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#5415 SUMMARY

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Abstract

Increasing age causes an increase in degenerative diseases. This degenerative disease is caused by antioxidants in the body unable to neutralize the increase in free radical concentration. One of the medicinal plants that is known to have antioxidant effects and is widely used in the community is Phaleria macrocarpa (Scheff.) Boerl. Flavonoids contained in fruit flesh have an antioxidant effect. At present there are still very limited clinical trials of Phaleria macrocarpa (Scheff.) Boerl. This study aimed to assess the antioxidant effects of dried extracts of Phaleria macrocarpa (Scheff.) Boerl fruit flesh in a range of doses given. This study was an experimental pre-post treatment without control in 30 healthy volunteers induced by glucose. Age of subjects ranged from 30-55 years. Data collection included anthropometric examination, malondialdehyde levels (baseline and administration dry extract dosages of 62.5 mg, 125 mg and 250 mg at 150 minutes after oral glucose induction. Data analysis using paired T test with p <0.05. Decreased malondialdehyde levels in the administration of extract doses of 62.5 mg, 125 mg and 250 mg by 40.9%, 22.9% and 18.3% compared to baselne malondialdehyde levels (1,608 nmol / ml). The results of statistical analysis using paired t test obtained p = 0,000 for all three doses compared to baseline levels. The dried fruit extract of the has an anti-oxidant effect, the extract dose of 62.5 mg has better antioxidant effectiveness than other doses. The effectiveness of antioxidant extract is not directly proportional to the dose.

Keywords: : Ekstrak, malondialdehid, Phaleria macrocarpa.

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Efek antioksidan ekstrak kering Phaleria macrocarpa (Scheff.) Boerl

Abstrak

Peningkatan usia menyebabkan peningkatan penyakit degeneratif. Penyakit ini disebabkan karena antioksidan yang ada didalam tubuh tidak mampu untuk menetralisir peningkatan konsentrasi radikal bebas. Salah satu tanaman obat yang diketahui mempunyai efek antioksidan dan banyak digunkan di masyarakat adalah Phaleria macrocarpa (Scheff.) Boerl. Flavonoid yang terkandung pada daging buah mempunyai efek sebagai antioksidan. Saat ini masih sangat terbatas uji klinis Phaleria macrocarpa (Scheff.) Boerl. maka penelitian ini bertujuan untuk menilai efek antioksidan ekstrak kering Phaleria macrocarpa (Scheff.) Boerl dalam beberapa kisaran dosis yang diberikan.Penelitian ini merupakan uji eksperimental pra-pasca perlakuan tanpa kontrol pada 30 sukarelawan sehat yang diinduksi dengan glukosa . Umur subjek berkisar dari 30-55 tahun Pengumpulan data meliputi pemeriksaan antropometri, kadar malondialdehid (baseline dan pemberian ekstrak kering dosis 62.5 mg, 125 mg dan 250 mg pada menit 150 setelah dilakukan induksi glukosa oral. Analisa data menggunakan paired T test dengan p <0.05 .Penurunan kadar malondialdehid pada pemberian ekstrak mahkota dewa dosis 62.5 mg, 125 mg dan 250 mg sebesar 40.9 %, 22.9% dan 18.3 % dibandingkan kadar malondialdehid baselne (1.608 nmol/ml). Hasil analisis statistik menggunakan paired t test didapatkan p=0.000 untuk ketiga dosis dibandingkan dengan kadar baseline.Ekstrak kering buah mahkota mempunyai efek antiooksidan, dosis

ekstrak 62.5 mg mempunyai efektifitas antioksidan lebih baik dibandingkan dengan dosis lainnya. Efektifitas antioksidan ekstrak tidak berbanding lurus dengan dosis.

Kata kunci: Ekstrak, malondialdehid, Phaleria macrocarpa.

Introduction

At present the development of medical science is so rapid that it raises an increase in one's life expectancy. Life expectancy is a leading indicator of a person's health quality [1] Life expectancy in Indonesia in 1999 was 66.2 years, in 2007 it increased to 70 years and in 2014 it became 71 years [1,2] causing an increase in risk for various degenerative diseases. Degenerative diseases found in many communities such as cancer, heart disease, diabetes, arthritis, liver disease and others.

This degenerative disease is caused by antioxidants in the body unable to neutralize the increase in free radical concentration. Free radicals are molecules which in their outer orbit have one or more unpaired electrons, they are very labile and very reactive so that they can cause damage to the cell components. Superoxide formed from hydroxyl radicals will initiate lipid peroxidation, which causes damage to the endothelial plasma membrane, lipoproteins and cell genetic carrier DNA. Hydrogen peroxide causes oxidative stress, one of which can be measured by using MDA. Lipid peroxidation can be detected indirectly by measuring plasma hydrolysis of lipoperoxidase to MDA form. [3] Diabetes mellitus (DM) is currently a worldwide health problem. The prevalence of DM in adults is estimated to increase year by year, in 2025 it is estimated to reach 5.4% of the entire adult population of the world. In Indonesia the prevalence of DM is based on data from the Indonesian Central Bureau of Statistics in 2003, estimated in urban areas at 14.7% and rural areas at 7.2% (of the total adult population of 133 million people). The number of diabetics in the world will increase to 366 million in 2030, the majority of the age affected by diabetes in developing countries is 45-64 years, while in developed countries it is 65 years. The prevalence of women affected by DM is higher than men. [4,5]

Diabetes mellitus is a degenerative disease that is currently a worldwide health problem. The prevalence of DM in adults is estimated to increase year by year, in 2025 it is estimated to reach 5.4% of the entire adult population of the world. The condition of hyperglycemia results in an increase in oxidative stress, where oxidative stress results from an imbalance between the formation of free radicals and antioxidants which is an important factor in the occurrence of blood vessel disorders. [6]

One of the medicinal plants that is known to have antioxidant effects and is widely used in the community is Phaleria macrocarpa (Scheff.) Boerl. This plant is found in Indonesia and is included in the divisions of Spermatophyta, Angiospermae subdivision, Dycotiledonae class, Celastrales nation, Thymelaceae tribe and Phaleria genus. [7] Plants from Papua are also known as Phaleria papuana Warb.Var.Wichannii (Val.) Back . Meanwhile, Chinese people prefer to call pau which means heirloom medicine. Empirically some people use it to treat diabetes mellitus. Some laboratory analysis studies show that Phaleria macrocarpa (Scheff.) Boerl. showed an antioxidant effect, especially in young fruit and Phaleria macrocarpa (Scheff.) Boerl. [8] because there are still very limited clinical trial data to see the antioxidant effects of Phaleria macrocarpa (Scheff.) Boerl so this study aims to assess the antioxidant effects of dried fruit pulp extract Phaleria macrocarpa (Scheff.) Boerl in a range of doses given. Based on the description above, it is necessary to conduct research that aims to determine the anti-oxidant effects of dried crown extract and the range of doses that provide the best antioxidant effect.

Methods

This study is a test of the dose range of antioxidant effects of dried extracts of crown god fruit in healthy volunteers. This study used an open clinical trial design (without comparison) with an increased dose. This research was conducted in the area of Angke Village, Tambora District, Jembatan Dua, West Jakarta in November 2018- February 2019.

This study used 30 healthy adult volunteers who fulfilled the following inclusion criteria: i) men and women aged 30-55 years, ii) Having a normal body weight (body mass index: ii)18.5- 25 kg / m2 iii) Not having chronic diseases and no history of chronic diseases in the family, especially diabetes mellitus, hypertension, heart disease, supported by laboratory tests showing routine blood tests, liver function (SGPT, alkaline phosphatase) and normal kidney function (creatinine levels), iv) no consume certain herbs or vitamin supplements 1 week before the study takes place / during the study takes place, v) in the previous 24 hours do not consume drinks containing caffeine, fruit juice or smoking, vi) Willing to take part in research and sign an informed consent. As exclusion criteria are: i) being pregnant or breastfeeding, ii) taking other drugs for the previous 1 week or during research that can affect blood glucose levels such as corticosteroid drugs, iii) Participating in other studies within 3 months before this study.

The treatment given is giving capsules containing dried extracts of MD fruit from Semarang, Central Java. At each visit given a glucose load of 400 calories (or 75 grams of glucose). The first visit was only given glucose load, whereas in the second visit 62.5 mg MD extract was given, wash out 1 week at the next visit was given 125 mg and the following week was given 250 mg MD extract Parameters used to measure malondialdehyde (MDA) levels.

Antioxidant effects were assessed by measuring malondialdehyde (MDA) levels by determining MDA levels in serum after reacting with TBA at hot temperatures in an acidic atmosphere. This reaction produces a solution that is red, then measured using a spectrophotometer. The reagents used are the MDA standard, TCA (trichloroacetic acid) and TBA (thiobarbituric acid). Before the research took place an accuracy and precision test of MDA examination was carried out. This research has passed the ethical review of the Triskati University Faculty of Medicine with no: 137 / KER / FK / I / 2019.

Results

A total of 40 subjects were examined for anthropometry (body weight, height), body mass index examination. In addition, laboratory tests (hematology, blood sugar, SGPT

and creatinine) were also carried out. Of the 40 subjects examined, only 30 people met the inclusion and exclusion criteria. Of the 30 volunteer subjects, 16 were women and 14 were men. Characteristic data from 30 subjects can be seen in table 1.

Table 1. Distribution of features characteristics of the subject (n=30)

Characteristic	X± SD (%) n= 30
Age (years)	41.40 ± 6.41
Sex	
Female	53.3
Male	46.7
Weight (kg)	56.05 ± 7.68
Height (m)	1.58 ± 0.09
Body mass index (BMI) kg/m2	22.29 ± 2.04

In this study the average age of 41.4 years was obtained. For the age range of healthy volunteer subjects aged years, with a range of 40-70 kilograms of weight, the range of body height was 143 to 174 cm and the range of body mass index was 18.66-24.84 kg / m2.

