect the oxidative stress in rats induced by toxic doses of paracetamol. **Method: *:*** Experimental research with control group design. Group P1, P2, and P3 were treatment group induced by paracetamol with a toxic dose of 2000 mg/kgBW and given extracts of longan peel (Dimocarpus longan L.) with multilevel doses, that were 25 mg/kgBW, 50 mg/kgBW, and 75 mg/kgBW wistar rat. Then, blood samples from each group were collected and measured plasma MDA levels and the animals were terminated, then their livers were taken for measurement of liver SOD enzyme activity. **Result:** One Way ANNOVA test showed a significant difference in plasma MDA ($p=0,000$) in the whole group of wistar rats. Significant differences were also shown on the examination of plasma MDA levels in the K1-K2, K2-P1, K2-P2, and K2-P3 groups ($p<0,05$). **Conclusion:** Longan peel extract had the effect of reducing plasma MDA levels of wistar rats induced by toxic doses of paracetamol compared to the control group.
Keywords: *Paracetamol, longan peel extract, MDA.*

INTRODUCTION

Oxidative stress is an imbalance between free radicals and antioxidants.[1] Increased free radicals in the body can be caused by various things, such as inflammation, exercise, smoking, environmental pollutants, radiation, certain drugs, pesticides, etc.[2] Increased free radicals which are not followed by an increase antioxidants in the body can increase oxidative stress. Oxidative stress can cause oxidative damage at the cellular, tissue or organ level. Oxidative damage to hepatocytes will lead to liver dysfunction. Oxidative stress is characterized by increased levels of malondialdehyde (MDA) which is the result of lipid peroxidation in the body.[3]

Antioxidants are compounds that can inhibit the oxidation reaction to scavenge free radicals and highly reactive molecules, resulting in cell damege will be inhibited.[3] Although the body can naturally cope with the increase in free radicals, but on certain conditions such as the relatively heavy physical exercise, inadequate endogenous antioxidants, so that the body needs antioxidants from outside the body.[4] Naturally antioxidants can controlled oxidative stress by inhibiting lipid peroxidation. Fruit peel is rich in natural antioxidants such as flavonoids, phenolics, anthocyanins, and carotenoids.[5] One of the fruit peels extract which has the highest antioxidant activity is longan peel.[5] It is interesting to be studied further about the antioxidant ability of longan peel extract to counteract or reduce free radicals that cause oxidative stress.

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This article will review the use paracetamol to induce free radicals that trigger oxidative stress in the rats. High dose of paracetamol will be metabolized by the cytochrome P-450 hepatic enzyme, producing the free radical N-acetyl-p-benzoquinoneimine (NAPQI). These free radicals will be controlled by the body's endogenous antioxidants but in toxic doses of paracetamol, endogenous antioxidants will go down resulting in the accumulation of free radicals.[6] Increased free radicals in the body that are not followed by an increase in antioxidants will create oxidative stress on the liver and cause the occurrence of lipid peroxidation in the form of an increase in plasma MDA levels.[7]

So far there are no studies that prove that longan peel has antioxidant effects that can affect the level of oxidative stress in rats induced by toxic doses of paracetamol. Considering the potential high antioxidant content in longan peel, researchers are interested to find out the effect of longan peel extract (*Dimocarpus longan L.*) on plasma MDA levels of wistar rats induced by toxic doses of paracetamol.

ETHICAL CLEARANCE

This research had been accepted and given an ethical clearance from Komisi Etik Penelitian Kesehatan (KEPK) Faculty of Medicine, Diponegoro University/RSUP Dr. Kariadi Semarang with ethical clearance number 65/EC/H/KEPK/FK-UNDIP/V/2019 on May, 21st 2019.

MATERIALS AND METHODS

Materials:

The materials used in the study was 1% CMC, 95% ethanol, 0,0518 M carbonate buffer, aquadest, chloroform: ethanol (3:5), 0,01 M ephinephrine solution, longan peel extract, paracetamol, phosphate buffer saline (PBS), trichloroacetic acid (TCA) 15%, tiobarbiturat acid (TBA) 0,37% in HCl 0,25 N, tetraetoksipropan (standard MDA).

