ORIGINAL ARTICLE

Antioxidant Effects of *Graptophyllum pictum* Leaf Extract on Malondialdehyde (MDA) Levels of Mice Induced By a Toxic Dose of Paracetamol

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Abstract:

Background: Antioxidants are important substances which possess the ability to protect the body from damage caused by free radicals-induced oxidative stress. A shift in the balance between oxidants and antioxidants promotes oxidative stress. *Graptophyllum pictum* (GP) contains substances that are efficacious as antioxidants. *Aim and Objectives:*To prove the antioxidant effects of GP leaf extract on Malondialdehyde (MDA) levels of mice induced by a toxic dose of paracetamol. *Material and Methods:* Thirty mice were randomly divided into five groups (n=6); Negative Control (NC) was without treatment, Positive Control (PC) with aquadest + paracetamol, T1: GP leaf extract 150 mg/kg BW + paracetamol, T2: GP leaf extract 300 mg/kg BW+ paracetamol, T3: GP leaf extract 600 mg/kg BW+ paracetamol. Extracts were given on the 1st to 10th day and paracetamol induction was performed on the 8th, 9th, and 10th days. On the 11th day, blood serum samples were collected and the level of MDA was then measured *Results:* T1, T2, T3 had the lower number of MDA levels when compared with the PC (3.625±0.374, 3.147±0.222, 2.574±0.319). One way Analysis of Variance (ANOVA) and *post hoc* test with Tukey showed that there was a significant difference between PC and T1, T2, T3 ($p<0.05$). *Conclusion:* GP extract has an antioxidant effect on the prevention of elevated MDA levels. A dose of 600 mg/kg BW was found to be the most effective in preventing elevation of MDA levels after a toxic dose of paracetamol was induced.

Keywords: Free Radicals, Malondialdehyde, *Graptophyllum pictum*, Paracetamol, Antioxidant

Introduction:

Antioxidant is an important substance in the body because of its function in protecting the cellular component from damage caused by free radicals induced by oxidative stress [1,2]. Antioxidant is present in the human body in order to compensate for the effects of oxidants [3]. It will neutralize free radical substances by sacrificing one of its electrons in order to create a stable molecule and break the free radical chain reaction [4]. Antioxidant is naturally produced by the body itself (endogenous) and also derived from food nutrient intake (exogenous). Based on its reaction, in-cell endogenous antioxidant is classified into enzymatic and non-enzymatic forms. In eukariotic organisms, major antioxidants, for example: superoxide dismutase, catalase and certain peroxides, are located in several parts of the cell and catalyze complex chain reactions to stabilize Reactive Oxygen Species (ROS) into stable molecules such as water and oxygent.A number of minor non-enzymatic antioxidants such as GSH, NADPH, thioredox in, vitamin E and C also participate in defence against ROS [5].

ROS is a product of free radical cellular oxygen reduction [6]. Free radical is a by-product of the metabolism within a normal cell which reduces oxygen and deliver Adenosine Triphosphate (ATP) in mitochondria and also generate free radicals [7]. A free radical is defined as an atom which has an unpaired electron and, thus,

becomes unstable and reactive [8]. Free radicals target important macromolecules in the body, damaging cells and disrupting homeostatis in the process. Oxidative stress is a term employed to describe the biological damaged caused by free radicals [6]. The target of free radicals are any molecules in the body, but the main targets are lipid, nucleatic acid, and protein [9].

An imbalance between oxidants and antioxidants can cause oxidative stress which, allied with a high level of ROS, Reactive Nitrogen Species (RNS) damages cell structure [3,5]. Oxidative stress can cause disease termed degeneration in nature. The World Health Organization (WHO) has declared that over 17 million people died every year because of degenerative diseases, including: cancer, neurodegenerative disorders, artherosclerosis, hypertension, ischemic heart disease, diabetes melitus and respiratory system disorders [3].

In order to prevent degenerative diseases, antioxidant is needed to compensate for the damage resulting from oxidative stress. Phenolic and polyphenol substances, which can be found in many natural resources such plants, possess antioxidant properties [10].

Graptophyllum pictum (GP) leaf contains flavonoids and tanins, substances which have potential as natural sources of antioxidant [11]. Flavonoid activity can inhibit enzymes which are involved in the creation of ROS, while tanin activity offers protection against free radicals, inhibit pro-oxidative enzyme and lipid peroxidation [12,13]. Therefore, the present study was conducted in order to establish the effect of preventing an increase in oxidative stress levels as a cause of degenerative disease.

