Effectivity of Insulin Leaf Extract (Tithonia Diversivolia) on Mice Malondialdehyde (MDA) Levels

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Abstract

Tithonia diversifolia popularly known in Indonesia as insulin leaves contain phenolic compounds such as chlorogenic acid and tryptophan. These compounds inhibit oxidative reactions and have a role in prevention of free radicals. Measurement of the malondialdehyd (MDA) level in the blood serum, can indicate the free radical amount in the body, so MDA can be classified as a biomarker of free radicals. The administrating a toxic dose of paracetamol may cause hepatotoxicity which could trigger an increase in free radicals and MDA in the body.

The purpose of this study is to identify the effectiveness of insulin leaf extract in preventing free radical escalation on mice induced by a toxic dose of paracetamol by monitoring its MDA levels. 38 mice were divided into 4 groups. A control negative group (not treated), control positive group (Aquadest + paracetamol), treatment group I (Insulin leaf extract 300mg/Kg BW+ paracetamol), treatment group II (Insulin leaf extract 500mg/Kg BW + paracetamol). Extracts were given for ten days and paracetamol induction was done on the eighth, ninth, and tenth day. On the eleventh day, mice blood serum was taken and then the level of MDA was measured using a spectrophotometer with a wavelength of 532 nm. The study showed the highest rate of MDA was obtained in the control positive group (2.97) followed by the treatment group II (2.43), the treatment group I (1.99) and control negative group (1.98). One Way Anova and test Tukey HSD, was found to have a significant difference between the control positive group and control negative group (p=0.025) and between control positive group and treatment group I (p=0.019).

To conclude, the Insulin leaf extract with a dose of 300mg/Kg of body weight was found to be the most effective in preventing elevation of MDA levels after a toxic dose of paracetamol was induced.

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Introduction

The free radicals are molecules or compounds that have unpaired electrons[1]. In unpaired conditions, these molecules are very reactive and attack cells in the body. Free radical compounds can arise on account of various complex chemical processes in the body in the form of byproducts of the cell oxidation process, cell metabolism, excessive exercise, infection, or when the body is exposed to environmental pollution such as motor vehicle fumes, cigarette smoke, pollutants, and solar radiation[2,3].

The situation where free radicals are present in the body is excessively referred to as oxidative stress. Oxidative stress that occurs in a long time in the body can cause several degenerative diseases such as coronary heart disease, diabetes mellitus, cancer, atherosclerosis, cataracts and so on[4]. Based on WHO data in 2005, the mortality rate due to degenerative diseases reached 17 million people worldwide[5].

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Compounds that can counteract free radicals are called antioxidants. Antioxidants are naturally present in the human body in the form of enzymes such as *Glutathione peroxidase* (GPx), *Catalase* (CAT), *Glutathione* (GSH) and *Superoxide dismutase* (SOD)[6]. However, in conditions of oxidative stress, the body needs additional antioxidants from outside that can be obtained from diet and vitamins[7].

Insulin leaves (*Smallanthus sonchifolius*) contain polyphenol antioxidant compounds in the form of *chlorogenic acid* and *trypthopan*. Polyphenol compounds are known to neutralize free radicals by reacting with unstable atomic or molecular structures[8,9].

Several studies have been conducted to determine the antioxidant activity of insulin leaves, one of which is *in vitro* research using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay method, and the result is 95% insulin leaf extract has potent antioxidant activity with IC_{50} values (50% Inhibitory Concentration) 149.63 ppm (parts per million) [10]. Extracts with IC_{50} values less than 200 ppm have potent antioxidant activity[11].

The administration of paracetamol toxic doses can cause hepatotoxicity which can trigger an increase in malondialdehyde (MDA) and free radicals in the body. It occurs because the metabolites of paracetamol in the form of *N-acetyl-p-benzoquinone imine* (NAPQI) produced by enzymes in the liver that are cytochrome P450 bind to liver cell protein and damage the mitochondria of liver cells so that MDA levels increase[12,13].

The *vivo* research which tests the effectiveness of insulin leaf extract against free radicals is still very little. Thus, this study is aimed to prove the effectiveness of insulin leaf extract to prevent elevated levels of MDA in mice that were induced by toxic doses of paracetamol.