Tools:

Centrifuge, glass tools, micropipette, water bath, Eppendorf tubes, tools volumetric, mortar, UV-VIS spectrophotometer, *sonde* oral, syringes, surgical tools, and weighing animals.

Experimental animals and study design

This study used a post-test experimental research design with control group design. The sample of this study was an experimental animals including 30 rats aged 2-3 months, with a bodyweight of 140-250 grams. Samples were divided into 5 groups; K1 group was a control and K2 group was a negative control group induced by paracetamol with a toxic dose of 2000 mg/kgBW. Group P1, P2, and P3 were treatment group induced by paracetamol with a toxic dose of 2000 mg/kgBW and given extracts of longan peel (*Dimocarpus longan L.*) with multilevel doses, that were 25 mg/kgBW, 50 mg/kgBW, and 75 mg/kgBW wistar rats. Then, blood samples from each group were collected and the plasma MDA levels were measured.

Longan peel (Dimocarpus longan L.) Extract Making

Longan peel (*Dimocarpus longan L.*) was washed and separated from the peel. Then, the longan peel thin was cut and dried in a room temperature. The Longan peel was then extracted by soxhlet method. First, all dried longan peels were weighed and a yield of 215,386 grams was obtained and then divided into two; 112,4163 grams and 102,9233 grams. Then, 112,4163 grams longan peel was being processed first. First, the marinade was made by inserting the longan peel into the erlen meyer tube and adding 96 ml of ethanol 96%. The longan peel that had been added by ethanol were stirred using a stirring rod until they were evenly mixed. After that, the tube was tightly closed and left for 24 hours in a dark place.

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Then, the longan peel was filtered and put into a porcelain cup and then evaporated in a water bath with a temperature of 70°C until the soxhlet disappeared and obtained a thick paste in the form of a paste. The same process was also done to make extracts from the 102,9233 grams longan peel. Next, the thick extracts that had been made were weighed and the total weight of the thick extracts measured was 16,43 grams. Finally, put the whole extracts into a sterile bottle.

Measurement of MDA

MDA levels were measured from blood plasma according to the method of thiobarbituric acid reactive substance (TBARs). 100 µL of the blood plasma was added 1 ml of trchloroacetic acid (TCA) 15% and 1 ml tiobarbiturat acid (TBA) of 0,37% in HCl 0,25 N. the solution was mixed homogenously heated by waterbath with 95°C temperature for 60 minutes. After cold, centrifuged at 3000 rpm for 15 minutes. Pink colored filtrate was measured absorbance at a wavelength of 545 nm using a UV-VIS spectrophotometer. MDA levels were calculated using the MDA standard curve with concentrations of 0; 0,125; 0,25; 0,5; 1 mM.

Statistcal Analysis

One-Way Analysis of Variance (ANOVA) and *Kruskal-Wallis* were used for statistical analysis. Statistical analysis was done using SPSS version 24.0 for windows.

RESULTS

Longan Peel Extract

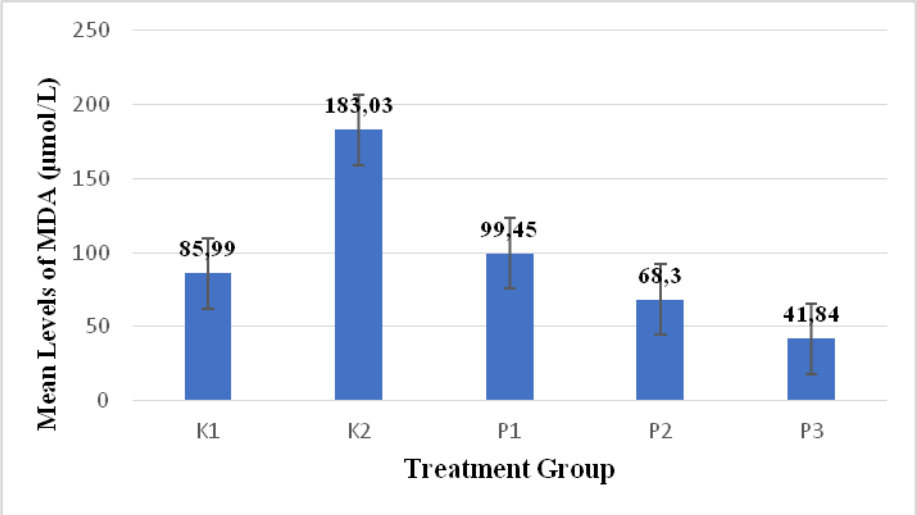
Yield is a ratio between quantity of extract resulting from extraction process. Yield obtained by compare the extract weight with dried and mashed up material multiplied 100%.[7] Yield of longan pericarp extraction can be seen in Tabel 1.