Material and Methods:

Animals:

Thirty 2-3 month old male mice, weighing 20-30 g, were used in this study. The subjects were acclimatized a week before initiating the experiment.

Experimental design:

This study constituted an experimental laboratory-based investigation with a post test only control group design with 30 adult male mice. It was approved by ethical clearence from the Universitas Airlangga Faculty of Dental Medicine and Health Research Ethical Clearance Commission with number 237/HRECC.FODM/X/2017.

Graptophyllum pictum (GP) leaf extract was made using maceration technique [14]. Thirty mice were selected randomly into five groups, each containing six animals. The Negative Control (NC) group was not the subject of any experiment, the Positive Control (PC) group was administered with aquadest and paracetamol (253.5 g/kg bw), the treatment group I (T1) was administered with GP leaf extract (150 mg/kg bw) and paracetamol (53.5 g/kg bw), the treatment group II (T2) was administered with GP leaf extract (300 mg/kg bw) and paracetamol (53.5 g/kg bw), the treatment group III (T3) was administered with GP leaf extract (600 mg/kg bw) and paracetamol (53.5 g/kg bw). GP leaf extract was administred orally over ten days. On the 8th, 9th and 10th days, paracetamol was administred orally one hour after the administering of GP leaf extract. Paracetemol was given on in three consecutive days in order to induce hepatotoxicity and increase free radicals. After the treatment, blood serum samples (0.5 ml) were collected from the heart on the 11th day.

Determination of MDA level:

The MDA level was determined using Thiobarbituric Acid Reactive Substance (TBARS) Assay [15].

Statistical Analysis:

Data was analyzed by means of SPSS® software (SPSS; IBM, Chicago, IL, USA). Data colleted was analysed using Analysis of Variance (ANOVA), followed by Post Hoc Tukey test. The result was expressed as Mean ± SD. p value ⩽ 0.05 which was regarded as significant.

Results:

Based on the results showed in Table 1, the concentration mean of MDA in the negative control group without any treatment was 2.421 nmol/mL. Meanwhile, the positive control group showed an increased MDA concentration value of 4.714 nmol/mL. The MDA concentration in T1 (3.625 nmol/ml) was lower compared to the positive control group (4.714 nmol/ml). In T1, MDA concentration showed a value of 3.147 nmol/ml which was lower compared to the positive control group (4.714 nmol/ml) and T2 (3.625 nmol/ml). In T3 (2.574 nmol/ml), MDA concentration was lower compared to the positive control group (4.714 nmol/ml), T1 (3.625 nmol/ml), T2 (3.147 nmol/ml) and close to the mean of the negative control group (2.421 nmol/ml).

The results of ANOVA showed significant differences ($p<0.05$) in MDA levels. Furthermore, Post Tukey's Post Hoc Test showed a significant difference ($p<0.05$) between the positive control groups and the treatment groups.

From the table, it can be seen that there were significant differences in MDA levels between positive control groups for treatment I, II, and III. This implied that GP leaf ethanol extract doses of 150 mg/kg BW, 300 mg/kg BW, and 600 mg/kg BW are able to prevent elevated levels of MDA.

Discussion:

Increased levels of free radicals can be triggered by the presence of toxic doses of paracetamol. Approximately 2-5% of a therapeutic dose of excreted paracetamol does not change in the urine, the remainder is metabolized in the liver. In a therapeutic dose, more than 80% of the paracetamol is metabolized into glucoronide and sulphate conjugate, with about 5-10% converted by the liver enzyme CYP450 into a highly reactive

Table 1: MDA Concentration in Mice Blood of Each Treatment Group

Groups	N	Mean ± SD	ANOVA				
			NC	PC	T1	T2	T3
NC	6	2.421 ± 0.183	-	0.000*	0.000*	0.004*	0.911
PC	6	4.714 ± 0.393		-	0.000*	0.000*	0.000*
T1	6	3.625 ± 0.374			-	0.088	0.000*
T2	6	3.147 ± 0.222				-	0.028*
T3	6	2.574 ± 0.319					-

Significant differences expressed with (*) at p value less than 0.05.

metabolite, N-acetyl-p-benzoquinoneimine (NAPQI) which is known for its hepatotoxicity [16]. This metabolite can be neutralized by the sulfihydryl glutathione group and excreted in urine as mercaptic acid [17]. The excess paracetamol is metabolized into NAPQI in excessive amounts and the production of the body's glutathione enzyme is unable to compensate. Therefore, NAPQI causes intracellular damage followed by liver necrosis and hepatotoxicity and oxidative stress [16, 18].