In this study, auto control was carried out. At the beginning of the study all subjects were tested for oral glucose tolerance (TTGO) with 75 grams of glucose given and samples taken in 150 minutes after glucose induction as baseline. One week then washout was carried out on the same subject with glucose induction and Phaleria macrocarpa extract (PME) at a dose of 62.5 mg, 125 mg and 250 mg with wash out 1 orange before treatment with different doses. MDA is at 150 minutes after glucose induction. The results of the average examination of MDA levels in the 150th minute can be seen in table 2.

Table 2. Level MDA baseline and EMD

	Level MDA (average ± SD)
Baseline	1.608 ± 0.509
PME 62.5 mg	0.950 ± 0.215
PME 125 mg	1.239 ± 0.230
PME 250 mg	1.313 ± 0.323

Table 3 shows the results of statistical analysis of baseline MDA levels, PME doses of 62.5 mg, 125 mg and 250 mg. From the results of statistical analysis using paired t test between PME baseline dose and MDA administration level after PME was given a significant difference ($p < 0.05$).

Table 3. Effect of Phaleria macrocarpa extract on level MDA

Variable

p

MDA baseline VS MDA PME 62.5 mg

0.000

MDA baseline VS MDA PME 125 mg

0.000

MDA baseline VS MDA PME 250 ng

0.000

p<0.05 significant difference (paired T test)

From Figure 1, it is seen that the MDA level decreases on a fairly large scale compared to the baseline at the PME dose of 62.5 mg, while the PMEdose of 125 mg and 250 mg shows a decrease in MDA levels but not too large compared to the 62.5 mg dose.

Figure 1. The effect of Phaleria macrocarpaextract on level MDA (nmol/ml) of subject baseline (1), and PME dose 62.5 mg (2), 125 mg (3) dan 250 mg (4)

Discussion

In this study using 30 volunteer subjects with an average age of 41.4 years, this was to show the anti-oxidant effect of PME . As is known that with age or aging associated with free radicals. Aging is a biological process that cannot be avoided and is related to biochemical and physiological changes that are gradual and spontaneous and increase the body's susceptibility to disease. Scientific studies have shown that the aging process causes a decrease in the body's ability to use calories from food, decrease hormone function, suppress enzyme function, and decrease the body's resistance to fight disease. Aging results from an accumulation of changes caused by reactions in the body that are started by high reactive molecules known as 'free radicals'. These changes that produce free radicals are believed to be a major cause of the aging process. [1,2]

Free radicals are defined as an atom, molecule, or component that contains unpaired electrons, so that it is generally unstable, has a short life, and is very reactive, which is produced due to the use of oxygen in the metabolic process. These free radicals are produced by normal body cells through metabolic processes, and also by external sources such as carcinogenic compounds and ionizing radiation. [1,3]

Free radicals can be produced from the results of the body's metabolism and external factors such as cigarette smoke, the results of ultra violet irradiation, radical trigger substances in foods and other pollutants. Disease caused by free radicals is chronic, ie it takes years for the disease to become apparent. Examples of diseases that are often associated with free radicals are heart attacks, cancer, cataracts and decreased kidney function. To prevent or reduce chronic disease because free radicals are needed antioxidants. The human body can neutralize these free radicals, only if the amount is excessive, the ability to neutralize it will decrease . [1,3]

Research on the chemical content of eggshell seeds and fruit flesh Phaleria macrocarpa (Scheff.) Boerl). showed that in hexane, ethyl acetate and methanol extract flavonoids, phenols, tannins, saponins and sterols / terpenes were obtained. The highest content is saponins (20.4%). [9]

Flavanoids are a natural antioxidant and have biological activities, including antioxidants that can inhibit various oxidation reactions and can act as reducing hydroxyl radicals, superoxide and peroxyl radicals. Extract the ethanol of young PM fruits has an inhibitory power of 78.48% and the old fruit has an 83.08% inhibitory effect, meaning that the old fruit PM has a greater antioxidant effect. [15,16]

This study used an oral glucose tolerance test procedure. In a hyperglycemic state it will produce free radicals. From table 3, the highest MDA levels were seen in 150 minutes after 45 gram glucose loading such as glucose tolerance test. MDA levels in the 150th minute will be taken as a baseline (without the administration of PME) and MDA levels will be measured again at the same time (150 minutes) with extracts of 62.5, 125 mg and 250 mg.

This research is an advanced study of the utilization of PME fruit flesh as an antihypoglycemic and antioxidant drug in patients with diabetes mellitus. This diabetes is characterized by relative or absolute deficiency of insulin secretion and / or resistance which causes chronic hyperglimeia and impaired carbohydrate, lipid and protein metabolism. Diabetes mellitus is known as an oxidative stress disorder that occurs due to an imbalance between the formation of free radicals and the natural antioxidant abilities of the body. Many studies have reported that oxidative stress plays a role in systemic inflammation, endothelial dysfunction, impaired pancreatic β cell secretion and impaired glucose utilization in peripheral tissues. This oxidative stress also plays an important role with complications that occur in diabetic patients. Sources of oxidative stress in diaberes include enzymatic, non-enzymatic and mitochontrial pathways. Increased oxidative stress in DM is influenced by many factors. The dominant factor is auto oxidation of glucose which causes an increase in free radicals. Increased extracellular glucose levels will induce dysregulation of reactive oxygen and nitrogen pathways. This situation will cause disruption of the vascular endothelium and the production of nitric oxid (NO). Superoxide, when joining NO on endothelial cells will produce perocynitrite which is a cytotoxic antioxidant. [17,18]

This research can be done in humans because PM is relatively safe and has been used for generations in Indonesian society. PM is extracted with water and ethanol in order to obtain active ingredients which are thought to be antioxidants. The volume of medicinal ingredients is less than that of PM meat powder, with a ratio of 1 gram of PM powder equal to 250 mg of dried extract of the god's crown (PME).

Oral acute toxicity testing of EMD conducted by Mariana [19] et al. Found that lethal dose (LD 50) PME was more than 1200 mg / kgBB and it was concluded that PME was relatively safe and no macroscopic abnormalities were found in all examined. Study

Sulistiyani [20] on mice found that LD 50 EMD was far above 1500 mg / kgBB and concluded that MD extract was classified as non-toxic.

From Figure 1, it is seen that the MDA level decreases on a fairly large scale compared to the baseline at the PME dose of 62.5 mg, while the PMEdose of 125 mg and 250 mg shows a decrease in MDA levels but not too large compared to the 62.5 mg dose. MDA levels were assessed at 150 minutes after glucose induction. The mean MDA baseline level was 1,608, and with a dose of 62.5 mg PME we found a decrease in MDA levels of 40.9% compared to the baseline. Giving an PME dose of 125 mg and 250 mg obtained a decrease in MDA levels of 22.9% and 18.3% compared to MDA baseline levels.

PME antioxidant activity was obtained from fenolic components and flavanoids contained in PM fruit. Karimi et al [21] reported that the content of the fruit consisted mainly of flavonoids and kaempferol, myricetin, naringin, quercetin. Correlation of flavanoid content in PM fruit with antioxidant activity may be caused by the presence of 3-hydroxyl groups in heterocyclic rings while additional hydroxyl or methoxyl groups at position 3.5, and 7 rings A and C appear to be of less importance. Highly active favonoids have ring B which is occupied by 3'4'-dihydroxy and / or 3-OH groups. [22-23]

Table 5 shows the results of statistical analysis of baseline MDA levels, PME doses of 62.5 mg, 125 mg and 250 mg. From the results of statistical analysis using paired t test between baseline and MDA administration level after PME was given a significant difference ($p < 0.05$).

The four main parts of PM plants that are often used in the community are the stem, leaves, shells of PM seeds and fruit. Research on the chemical content of shells and fruit flesh on PM obtained alkaloid compounds, saponins, flavonoids and polyphenols. Besides having an antioxidant effect on flavonoids in PM fruit can increase insulin expenditure by changing Ca2+ metabolism and can regenerate Langerhans island especially β.Flavonoid cells contained in PM as antioxidants which will protect the damage of pancreatic cells from free radicals.

Complaints found in the subject are nausea and fullness on 250 mg PME, this is probably due to the high saponin content (20.4%) in the PM fruit which can cause gastrointestinal irritation. In addition, it was also seen the effect of reducing blood pressure in subjects with 125 mg or 250 mg PME doses, this effect was caused by the presence of PM fruit action mechanism such as ACE receptor inhibitors.

The main limitation of this study is design as pre-post test experimental study without controls, so that further studies should be conducted with the standards of a good clinical trial.

Conclusion

Phaleria macrocarpa extract has antioxidant effects.The PME dose of 62.5 mg has better antioxidant efficacy than the dose 125 mg or 250 mg

Conflict of Interest

All authors state whether there was no conflict of interest in this article .

Acknowledgement

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Title and Abstract

Title	Effect of *Phaleria macrocarpa* (Scheff.) Boerl Dry Extract to the Level of Malondialdehyde

Abstract

Increased age causes an increase in degenerative diseases. Antioxidants in the body unable to neutralize the increased concentration of free radicals. The flesh of the *Phaleria macrocarpa* (Scheff.) Boerl contains flavonoids which have antioxidant effects. At present, there are still very limited clinical trials of *Phaleria macrocarpa* (Scheff.) Boerl. This study was an experimental pretest and posttest involving 30 healthy volunteers receiving glucose loads in November 2018–February 2019 in Jakarta. This study aimed to assess the antioxidant effect of *Phaleria macrocarpa* (Scheff.) Boerl dry fruit extract in various dosage ranges. Subjects of this study aged 30–55 years. The data collection included anthropometric examination and malondialdehyde levels before and after administration of dry fruit extract doses of 62.5 mg, 125 mg, and 250 mg at 150 minutes after oral glucose induction. Data analysis using a paired t test with $p<0.05$. Decreased levels of malondialdehyde in the administration of *Phaleria macrocarpa* (Scheff.) Boerl with a dose of 62.5 mg, 125 mg, and 250 mg by 40.9%, 22.9%, and 18.3% compared to the baseline malondialdehyde level (1,608 nmol/mL). Statistical analysis using a paired t test showed $p=0.000$ for all three doses compared with baseline levels. Dry fruit extract of *Phaleria macrocarpa* (Scheff.) Boerl has an antioxidant effect; the antioxidant effect of the extract is not directly proportional to the dose.