Materials and methods

This research is an experimental laboratory study using a *post-test only control group design*[6]. This study was approved by the Health Research Ethics Commission (HREC) in the Faculty of Dentistry, Airlangga University, with the registration number: 253 / KKEPK.FKG / X / 2016. This research was carried out at the Biochemical Laboratory of the Faculty of Medicine, Airlangga University in October-November 2016. The process of making insulin leaf extract was carried out in the Integrated Service Unit (ISU) of Materia Medika Batu, Malang.

This study used 40 mice which were categorized into 4 groups: control negative group (the group that was not given any treatment), control positive group (the group given aquades *ad libitum* 0.2 ml/20 grBB and induced paracetamol 253.5 mg / kgBW), treatment group I (given 300 mg/kg BW insulin leaf extract and induced paracetamol 253.5 mg/kg BW), and treatment group II (given 500 mg/kg BW insulin leaf extract and induced paracetamol 253.5 mg/Kg BW). Insulin leaf extract in treatment groups I and II were given every day for 10 days, while the induction of paracetamol in the control positive group, treatment group I and treatment group II were given on days 8, 9, 10. On day 11 animals were sacrificed and carried out taking blood from the heart as much as 0.5 ml[14].

Determining MDA levels can be obtained by measuring MDA levels from blood serum taken from the heart of mice by using a UV-VIS spectrophotometer (Boeco) with a wavelength of 532 nm.

Results

Results of observations of the mean and standard deviation (SD) of MDA levels in the 4 groups are presented in Table 1 below:

Groups	Mean	SD
Control nagetive	1.98	0.29
Control positive	2.97	1.11
Treatment I	1.99	0.41
Treatment II	2.43	0.55

Table 1. Mean and SD of MDA levels in each group.

The mean and SD of MDA levels in each group, namely the control negative group, control positive group, treatment group I and treatment group II can be described in the bar diagram as follows in Figure 1.

Based on table 1 and figure 1, the highest average of MDA level was in the control positive group namely the group given aquades *ad libitum* 0.2 ml / 20 grBB and paracetamol induced 253.5 mg/kg BW (2.97), followed sequentially by treatment group II namely the group treated

given 95% 500 mg/kg BW insulin leaf extract and paracetamol induced 253.5 mg/kg BW (2.43), and treatment I was the group given 95% 300 mg/kg BW (1.99) insulin leaf extract, with the lowest MDA levels in the control negative group namely the group without treatment (1.98).

MDA level normality test results were tested by using *Kolmogorov Smirnov* 1 sample at alpha = 0.05 indicating a value of p = 0.258 (p> 0.05) which means that MDA levels were normally distributed. Homogeneity test data using *Levene test*, at alpha= 0.05 shows the value of p= 0.242 (p> 0.05) which means the data were distributed homogeneously. After knowing the data are normally distributed and homogeneous, Anova One Way test is then performed at alpha = 0.05 showing p = 0.013 (p> 0.05) which means that there are significant differences in MDA levels between groups.

To identify groups that had significantly different MDA levels then tested using Tukey HSD. The results are as follows in Table 2.

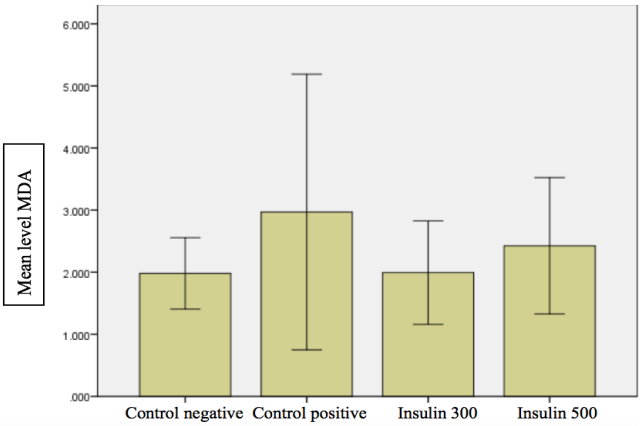

Figure 1. Mean and SD of MDA levels in each group.