Hepatotoxicity leads to production of toxic free radicals, such as peroxynitrite from superoxidant reactions and nitric oxide [19]. Formed peroxy-nitrite can cause lipid peroxidation which also causes damage to the liver. Lipid peroxidation is described as both a free radical oxidation process and nonradical species attacking lipids containing double carbon bonds, especially Polyunsaturated Fatty Acids (PUFAs) [20]. In lipid peroxidation, H^+ ion in fat produces radical fatty acids. This leads to the occurrence of chained free radicals reaction and triggers a large amount of peroxidation [21]. Lipid peroxidation can be determined based on MDA levels, since MDA is one of the secondary oxidation products of lipid peroxidation [22]. Therefore, antioxidant is needed to prevent elevated levels of MDA.

GP leaf extract contains flavonoid and tannin that possess the ability to prevent elevation of MDA by reducing free radicals [11]. The presence of flavonoids and tannins is suspected to induce antioxidants in GP leaf activity. This hypothesis is supported by the fact that flavonoid is a class of phenolic compounds that can reduce free radicals. Flavonoids' antioxidant activity may inhibit the enzymes involved in ROS formation [12]. Flavonoids in GP leaf can neutralize free radicals by donating H^+ ions, with the result that free radicals regenerated into stable H_2O [23]. In addition, flavonoids act as intracellular antioxidants by inhibiting free radical-producing enzymes such as xanthine oxidase, lipoxigenase, protein kinase C, cyclooxygenase, microsomal monoxygenase, mitochondrial sucoxyase and NADPH oxidase [24]. Tannin activities as an antioxidant counteract free radicals, inhibiting pro-oxidative enzymes and lipid peroxidation [13]. Tannins also can reduce the oxidizing power and activity of free radicals [25].

The results of this study showed that MDA levels in the positive control group were the highest. High levels of MDA are proportional to the increase in oxidative stress and free radicals in the body. The positive control group showed high levels of MDA which is due to the induction of paracetamol toxic dose resulting in increased NAPQI and free radicals in the body. This is because of the absence of any active ingredients capable of inhibiting the increase of free radicals and oxidative stress from NAPQI [26].

MDA levels were observed in the blood serum of each group. In general, a blood test is used to measure MDA levels resulting from lipid peroxidation with the assumption that blood can accurately represent lipid peroxidation in organ tissues. Supported by previous research, the blood levels of MDA were correlated with MDA levels in the liver. Therefore, blood tests were assumed relevant to measure MDA levels in this research [27].

Lower MDA concentrations in treatment groups I, II, and III compared to the positive controls were due to the provision of GP leaf extract containing flavonoid and tannin compounds that promote antioxidant activity [13]. Therefore, the presence

of NAPQI and free radicals resulting from paracetamol induction toxic dose can be neutralized.

The concentration of GP leaf extract is proportional to its antioxidant activity. Consequently, in the treatment group III, the lowest level of MDA was found compared to that in treatment groups I and II. In the negative control group, the detected MDA levels were low because the absence of paracetamol induction at toxic doses leads to zero production of NAPQI and excessive free radicals [22]. The MDA levels of this group represent those of mice under normal circumstances.

The decrease in MDA levels in treatment groups I, II and III showed that GP leaf has antioxidant effects in preventing elevated MDA levels. This hypothesis is supported by the MDA results in treatment group III which was close to the MDA levels in the negative control. Thus, it can be said that the most effective dose to prevent elevated MDA levels is 600 mg/kg BW doses used in treatment group III.

Conclusion:

GP leaf ethanol extract at doses of 150 mg/kg BW, 300 mg/kg BW, and 600 mg/kg BW has antioxidant effects in preventing increased MDA levels. A dose of 600 mg/kg BW is the most effective as a means of forestalling elevated MDA levels after paracetamol toxic dose induction.

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