EFEK ANTIOKSIDAN EKSTRAK KERING *PHALERIA MACROCARPA* (SCHEFF.) BOERL TERHADAP KADAR MALONDIALDEHID

Pertambahan usia menyebabkan peningkatan penyakit degeneratif. Antioksidan dalam tubuh tidak mampu menetralkan peningkatan konsentrasi radikal bebas. Daging buah *Phaleria macrocarpa* (Scheff.) Boerl mengandung flavonoid mempunyai efek antioksidan. Saat ini masih sangat terbatas uji klinis *Phaleria macrocarpa* (Scheff.) Boerl. Penelitian ini merupakan uji eksperimental sebelum dan sesudah perlakuan pada 30 sukarelawan sehat yang diinduksi dengan glukosa yang dilaksanakan pada bulan November 2018–Februari

2019 di Jakarta. Penelitian ini bertujuan menilai efek antioksidan ekstrak kering buah *Phaleria macrocarpa* (Scheff.) Boerl dalam beberapa kisaran dosis yang diberikan. Subjek penelitian berusia 30–55 tahun. Pengumpulan data meliputi pemeriksaan antropometri serta kadar malondialdehid sebelum dan sesudah pemberian ekstrak kering dosis 62,5 mg, 125 mg, dan 250 mg pada menit 150 setelah dilakukan induksi glukosa oral. Analisis data menggunakan uji t berpasangan dengan p<0,05. Penurunan kadar malondialdehid pada pemberian ekstrak *Phaleria macrocarpa* (Scheff.) Boerl dosis 62,5 mg, 125 mg, dan 250 mg sebesar 40,9%, 22,9%, dan 18,3% dibanding dengan kadar malondialdehid sebelum pemberian (1.608 nmol/mL). Analisis statistik menggunakan uji t berpasangan didapatkan p=0,000 untuk ketiga dosis dibanding dengan kadar awal. Ekstrak kering buah *Phaleria macrocarpa* (Scheff.) Boerl mempunyai efek antioksidan; efek antioksidan ekstrak tidak berbanding lurus dengan dosis.

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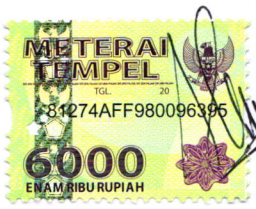

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Antioxidant effect of Phaleria macrocarpa (Scheff.) Boerl dry extract

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Abstract
Increasing age causes an increase in degenerative diseases. This degenerative disease is caused by antioxidants in the body unable to neutralize the increase in free radical concentration. One of the medicinal plants that is known to have antioxidant effects and is widely used in the community is Phaleria macrocarpa (Scheff.) Boerl. Flavonoids contained in fruit flesh have an antioxidant effect. At present there are still very limited clinical trials of Phaleria macrocarpa (Scheff.) Boerl. This study aimed to assess the antioxidant effects of dried extracts of Phaleria macrocarpa (Scheff.) Boerl fruit flesh in a range of doses given. This study was an experimental pre-post treatment without control in 30 healthy volunteers induced by glucose. Age of subjects ranged from 30-55 years. Data collection included anthropometric examination, malondialdehyde levels (baseline and administration dry extract dosages of 62.5 mg, 125 mg and 250 mg at 150 minutes after oral glucose induction. Data analysis using paired T test with $p < 0.05$. Decreased malondialdehyde levels in the administration of extract doses of 62.5 mg, 125 mg and 250 mg by 40.9%, 22.9% and 18.3% compared to baselne malondialdehyde levels (1,608 nmol / ml). The results of statistical analysis using paired t test obtained p = 0,000 for all three doses compared to baseline levels. The dried fruit extract of the has an anti-oxidant effect, the extract dose of 62.5 mg has better antioxidant effectiveness than other doses. The effectiveness of antioxidant extract is not directly proportional to the dose.

Keywords: : Ekstrak, malondialdehid, Phaleria macrocarpa.

Efek antioksidan ekstrak kering Phaleria macrocarpa (Scheff.) Boerl

Abstrak

Peningkatan usia menyebabkan peningkatan penyakit degeneratif. Penyakit ini disebabkan karena antioksidan yang ada didalam tubuh tidak mampu untuk menetralisir peningkatan konsentrasi radikal bebas. Salah satu tanaman obat yang diketahui mempunyai efek antioksidan dan banyak digunkan di masyarakat adalah Phaleria macrocarpa (Scheff.) Boerl. Flavonoid yang terkandung pada daging buah mempunyai efek sebagai antioksidan. Saat ini masih sangat terbatas uji klinis Phaleria macrocarpa (Scheff.) Boerl. maka penelitian ini bertujuan untuk menilai efek antioksidan ekstrak kering Phaleria macrocarpa (Scheff.) Boerl dalam beberapa kisaran dosis yang diberikan.Penelitian ini merupakan uji eksperimental pra-pasca perlakuan tanpa kontrol pada 30 sukarelawan sehat yang diinduksi dengan glukosa . Umur subjek berkisar dari 30-55 tahun Pengumpulan data meliputi pemeriksaan antropometri, kadar malondialdehid (baseline dan pemberian ekstrak kering dosis 62.5 mg, 125 mg dan 250 mg pada menit 150 setelah dilakukan induksi glukosa oral. Analisa data menggunakan paired T test dengan $p < 0.05$ .Penurunan kadar malondialdehid pada pemberian ekstrak mahkota dewa dosis 62.5 mg, 125 mg dan 250 mg sebesar 40.9 %, 22.9% dan 18.3 % dibandingkan kadar malondialdehid baselne (1.608 nmol/ml). Hasil analisis statistik menggunakan paired t test didapatkan $p=0.000$ untuk ketiga dosis dibandingkan dengan kadar baseline.Ekstrak kering buah mahkota mempunyai efek antiooksidan, dosis ekstrak 62.5 mg mempunyai efektifitas antioksidan lebih baik dibandingkan dengan dosis lainnya. Efektifitas antioksidan ekstrak tidak berbanding lurus dengan dosis.

Kata kunci: Ekstrak, malondialdehid, Phaleria macrocarpa.

Introduction

At present the development of medical science is so rapid that it raises an increase in one's life expectancy. Life expectancy is a leading indicator of a person's health quality [1] Life expectancy in Indonesia in 1999 was 66.2 years, in 2007 it increased to 70 years and in 2014 it became 71 years [1,2] causing an increase in risk for various degenerative diseases. Degenerative diseases found in many communities such as cancer, heart disease, diabetes, arthritis, liver disease and others.

This degenerative disease is caused by antioxidants in the body unable to neutralize the increase in free radical concentration. Free radicals are molecules which in their outer orbit have one or more unpaired electrons, they are very labile and very reactive so that they can cause damage to the cell components. Superoxide formed from hydroxyl radicals will initiate lipid peroxidation, which causes damage to the endothelial plasma membrane, lipoproteins and cell genetic carrier DNA. Hydrogen peroxide causes oxidative stress, one of which can be measured by using MDA. Lipid peroxidation can be detected indirectly by measuring plasma hydrolysis of lipoperoxidase to MDA form. [3] Diabetes mellitus (DM) is currently a worldwide health problem. The prevalence of DM in adults is estimated to increase year by year, in 2025 it is estimated to reach 5.4% of the entire adult population of the world. In Indonesia the prevalence of DM is based on data from the Indonesian Central Bureau of Statistics in 2003, estimated in urban areas at 14.7% and rural areas at 7.2% (of the total adult population of 133 million people). The number of diabetics in the world will

increase to 366 million in 2030, the majority of the age affected by diabetes in developing countries is 45-64 years, while in developed countries it is 65 years. The prevalence of women affected by DM is higher than men. [4,5]

Diabetes mellitus is a degenerative disease that is currently a worldwide health problem. The prevalence of DM in adults is estimated to increase year by year, in 2025 it is estimated to reach 5.4% of the entire adult population of the world. The condition of hyperglycemia results in an increase in oxidative stress, where oxidative stress results from an imbalance between the formation of free radicals and antioxidants which is an important factor in the occurrence of blood vessel disorders. [6]

One of the medicinal plants that is known to have antioxidant effects and is widely used in the community is Phaleria macrocarpa (Scheff.) Boerl. This plant is found in Indonesia and is included in the divisions of Spermatophyta, Angiospermae subdivision, Dycotiledonae class, Celastrales nation, Thymelaceae tribe and Phaleria genus. [7] Plants from Papua are also known as Phaleria papuana Warb.Var.Wichannii (Val.) Back . Meanwhile, Chinese people prefer to call pau which means heirloom medicine. Empirically some people use it to treat diabetes mellitus. Some laboratory analysis studies show that Phaleria macrocarpa (Scheff.) Boerl. showed an antioxidant effect, especially in young fruit and Phaleria macrocarpa (Scheff.) Boerl. [8] because there are still very limited clinical trial data to see the antioxidant effects of Phaleria macrocarpa (Scheff.) Boerl so this study aims to assess the antioxidant effects of dried fruit pulp extract Phaleria macrocarpa (Scheff.) Boerl in a range of doses given. Based

on the description above, it is necessary to conduct research that aims to determine the anti-oxidant effects of dried crown extract and the range of doses that provide the best antioxidant effect.