Groups	Control negative	Control positive	Treatment I	Treatment II
Control negative	-	0.025*	1.000	0.535
Control positive	0.025*	-	0.019*	0.361
Treatment I	1.000	0.019*	-	0.514
Treatment II	0.535	0.631	0.514	-

Table 2. Tukey HSD test results for MDA levels between groups.

Discussion

The current study aimed to determine the effectiveness of ethanol extract of insulin leaves in preventing the increase of free radicals in mice induced by toxic doses of paracetamol, by observing MDA (Malondialdehyde) levels in the blood serum of mice. In this study, level profile of MDA was used, because MDA is a biological biomarker of lipid peroxide metabolites to assess the level of oxidative stress[15].

In the results of this study, it is clear in table 1 and figure 1 that based on the analysis of MDA levels using spectrophotometry there was a significant increase in MDA levels with p<0.025 between the control negative group with the control positive group. It can be explained that in the control positive group there has been an increase in MDA levels in the blood serum of mice. An increase in MDA levels is an indicator of oxidative stress as a result of administering toxic doses of paracetamol[16]. It is abvious that the increase in MDA levels in the positive control group that is induced using paracetamol 253.5 mg/kg BW without the administration of Insulin leaf ethanol extract will cause an excess of NAPQI metabolites due to the administration of toxic doses of paracetamol which can result in a bond between macromolecules of liver cell protein and reduces O2 to O2 *, so that it becomes reactive radicals (ROS) which then oxidize phospholipids by initiation, propagation, and termination. Furthermore, peroxyl radicals are rearranged through the cyclicisation reaction in endoperoxide (malondialdehyde precursor) with the final product of the peroxidation process being MDA[17,18].

There was also a significant difference with p <0.019 between the control positive group and the treatment group I. In the control positive group the mean of MDA level was 2.97 nmol/ml, whereas in the treatment group I that were given insulin leaf extract 300 mg/Kg BW for 10 days in a row and on days 8, 9 and 10 were induced with paracetamol 253.5 mg/kg BW, a decrease in MDA levels significant from 2.97 nmol/ml to 1.99 nmol/ml in the control positive group who were not given insulin leaf extract (2.97 nmol/mL). This is because *chlorogenic acid* and *tryptophan* contained in insulin leaves act as antioxidants and prevent the accumulation of free radicals *N-acetyl-p-benzoquinone imine* (NAPQI) which is reactive by improving the levels of *glutathione* (GSH) which will help the NAPQI conjugation process to become a non-reactive and more stable compound. *Chlorogenic acid* is a derivative of *cinnamic acid* which has a biological effect which is mostly related to antioxidants and

anti-inflammatory activity [19].

In treatment group II that were given 500 mg/Kg BW insulin leaf extract, there was no significant decrease in MDA levels p<0.361 when compared with control positive that is from the average MDA level of the positive control group 2.97 nmol/ml to 2.43 nmol/ml. It could be explained that insulin leaf extract with a dose of 500 mg/Kg BW was no more effective in preventing an increase in MDA levels than insulin extract at a dose of 300 mg/Kg BW. Experiments of mice also experienced 2 deaths in the control positive group and treatment group II that were given 500 mg/Kg BW insulin leaf extract, whereas in the treatment group I were given 300mg/Kg BW insulin leaf extract there were no dead mice, it could have been possible because insulin leaves contain *Sesquiterpene Lactones* (STLs). They are identified as toxic compounds and have toxic effects on the kidneys and liver of experimental animals at high doses[20]. Another possibility is that the activity of phenolic compounds contained in insulin leaves will decrease in the administration of extracts with higher doses. This is due to an increase in concentrations of extracts at high doses which results in phenolic compounds being difficult to be absorbed by the body so that antioxidant activity decreases[21].

In this study, it is possible that the *Sesquiterpene Lactones* (STLs) compounds and increased concentrations of extracts can cause the ineffectiveness of high-dose insulin leaf extract in preventing the increase in MDA levels and causing the death of mice samples in treatment group II.

It can be concluded from the results of this study that the 300 mg/Kg BW dose of insulin leaf extract is most effective in preventing the increase in MDA levels after the toxic dose of paracetamol is induced.

Declaration of Interest

All authors state that they have no conflicts of interest.

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