Tabel 1. Total Yield of Longan Pericarp Extraction

Solvent	Material	Dried weight (gram)	Extract weight (gram)	Yield (%)
Etanol 96%	Longan pericarp	215,3386	16,43	7,62

MDA Plasma Levels

Data analysis of MDA plasma levels can be seen in Graph 1.



Graph 1. Mean Levels of MDA

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One Way ANOVA parametric test shows signifficant difference between groups (p=0,000). Then further data analysis will be continued using the Post Hoc Test to assess meaningful differences between the groups.

Tabel 2. Post Hoc Result for MDA Plasma Levels

Groups (n=6)	MDA Plasma Levels (µmol/L)	K1	K2	P1	P2
K1	85,99 ± 39,46	-	-	-	-
K2	183,03 ± 46,40	0,000*	-	-	-
P1	99,45 ± 23,82	0,947	0,001*	-	-
P2	68,30 ± 26,39	0,870	0,000*	0,458	-
P3	41,84 ± 10,99	0,150	0,000*	0,033*	0,612

Information: p<0.05; Significant

Based on the Post Hoc Test, the result showed that there were significant differences between the K1 group with K2 (p<0,001), K2 with P1 (p= 0,001), K2 with P2 (p<0,001), K2 with P3 (p<0,001) which meant thete were significant differences in the group induced paracetamol and given longan peel extract with 25 mg/kgBW dose (P1), 50 mg/kgBW dose (P2) and 75 mg/kgBW dose (P3).

DISCUSSION

This study aimed to determine the MDA plasma levels of paracetamol-induced wistar rats in the given of longan peel extract. The results showed that the paracetamol induced group had higher MDA plasma levels of than the normal control group. This was in accordance with previous studies where toxic dose of paracetamol 2000mg / kgBW in wistar rats can increase MDA plasma levels.[8,9] The results of the administration of longan peel extract in all treatment groups P1, P2 and P3 with a dose of longan skin extract by 25, 50, and 75 mg / kgBW can significantly reduce plasma MDA levels (p <0.05) after the toxic dose of paracetamol was induced (2000 mg / kgBB) when compared to the negative control group (K2), which only induced toxic doses of paracetamol without prior extract of the longan peel. However, it did not different significantly when compared to the control group that was not given any treatment (K1). This means that longan peel extract can reduce MDA levels in rats that are increased due to the induction of toxic dose paracetamol (K2) to almost the equivalent of mice that were not given any treatment (K1).

Longan peel extract contains secondary antioxidants in the form of flavonoids and phenolics that work by inhibiting the formation of reactive oxygen compounds by capturing these radical compounds, thus preventing chain reactions that lead to cell damage.[11,12] Phenolic compounds and flavonoids are hydrogen donors for free radicals, formed during the fat peroxidation process.[10] Antioxidants suppress the formation of hydroperoxy radicals in the initial phase of fat peroxidation through the breakdown of chain reactions.[13] Based on data analysis all the doses above can significantly reduce MDA plasma levels but did not find significant differences between groups P1, P2, and P3. However, when we observed from the means of MDA plasma levels difference between the groups, and from the average p value obtained, the P3 group given a longan peel extract with a dose of 75 mg/kgBW was the best result.

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CONCLUSIONS

From this study we can conclude that longan peel extract can reduce MDA plasma levels in paracetamol-induced wistar rats. The best dose of longan peel extract that can reduce MDA levels of wistar rats was 75 mg/kgBW.

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