Methods

This study is a test of the dose range of antioxidant effects of dried extracts of crown god fruit in healthy volunteers. This study used an open clinical trial design (without comparison) with an increased dose. This research was conducted in the area of Angke Village, Tambora District, Jembatan Dua, West Jakarta in November 2018- February 2019.

This study used 30 healthy adult volunteers who fulfilled the following inclusion criteria: i) men and women aged 30-55 years, ii) Having a normal body weight (body mass index: ii)18.5- 25 kg / m2 iii) Not having chronic diseases and no history of chronic diseases in the family, especially diabetes mellitus, hypertension, heart disease, supported by laboratory tests showing routine blood tests, liver function (SGPT, alkaline phosphatase) and normal kidney function (creatinine levels), iv) no consume certain herbs or vitamin supplements 1 week before the study takes place / during the study takes place, v) in the previous 24 hours do not consume drinks containing caffeine, fruit juice or smoking, vi) Willing to take part in research and sign an informed consent. As exclusion criteria are: i) being pregnant or breastfeeding, ii) taking other drugs for the previous 1 week or during research that can affect blood glucose levels such as corticosteroid drugs, iii) Participating in other studies within 3 months before this study.

The treatment given is giving capsules containing dried extracts of MD fruit from Semarang, Central Java. At each visit given a glucose load of 400 calories (or 75 grams of glucose). The first visit was only given glucose load, whereas in the second visit 62.5 mg MD extract was given, wash out 1 week at the next visit was given 125 mg and the following week was given 250 mg MD extract Parameters used to measure malondialdehyde (MDA) levels.

Antioxidant effects were assessed by measuring malondialdehyde (MDA) levels by determining MDA levels in serum after reacting with TBA at hot temperatures in an acidic atmosphere. This reaction produces a solution that is red, then measured using a spectrophotometer. The reagents used are the MDA standard, TCA (trichloroacetic acid) and TBA (thiobarbituric acid). Before the research took place an accuracy and precision test of MDA examination was carried out. This research has passed the ethical review of the Triskati University Faculty of Medicine with no: 137 / KER / FK / I / 2019.

Results

A total of 40 subjects were examined for anthropometry (body weight, height), body mass index examination. In addition, laboratory tests (hematology, blood sugar, SGPT and creatinine) were also carried out. Of the 40 subjects examined, only 30 people met the inclusion and exclusion criteria. Of the 30 volunteer subjects, 16 were women and 14 were men. Characteristic data from 30 subjects can be seen in table 1.

Table 1. Distribution of features characteristics of the subject (n=30)

Characteristic	X± SD n= 30	(%)
Age (years)	41.40 ± 6.41	
Sex		
Female		53.3
Male		46.7
Weight (kg)	56.05 ± 7.68	
Height (m)	1.58 ± 0.09	
Body mass index (BMI) kg/m2	22.29 ± 2.04	

In this study the average age of 41.4 years was obtained. For the age range of healthy volunteer subjects aged years, with a range of 40-70 kilograms of weight, the range of body height was 143 to 174 cm and the range of body mass index was 18.66-24.84 kg / m2.

In this study, auto control was carried out. At the beginning of the study all subjects were tested for oral glucose tolerance (TTGO) with 75 grams of glucose given and samples taken in 150 minutes after glucose induction as baseline. One week then washout was carried out on the same subject with glucose induction and Phaleria macrocarpa extract (PME) at a dose of 62.5 mg, 125 mg and 250 mg with wash out 1 orange before treatment with different doses. MDA is at 150 minutes after glucose induction. The results of the average examination of MDA levels in the 150th minute can be seen in table 2.

Table 2. Level MDA baseline and EMD

	Level MDA (average ± SD)
Baseline	1.608 ± 0.509
PME 62.5 mg	0.950 ± 0.215
PME 125 mg	1.239 ± 0.230
PME 250 mg	1.313 ± 0.323

Table 3 shows the results of statistical analysis of baseline MDA levels, PME doses of 62.5 mg, 125 mg and 250 mg. From the results of statistical analysis using paired t test between PME baseline dose and MDA administration level after PME was given a significant difference ($p < 0.05$).

Table 3. Effect of Phaleria macrocarpa extract on level MDA

Variable	p
MDA baseline VS MDA PME 62.5 mg	0.000
MDA baseline VS MDA PME 125 mg	0.000
MDA baseline VS MDA PME 250 ng	0.000

$p<0.05$ significant difference (paired T test)

From Figure 1, it is seen that the MDA level decreases on a fairly large scale compared to the baseline at the PME dose of 62.5 mg, while the PMEdose of 125 mg and 250 mg shows a decrease in MDA levels but not too large compared to the 62.5 mg dose.

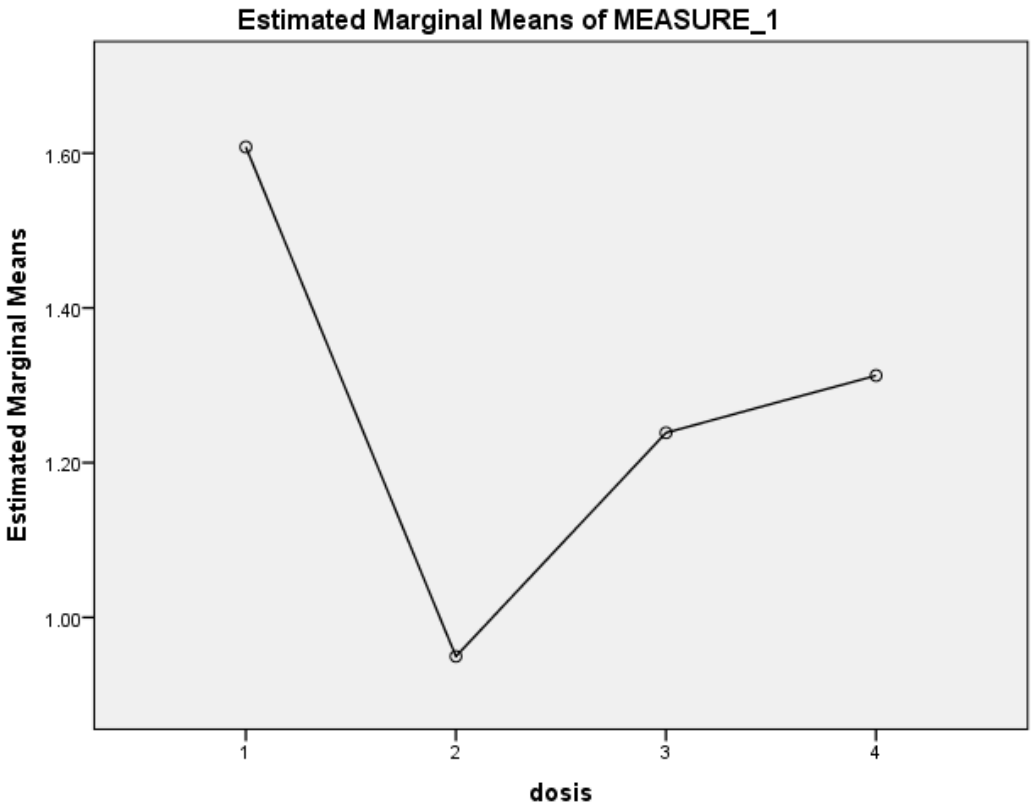

Figure 1. The effect of Phaleria macrocarpaextract on level MDA (nmol/ml) of subject baseline (1), and PME dose 62.5 mg (2), 125 mg (3) dan 250 mg (4)

Discussion

In this study using 30 volunteer subjects with an average age of 41.4 years, this was to show the anti-oxidant effect of PME . As is known that with age or aging associated with free radicals. Aging is a biological process that cannot be avoided and is related to biochemical and physiological changes that are gradual and spontaneous and increase the body's susceptibility to disease. Scientific studies have shown that the aging process causes a

decrease in the body's ability to use calories from food, decrease hormone function, suppress enzyme function, and decrease the body's resistance to fight disease. Aging results from an accumulation of changes caused by reactions in the body that are started by high reactive molecules known as 'free radicals'. These changes that produce free radicals are believed to be a major cause of the aging process. [1,2]

Free radicals are defined as an atom, molecule, or component that contains unpaired electrons, so that it is generally unstable, has a short life, and is very reactive, which is produced due to the use of oxygen in the metabolic process. These free radicals are produced by normal body cells through metabolic processes, and also by external sources such as carcinogenic compounds and ionizing radiation. [1,3]

Free radicals can be produced from the results of the body's metabolism and external factors such as cigarette smoke, the results of ultra violet irradiation, radical trigger substances in foods and other pollutants. Disease caused by free radicals is chronic, ie it takes years for the disease to become apparent. Examples of diseases that are often associated with free radicals are heart attacks, cancer, cataracts and decreased kidney function. To prevent or reduce chronic disease because free radicals are needed antioxidants. The human body can neutralize these free radicals, only if the amount is excessive, the ability to neutralize it will decrease . [1,3]

Research on the chemical content of eggshell seeds and fruit flesh Phaleria macrocarpa (Scheff.) Boerl). showed that in hexane, ethyl acetate

and methanol extract flavonoids, phenols, tannins, saponins and sterols / terpenes were obtained. The highest content is saponins (20.4%). [9]

Flavanoids are a natural antioxidant and have biological activities, including antioxidants that can inhibit various oxidation reactions and can act as reducing hydroxyl radicals, superoxide and peroxyl radicals. Extract the ethanol of young PM fruits has an inhibitory power of 78.48% and the old fruit has an 83.08% inhibitory effect, meaning that the old fruit PM has a greater antioxidant effect. [15,16]

This study used an oral glucose tolerance test procedure. In a hyperglycemic state it will produce free radicals. From table 3, the highest MDA levels were seen in 150 minutes after 45 gram glucose loading such as glucose tolerance test. MDA levels in the 150th minute will be taken as a baseline (without the administration of PME) and MDA levels will be measured again at the same time (150 minutes) with extracts of 62.5, 125 mg and 250 mg.

This research is an advanced study of the utilization of PME fruit flesh as an antihypoglycemic and antioxidant drug in patients with diabetes mellitus. This diabetes is characterized by relative or absolute deficiency of insulin secretion and / or resistance which causes chronic hyperglimeia and impaired carbohydrate, lipid and protein metabolism. Diabetes mellitus is known as an oxidative stress disorder that occurs due to an imbalance between the formation of free radicals and the natural antioxidant abilities of the body. Many studies have reported that oxidative stress plays a role in systemic inflammation, endothelial dysfunction, impaired pancreatic β cell

secretion and impaired glucose utilization in peripheral tissues. This oxidative stress also plays an important role with complications that occur in diabetic patients. Sources of oxidative stress in diaberes include enzymatic, non-enzymatic and mitochontrial pathways. Increased oxidative stress in DM is influenced by many factors. The dominant factor is auto oxidation of glucose which causes an increase in free radicals. Increased extracellular glucose levels will induce dysregulation of reactive oxygen and nitrogen pathways. This situation will cause disruption of the vascular endothelium and the production of nitric oxid (NO). Superoxide, when joining NO on endothelial cells will produce perocynitrite which is a cytotoxic antioxidant. [17,18]

This research can be done in humans because PM is relatively safe and has been used for generations in Indonesian society. PM is extracted with water and ethanol in order to obtain active ingredients which are thought to be antioxidants. The volume of medicinal ingredients is less than that of PM meat powder, with a ratio of 1 gram of PM powder equal to 250 mg of dried extract of the god's crown (PME).

Oral acute toxicity testing of EMD conducted by Mariana [19] et al. Found that lethal dose (LD 50) PME was more than 1200 mg / kgBB and it was concluded that PME was relatively safe and no macroscopic abnormalities were found in all examined. Study Sulistiyani [20] on mice found that LD 50 EMD was far above 1500 mg / kgBB and concluded that MD extract was classified as non-toxic.

From Figure 1, it is seen that the MDA level decreases on a fairly large scale compared to the baseline at the PME dose of 62.5 mg, while the PMEdose of 125 mg and 250 mg shows a decrease in MDA levels but not too large compared to the 62.5 mg dose. MDA levels were assessed at 150 minutes after glucose induction. The mean MDA baseline level was 1,608, and with a dose of 62.5 mg PME we found a decrease in MDA levels of 40.9% compared to the baseline. Giving an PME dose of 125 mg and 250 mg obtained a decrease in MDA levels of 22.9% and 18.3% compared to MDA baseline levels.

PME antioxidant activity was obtained from fenolic components and flavanoids contained in PM fruit. Karimi et al [21] reported that the content of the fruit consisted mainly of flavonoids and kaempferol, myricetin, naringin, quercetin. Correlation of flavanoid content in PM fruit with antioxidant activity may be caused by the presence of 3-hydroxyl groups in heterocyclic rings while additional hydroxyl or methoxyl groups at position 3.5, and 7 rings A and C appear to be of less importance. Highly active favonoids have ring B which is occupied by 3'4'-dihydroxy and / or 3-OH groups. [22-23]

Table 5 shows the results of statistical analysis of baseline MDA levels, PME doses of 62.5 mg, 125 mg and 250 mg. From the results of statistical analysis using paired t test between baseline and MDA administration level after PME was given a significant difference ($p < 0.05$).

The four main parts of PM plants that are often used in the community are the stem, leaves, shells of PM seeds and fruit. Research on the chemical

content of shells and fruit flesh on PM obtained alkaloid compounds, saponins, flavonoids and polyphenols. Besides having an antioxidant effect on flavonoids in PM fruit can increase insulin expenditure by changing Ca2+ metabolism and can regenerate Langerhans island especially β.Flavonoid cells contained in PM as antioxidants which will protect the damage of pancreatic cells from free radicals.

Complaints found in the subject are nausea and fullness on 250 mg PME, this is probably due to the high saponin content (20.4%) in the PM fruit which can cause gastrointestinal irritation. In addition, it was also seen the effect of reducing blood pressure in subjects with 125 mg or 250 mg PME doses, this effect was caused by the presence of PM fruit action mechanism such as ACE receptor inhibitors.

The main limitation of this study is design as pre-post test experimental study without controls, so that further studies should be conducted with the standards of a good clinical trial.

Conclusion

Phaleria macrocarpa extract has antioxidant effects.The PME dose of 62.5 mg has better antioxidant efficacy than the dose 125 mg or 250 mg

Conflict of Interest

All authors state whether there was no conflict of interest in this article .

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2. Bukti konfirmasi submit artikel dari jurnal GMHC

(10 April 2020)

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Meiyanti Meiyanti <meiyanti@trisakti.ac.id>

[GMHC] Submission Acknowledgement for Manuscript No.#5415

1 message

gmhc. unisba <gmhc.unisba@gmail.com> Fri, Apr 10, 2020 at 11:27 PM
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[GMHC] Revisions Required for Manuscript No.#5415

1 message

gmhc. unisba <gmhc.unisba@gmail.com> Mon, Apr 13, 2020 at 1:46 PM
To: dr Meiyanti Meiyanti <meiyanti@trisakti.ac.id>

Dear
Mrs. dr Meiyanti Meiyanti

We have reached a decision regarding your submission to Global Medical & Health Communication (GMHC) No.#5415, "Antioxidant effect of Phaleria macrocarpa (Scheff.) Boerl dry extract".

We decide that your article need some revision.
The revised file have to been submitting in **2 (two) weeks**.

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GLOBAL MEDICAL AND HEALTH COMMUNICATION
MANUSCRIPT ASSESSMENT FORM

Manuscript ID: 5415

Manuscript Title: Antioxidant effect of Phaleria macrocarpa (Scheff.) Boerl dry extract

No.	Assessment Points	Reviewer's Suggestions
1	Is the writing format and systematics in conformity with the introduction, method, result, and discussion (IMRAD) guidelines?	
2	Has the manuscript been using good and correct English spelling?	
3	Is the title concise and can clearly illustrate the contents of the manuscript?	
4	Is the abstract sufficiently concise, proportional, IMRAD, and can clearly illustrate the contents of the manuscript?	
5	Does the introduction clearly describe the problem, scope, and purpose of the study?	
6	Has the methods been clearly written so that the experiment can be repeated and consider the ethical aspect?	
7	Are the statistical methods/statistical tests used sufficiently clear, detailed, and appropriate?	
8	Are the results arranged in detail in the form of tables or drawings, and given easy-to-understand information?	
9	Is there a correlation between the results obtained with basic concepts and/or hypotheses?	
10	Are conclusions brief and clear?	

Decision	Check (X)
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- Introduction terlalu panjang
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Bdg, 12 April 2020

Efek antioksidan ekstrak kering Phaleria macrocarpa (Scheff.) Boerl

Abstrak

Peningkatan usia menyebabkan peningkatan penyakit degeneratif. Penyakit ini disebabkan karena antioksidan yang ada didalam tubuh tidak mampu untuk menetralisir peningkatan konsentrasi radikal bebas. Salah satu tanaman obat yang diketahui mempunyai efek antioksidan dan banyak digunkan di masyarakat adalah Phaleria macrocarpa (Scheff.) Boerl. Flavonoid yang terkandung pada daging buah mempunyai efek sebagai antioksidan. Saat ini masih sangat terbatas uji klinis Phaleria macrocarpa (Scheff.) Boerl, maka penelitian ini bertujuan untuk menilai efek antioksidan ekstrak kering Phaleria macrocarpa (Scheff.) Boerl dalam beberapa kisaran dosis yang diberikan.Penelitian ini merupakan uji eksperimental pra-pasca perlakuan tanpa kontrol pada 30 sukarelawan sehat yang diinduksi dengan glukosa. Umur subjek berkisar dari 30-55 tahun Pengumpulan data meliputi pemeriksaan antropometri, kadar malondialdehid (baseline dan pemberian ekstrak kering dosis 62.5 mg, 125 mg dan 250 mg pada menit 150 setelah dilakukan induksi glukosa oral. Analisa data menggunakan paired T test dengan $p < 0.05$ .Penurunan kadar malondialdehid pada pemberian ekstrak mahkota dewa dosis 62,5 mg, 125. mg,dan 250 mg sebesar 40,9 %, 22,9% dan 18,3 % dibandingkan kadar malondialdehid baseline (1.608 nmol/ml). Hasil analisis statistik menggunakan paired t test didapatkan p=0.000 untuk ketiga dosis dibandingkan dengan kadar baseline.Ekstrak kering buah mahkota mempunyai efek antiooksidan, dosis ekstrak 62,5 mg mempunyai efektifitas antioksidan lebih baik dibandingkan dengan dosis lainnya. Efektifitas antioksidan ekstrak tidak berbanding lurus dengan dosis.

Kata kunci: Ekstrak, malondialdehid, Phaleria macrocarpa

Introduction

At present the development of medical science is so rapid that it raises an increase in one's life expectancy. Life expectancy is a leading indicator of a person's health quality.[1] Life expectancy in Indonesia in 1999 was 66.2 years, in 2007 it increased to 70 years and in 2014 it became 71 years[1,2] causing an increase in risk for various degenerative diseases. Degenerative diseases found in many communities such as cancer, heart disease, diabetes, arthritis, liver disease and others.

This degenerative disease is caused by antioxidants in the body unable to neutralize the increase in free radical concentration. Free radicals are molecules which in their outer orbit have one or more unpaired electrons, they are very labile and very reactive so that they can cause damage to the cell components. Superoxide formed from hydroxyl radicals will initiate lipid peroxidation, which causes damage to the endothelial plasma membrane, lipoproteins and cell genetic carrier DNA. Hydrogen peroxide causes oxidative stress, one of which can be measured by using MDA. Lipid peroxidation can be detected indirectly by measuring plasma hydrolysis of lipoperoxidase to MDA form.[3] Diabetes mellitus (DM) is currently a worldwide health problem. The prevalence of DM in adults is estimated to increase year by year, in 2025 it is estimated to reach 5.4% of the entire adult population of the world. In Indonesia the prevalence of DM is based on data from the Indonesian Central Bureau of Statistics in 2003, estimated in urban areas at 14.7% and rural areas at 7.2% (of the total adult population of 133 million people). The number of diabetics in

the world will increase to 366 million in 2030, the majority of the age affected by diabetes in developing countries is 45-64 years, while in developed countries it is 65 years. The prevalence of women affected by DM is higher than men. (4,5)

Diabetes mellitus is a degenerative disease that is currently a worldwide health problem. The prevalence of DM in adults is estimated to increase year by year, in 2025 it is estimated to reach 5.4% of the entire adult population of the world. The condition of hyperglycemia results in an increase in oxidative stress, where oxidative stress results from an imbalance between the formation of free radicals and antioxidants which is an important factor in the occurrence of blood vessel disorders. (6)

One of the medicinal plants that is known to have antioxidant effects and is widely used in the community is Phaleria macrocarpa (Scheff.) Boerl. This plant is found in Indonesia and is included in the divisions of Spermatophyta, Angiospermae subdivision, Dycotiledonae class, Celastrales nation, Thymelaceae tribe and Phaleria genus. (7) Plants from Papua are also known as Phaleria papuana Warb.Var.Wichannii (Val.) Back.). Meanwhile, Chinese people prefer to call pau which means heirloom medicine. Empirically some people use it to treat diabetes mellitus. Some laboratory analysis studies show that Phaleria macrocarpa (Scheff.) Boerl. showed an antioxidant effect, especially in young fruit and Phaleria macrocarpa (Scheff.) Boerl. (8) because there are still very limited clinical trial data to see the antioxidant effects of Phaleria macrocarpa (Scheff.) Boerl so this study aims to assess the antioxidant effects of dried fruit pulp

extract Phaleria macrocarpa (Scheff.) Boerl in a range of doses given. Based on the description above, it is necessary to conduct research that aims to determine the anti-oxidant effects of dried crown extract and the range of doses that provide the best antioxidant effect.

Methods

This study is a test of the dose range of antioxidant effects of dried extracts of crown god fruit in healthy volunteers. This study used an open clinical trial design (without comparison) with an increased dose. This research was conducted in the area of Angke Village, Tambora District, Jembatan Dua, West Jakarta in November 2018–February 2019.

This study used 30 healthy adult volunteers who fulfilled the following inclusion criteria: i) men and women aged 30-55 years, ii) Having a normal body weight (body mass index: ii)18.5–25 kg / m^2 iii) Not having chronic diseases and no history of chronic diseases in the family, especially diabetes mellitus, hypertension, heart disease, supported by laboratory tests showing routine blood tests, liver function (SGPT, alkaline phosphatase) and normal kidney function (creatinine levels), iv) no consume certain herbs or vitamin supplements 1 week before the study takes place / during the study takes place, v) in the previous 24 hours do not consume drinks containing caffeine, fruit juice or smoking, vi) Willing to take part in research and sign an informed consent. As exclusion criteria are: i) being pregnant or breastfeeding, ii) taking other drugs for the previous 1 week or during research that can affect blood glucose levels such

as corticosteroid drugs, iii) Participating in other studies within 3 months before this study.

The treatment given is giving capsules containing dried extracts of MD fruit from Semarang, Central Java. At each visit given a glucose load of 400 calories (or 75 grams of glucose). The first visit was only given glucose load, whereas in the second visit 62.5 mg MD extract was given, wash out 1 week at the next visit was given 125 mg and the following week was given 250 mg MD extract. Parameters used to measure malondialdehyde (MDA) levels.

Antioxidant effects were assessed by measuring malondialdehyde (MDA) levels by determining MDA levels in serum after reacting with TBA at hot temperatures in an acidic atmosphere. This reaction produces a solution that is red, then measured using a spectrophotometer. The reagents used are the MDA standard, TCA (trichloroacetic acid) and TBA (thiobarbituric acid). Before the research took place an accuracy and precision test of MDA examination was carried out. This research has passed the ethical review of the Triskati University Faculty of Medicine with no: 137 / KER / FK / I / 2019.

Results

A total of 40 subjects were examined for anthropometry (body weight, height), body mass index examination. In addition, laboratory tests (hematology, blood sugar, SGPT and creatinine) were also carried out. Of

the 40 subjects examined, only 30 people met the inclusion and exclusion criteria. Of the 30 volunteer subjects, 16 were women and 14 were men. Characteristic data from 30 subjects can be seen in table 1.

Table 1. Distribution of features characteristics of the subject (n=30)

Characteristic	X± SD n= 30	(%)
Age (years)	41.40 ± 6.41	
Sex		
Female		53.3
Male		46.7
Weight (kg)	56.05 ± 7.68	
Height (m)	1.58 ± 0.09	
Body mass index (BMI) kg/m2	22.29 ± 2.04	

In this study the average age of 41.4 years was obtained. For the age range of healthy volunteer subjects aged years, with a range of 40-70 kilograms of weight, the range of body height was 143 to 174 cm and the range of body mass index was 18.66-24.84 kg / m2.

In this study, auto control was carried out. At the beginning of the study all subjects were tested for oral glucose tolerance (TTGO) with 75 grams of glucose given and samples taken in 150 minutes after glucose induction as baseline. One week then washout was carried out on the same subject with glucose induction and Phaleria macrocarpa extract (PME) at a dose of 62.5 mg, 125 mg and 250 mg with wash out 1 orange before treatment with different doses. MDA is at 150 minutes after glucose induction. The results

of the average examination of MDA levels in the 150th minute can be seen in table 2.

Table 2. Level MDA baseline and EMD

	Level MDA (Average ± SD)
Baseline	1.608 ± 0.509
PME 62.5 mg	0.950 ± 0.215
PME 125 mg	1.239 ± 0.230
PME 250 mg	1.313 ± 0.323

Table 3 shows the results of statistical analysis of baseline MDA levels, PME doses of 62.5 mg, 125 mg and 250 mg. From the results of statistical analysis using paired t test between PME baseline dose and MDA administration level after PME was given a significant difference ($p < 0.05$).

Table 3. Effect of Phaleria macrocarpa extract on level MDA

Variable	p
MDA baseline VS MDA PME 62.5 mg	0.000
MDA baseline VS MDA PME 125 mg	0.000
MDA baseline VS MDA PME 250 ng	0.000

p<0.05 significant difference (paired T test)

From Figure 1, it is seen that the MDA level decreases on a fairly large scale compared to the baseline at the PME dose of 62.5 mg, while the

PMEdose of 125 mg and 250 mg shows a decrease in MDA levels but not too large compared to the 62.5 mg dose.

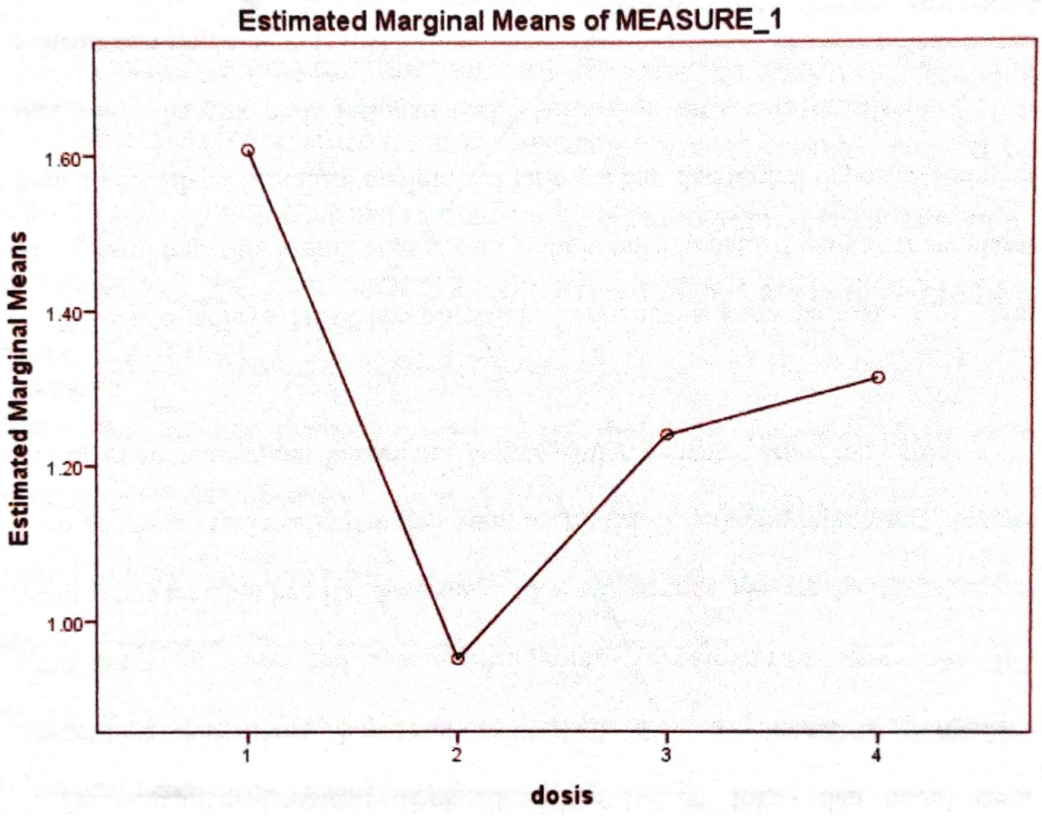

Figure 1. The effect of Phaleria macrocarpaextract on level MDA (nmol/ml) of subject baseline (1), and PME dose 62.5 mg (2), 125 mg (3) dan 250 mg (4)

Discussion

In this study using 30 volunteer subjects with an average age of 41.4 years, this was to show the anti-oxidant effect of PME . As is known that with age or aging associated with free radicals. Aging is a biological process that cannot be avoided and is related to biochemical and physiological changes that are gradual and spontaneous and increase the body's susceptibility to disease. Scientific studies have shown that the aging process causes a decrease in the body's ability to use calories from food, decrease hormone function, suppress enzyme function, and decrease the body's resistance to fight disease. Aging results from an accumulation of changes caused by reactions in the body that are started by high reactive molecules known as 'free radicals'. These changes that produce free radicals are believed to be a major cause of the aging process. [1,2]

Free radicals are defined as an atom, molecule, or component that contains unpaired electrons, so that it is generally unstable, has a short life, and is very reactive, which is produced due to the use of oxygen in the metabolic process. These free radicals are produced by normal body cells through metabolic processes, and also by external sources such as carcinogenic compounds and ionizing radiation. [1,3]

Free radicals can be produced from the results of the body's metabolism and external factors such as cigarette smoke, the results of ultra violet irradiation, radical trigger substances in foods and other pollutants. Disease caused by free radicals is chronic, ie it takes years for the disease to become apparent. Examples of diseases that are often associated with free radicals are heart attacks, cancer, cataracts and

decreased kidney function. To prevent or reduce chronic disease because free radicals are needed antioxidants. The human body can neutralize these free radicals, only if the amount is excessive, the ability to neutralize it will decrease. (1,3)

Research on the chemical content of eggshell seeds and fruit flesh Phaleria macrocarpa (Scheff.) Boerl). showed that in hexane, ethyl acetate and methanol extract flavonoids, phenols, tannins, saponins and sterols / terpenes were obtained. The highest content is saponins (20.4%). (9)

Flavanoids are a natural antioxidant and have biological activities, including antioxidants that can inhibit various oxidation reactions and can act as reducing hydroxyl radicals, superoxide and peroxyl radicals. Extract the ethanol of young PM fruits has an inhibitory power of 78.48% and the old fruit has an 83.08% inhibitory effect, meaning that the old fruit PM has a greater antioxidant effect. (15,16)

This study used an oral glucose tolerance test procedure. In a hyperglycemic state it will produce free radicals. From table 3, the highest MDA levels were seen in 150 minutes after 45 gram glucose loading such as glucose tolerance test. MDA levels in the 150th minute will be taken as a baseline (without the administration of PME) and MDA levels will be measured again at the same time (150 minutes) with extracts of 62.5, 125 mg and 250 mg.

This research is an advanced study of the utilization of PME fruit flesh as an antihypoglycemic and antioxidant drug in patients with diabetes mellitus. This diabetes is characterized by relative or absolute deficiency of

insulin secretion and / or resistance which causes chronic hyperglimeia and impaired carbohydrate, lipid and protein metabolism. Diabetes mellitus is known as an oxidative stress disorder that occurs due to an imbalance between the formation of free radicals and the natural antioxidant abilities of the body. Many studies have reported that oxidative stress plays a role in systemic inflammation, endothelial dysfunction, impaired pancreatic β cell secretion and impaired glucose utilization in peripheral tissues. This oxidative stress also plays an important role with complications that occur in diabetic patients. Sources of oxidative stress in diaberes include enzymatic, non-enzymatic and mitochontrial pathways. Increased oxidative stress in DM is influenced by many factors. The dominant factor is auto oxidation of glucose which causes an increase in free radicals. Increased extracellular glucose levels will induce dysregulation of reactive oxygen and nitrogen pathways. This situation will cause disruption of the vascular endothelium and the production of nitric oxid (NO). Superoxide, when joining NO on endothelial cells will produce perocynitrite which is a cytotoxic antioxidant. (17,18)

This research can be done in humans because PM is relatively safe and has been used for generations in Indonesian society. PM is extracted with water and ethanol in order to obtain active ingredients which are thought to be antioxidants. The volume of medicinal ingredients is less than that of PM meat powder, with a ratio of 1 gram of PM powder equal to 250 mg of dried extract of the god's crown (PME).

Oral acute toxicity testing of EMD conducted by Mariana [19] et al. Found that lethal dose (LD 50) PME was more than 1200 mg / kgBB and it was concluded that PME was relatively safe and no macroscopic abnormalities were found in all examined. Study Sulistiyani [20] on mice found that LD 50 EMD was far above 1500 mg / kgBB and concluded that MD extract was classified as non-toxic.

From Figure 1, it is seen that the MDA level decreases on a fairly large scale compared to the baseline at the PME dose of 62.5 mg, while the PME dose of 125 mg and 250 mg shows a decrease in MDA levels but not too large compared to the 62.5 mg dose. MDA levels were assessed at 150 minutes after glucose induction. The mean MDA baseline level was 1,608, and with a dose of 62.5 mg PME we found a decrease in MDA levels of 40.9% compared to the baseline. Giving an PME dose of 125 mg and 250 mg obtained a decrease in MDA levels of 22.9% and 18.3% compared to MDA baseline levels.

PME antioxidant activity was obtained from fenolic components and flavanoids contained in PM fruit. Karimi et al [21] reported that the content of the fruit consisted mainly of flavonoids and kaempferol, myricetin, naringin, quercetin. Correlation of flavanoid content in PM fruit with antioxidant activity may be caused by the presence of 3-hydroxyl groups in heterocyclic rings while additional hydroxyl or methoxyl groups at position 3.5, and 7 rings A and C appear to be of less importance. Highly active favonoids have ring B which is occupied by 3'4'-dihydroxy and / or 3-OH groups. [22,23]

Table 5 shows the results of statistical analysis of baseline MDA levels, PME doses of 62.5 mg, 125 mg and 250 mg. From the results of statistical analysis using paired t test between baseline and MDA administration level after PME was given a significant difference ($p < 0.05$).

The four main parts of PM plants that are often used in the community are the stem, leaves, shells of PM seeds and fruit. Research on the chemical content of shells and fruit flesh on PM obtained alkaloid compounds, saponins, flavonoids and polyphenols. Besides having an antioxidant effect on flavonoids in PM fruit can increase insulin expenditure by changing Ca2+ metabolism and can regenerate Langerhans island especially β.Flavonoid cells contained in PM as antioxidants which will protect the damage of pancreatic cells from free radicals.

Complaints found in the subject are nausea and fullness on 250 mg PME, this is probably due to the high saponin content (20.4%) in the PM fruit which can cause gastrointestinal irritation. In addition, it was also seen the effect of reducing blood pressure in subjects with 125 mg or 250 mg PME doses, this effect was caused by the presence of PM fruit action mechanism such as ACE receptor inhibitors.

The main limitation of this study is design as pre-post test experimental study without controls, so that further studies should be conducted with the standards of a good clinical trial.

Conclusion

Phaleria macrocarpa extract has antioxidant effects. The PME dose of 62.5 mg has better antioxidant efficacy than the dose 125 mg or 250 mg

Conflict of Interest

All authors state whether there was no conflict of interest in this article .

Acknowledgement

The investigators thank the Faculty of Medicine Trisakti University and Trisakti University Research Institute (Lembaga Penelitian Universitas Trisakti) for the funding this study. Thanks are also due to the DS Foundation and all subjects are agreed to participate in this study.

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4. Bukti konfirmasi review dan hasil review kedua

(19 April 2020)

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Meiyanti Meiyanti <meiyanti@trisakti.ac.id>

[GMHC] Revisions Required for Manuscript No.#5415 (2nd)

1 message

gmhc. unisba <gmhc.unisba@gmail.com> Sun, Apr 19, 2020 at 3:54 PM
To: Meiyanti Meiyanti <meiyanti@trisakti.ac.id>

Dear
Mrs. dr. Meiyanti Meiyanti

We have reached a decision regarding your submission to Global Medical & Health Communication (GMHC) No.#5415, "Antioxidant effect of Phaleria macrocarpa (Scheff.) Boerl dry extract".

We decide that your article need some revision (file attached).
The revised file have to been submitting in 2 (two) weeks.

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Manuscript Title: Antioxidant effect of Phaleria macrocarpa (Scheff.) Boerl dry extract		
No.	**Assessment Points**	**Reviewer's Suggestions**
1	Is the writing format and systematics in conformity with the introduction, method, result, and discussion (IMRAD) guidelines?	References need update. Some are more than 10 years old.
2	Has the manuscript been using good and correct English spelling?	Some grammatical errors
3	Is the title concise and can clearly illustrate the contents of the manuscript?	Yes
4	Is the abstract sufficiently concise, proportional, IMRAD, and can clearly illustrate the contents of the manuscript?	No date and place of study
5	Does the introduction clearly describe the problem, scope, and purpose of the study?	Too long.
6	Has the methods been clearly written so that the experiment can be repeated and consider the ethical aspect?	Yes. Sentences are too long.
7	Are the statistical methods/statistical tests used sufficiently clear, detailed, and appropriate?	No statistical analysis stated
8	Are the results arranged in detail in the form of tables or drawings, and given easy-to-understand information?	yes
9	Is there a correlation between the results obtained with basic concepts and/or hypotheses?	Yes.
10	Are conclusions brief and clear?	yes

Decision	**Check (X)**
• Manuscript can be published without revisions	
• Manuscript can be published with **major** revisions	X
• Manuscript can be published with **minor** revisions	
• Manuscript can not be published	

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5. Bukti konfirmasi resubmit artikel yang sudah direvisi oleh penulis dan artikel yang diresubmit

(21 April 2020)

Antioxidant Effect of *Phaleria macrocarpa* (Scheff.) Boerl Dry Extract to The Level of Malondialdehyde

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Abstract
Increased age causes an increase in degenerative diseases. This disease is caused by antioxidants in the body unable to neutralize the increased concentration of free radicals. The flesh of the *Phaleria macrocarpa (Scheff.) Boerl* contains flavonoids which have antioxidant effects. At present there are still very limited clinical trials of *Phaleria macrocarpa (Scheff.) Boerl*. This research was an experimental pre- and post-test involving 30 healthy volunteer receiving glucose loads in November 2018-February 2019 in Jakarta. The aim of this study was to assess the antioxidant effect of *Phaleria macrocarpa (Scheff.) Boerl* dry fruit extract in various dosage ranges. Research subjects aged 30-55 years The data collection included anthropometric examination, baseline malondialdehyde levels and administration of dry fruit extract doses of 62.5 mg, 125 mg and 250 mg at 150 minutes after oral glucose induction. Data analysis using paired T test with p <0.05. Decreased levels of malondialdehyde in the administration of *Phaleria macrocarpa (Scheff.) Boerl* with a dose of 62.5 mg, 125 mg and 250 mg by 40.9%, 22.9% and 18.3% compared to the baseline malondialdehyde level (1,608 nmol / mL). Statistical analysis using paired T test showed p = 0,000 for all three doses compared with baseline levels. Dry fruit extract of *Phaleria macrocarpa (Scheff.) Boerl* has an anti-oxidant effect, the extract dose of 62.5 mg has an antioxidant effect better than other doses. The antioxidant effect of the extract is not directly proportional to the dose.

Keywords: : Extract, malondialdehyde , *Phaleria macrocarpa*.

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Efek Antioksidan Ekstrak Kering *Phaleria macrocarpa* (Scheff.) Boerl Terhadap Kadar Malondialdehid

Abstrak

Peningkatan usia menyebabkan peningkatan penyakit degeneratif. Penyakit ini disebabkan oleh antioksidan yang ada di dalam tubuh tidak mampu menetralisir peningkatan konsentrasi radikal bebas. Daging buah *Phaleria macrocarpa (Scheff.) Boerl* mengandung flavonoid mempunyai efek antioksidan. Saat ini masih sangat terbatas uji klinis *Phaleria macrocarpa (Scheff.) Boerl*. Penelitian ini merupakan uji eksperimental sebelum dan sesudah perlakuan pada 30 sukarelawan sehat yang diinduksi dengan glukosa dilaksanakan pada bulan November 2018-Februari 2019 di Jakarta. Penelitian ini bertujuan menilai efek antioksidan ekstrak kering *Phaleria macrocarpa (Scheff.) Boerl* dalam beberapa kisaran dosis yang diberikan. Subjek penelitian berusia 30-55 tahun Pengumpulan data meliputi pemeriksaan antropometri, kadar malondialdehid sebelum dan pemberian ekstrak kering dosis 62,5 mg, 125 mg dan 250 mg pada menit 150 setelah dilakukan induksi glukosa oral. Analisis data menggunakan uji T berpasangan dengan p <0,05. Penurunan kadar malondialdehid pada pemberian ekstrak *Phaleria macrocarpa (Scheff.) Boerl* dosis 62,5 mg, 125 mg dan 250 mg sebesar 40,9%, 22,9% dan 18,3% dibandingkan kadar malondialdehid sebelum pemberian (1.608 nmol/mL). Hasil analisis statistik menggunakan uji T berpasangan didapatkan p=0.000 untuk ketiga dosis dibandingkan dengan kadar awal. Ekstrak kering buah *Phaleria macrocarpa (Scheff.) Boerl* mempunyai efek antiooksidan, dosis ekstrak 62,5 mg mempunyai efek antioksidan lebih baik dibandingkan dengan dosis lainnya. Efek antioksidan ekstrak tidak berbanding lurus dengan dosis.

Kata kunci: Ekstrak, malondialdehid, *Phaleria macrocarpa*

Introduction

At present the development of medical science is so rapid that it raises an increase in one's life expectancy. Life expectancy is the leading indicator of a person's health quality.[1] In 1999 life expectancy in Indonesia was 66.2 years, in 2007 it increased to 70 years and in 2014 it became 71 years[1,2] causing an increase in risk for various degenerative diseases. Degenerative diseases found in many communities such as cancer, heart disease, diabetes, arthritis, liver disease and others.

This degenerative disease is caused by inability of antioxidants in the body to neutralize the increase in free radical concentration. Free radicals are molecules which in their outer orbit have one or more unpaired electrons, they are very labile and very reactive so that they can cause damage to the cell components. Superoxide formed from hydroxyl radicals will initiate lipid peroxidation, which causes damage to the endothelial plasma membrane, lipoproteins and cell genetic carrier DNA. Hydrogen peroxide causes oxidative stress, one of which can be measured by using MDA. Lipid peroxidation can be detected indirectly by measuring plasma hydrolysis of lipoperoxidase to MDA form.[3,4]

Diabetes mellitus (DM) is currently a worldwide health problem. In 2011 around 366 million adult suffered from DM, and it was estimated in year 2030 there would a very large increase of up to 551 millions patients with DM.[5] The condition of hyperglycemia results in an increase in oxidative stress, where it results from an imbalance between the formation of free radicals and antioxidants which is an important factor in the occurrence of

blood vessel disorders. Previous clinical studies report that oxidative stress play a major role in the pathogenesis and development of complications of DM.[3,4,6]

One of the medicinal plants that is known to have antioxidant effects and is widely used in the community is *Phaleria macrocarpa (Scheff.) Boerl* (PM). Some laboratory analysis studies show that PM showed an antioxidant effect, especially in young fruit and leaves.[7,8] Currently clinical trial are conducted very rare to prove the antioxidant effects of PM, so this study aims to assess the antioxidant effects of dried fruit pulp extract PM in a range of doses given. Based on the description above, it is necessary to conduct research that aims to determine the anti-oxidant effects of dried extract and the range of doses that provide the best antioxidant effect.

Methods

This study is a test of the dose range of antioxidant effects of dry extract of PM pericarp (PME) in healthy volunteers. This study used an open clinical trial design (without comparison) with an increased dose. This research was conducted in the area of Angke Village, Tambora District, Jembatan Dua, West Jakarta in November 2018-February 2019.

This study used 30 healthy adult volunteers who fulfilled the following inclusion criteria: i) men and women aged 30-55 years, ii) having a normal body weight (body mass index: 18.5-25 kg/m^2), iii) not having chronic diseases and no history of chronic diseases in the family, especially diabetes mellitus, hypertension, heart disease, supported by laboratory tests showing routine blood tests, liver function (SGPT, alkaline phosphatase) and normal

kidney function (creatinine levels), iv) no consume certain herbs or vitamin supplements a week before the study takes place/during the study takes place, v) in the previous 24 hours do not consume drinks containing caffeine, fruit juice or smoking, vi) willing to take part in research and sign an informed consent. Exclusion criteria are consisted of : i) being pregnant or breastfeeding, ii) taking other drugs for the previous a week or during research that can affect blood glucose levels such as corticosteroid drugs, iii) participating in other studies within 3 months before this study.

The PM pericarp (without seed coat and seed) was obtained from plant nurseries in the region of Semarang, Central Java. At each visit given a glucose load of 400 calories (or 75 grams of glucose). The first visit was only given glucose load, whereas in the second visit 62,5 mg PME was given, wash out 1 week at the next visit was given 125 mg and the following week was given 250 mg PME parameters used to measure malondialdehyde (MDA) levels.

Antioxidant effects were assessed by measuring MDA levels by determining MDA levels in serum after reacting with TBA at hot temperatures in an acidic atmosphere. This reaction produces a solution that is red, then measured using a spectrophotometer. The reagents used are the MDA standard, TCA (trichloroacetic acid) and TBA (thiobarbituric acid). Before the research took place an accuracy and precision test of MDA examination was carried out. This research has passed the ethical review of the Triskati University Faculty of Medicine with no: 137/KER/FK / I /2019.

Results

A total of 40 subjects were examined for anthropometry (body weight, height), body mass index examination. In addition, laboratory tests (hematology, blood sugar, SGPT and creatinine) were also carried out. Of the 40 subjects examined, only 30 people met the inclusion and exclusion criteria. Of the 30 volunteer subjects, 16 were women and 14 were men. Characteristic data from 30 subjects can be seen in Table 1.

Table 1 Distribution of Features Characteristics of the Subject (n=30)

Characteristic	**X± SD** **n= 30**
Age (years)	41.40 ± 6.41
Sex	
Female	16
Male	14
Weight (kg)	56.05 ± 7.68
Height (m)	1.58 ± 0.09
Body mass index (BMI) kg/m2	22.29 ± 2.04

In this study the average age of 41.4 years was obtained. For the age range of healthy volunteer subjects aged years, with a range of 40-70 kilograms of weight, the range of body height was 143 to 174 cm and the range of body mass index was 18.66 to 24.84 kg /m^2.

In this study, autocontrol was carried out. At the beginning of the study all subjects were tested for oral glucose tolerance (TTGO) with 75 grams of

glucose given and samples taken in 150 minutes after glucose induction as baseline. One week then washout was carried out on the same subject with glucose induction and PME at a dose of 62.5 mg, 125 mg and 250 mg with wash out one week before treatment with different doses. MDA was at 150 minutes after glucose induction. The results of the average examination of MDA levels in the 150th minute can be seen in Table 2.

Table 2 Level MDA Baseline and PME

	Level MDA (average ± SD)
Baseline	1.608 ± 0.509
PME 62.5 mg	0.950 ± 0.215
PME 125 mg	1.239 ± 0.230
PME 250 mg	1.313 ± 0.323

Table 3 shows the results of statistical analysis of baseline MDA levels, PME doses of 62.5 mg, 125 mg and 250 mg. From the results of statistical analysis using paired t test between PME baseline dose and MDA administration level after PME was given a significant difference ($p < 0.05$).

Table 3 Effect PME on Level MDA

Variable	**p**
MDA baseline VS MDA PME 62.5 mg	0.000
MDA baseline VS MDA PME 125 mg	0.000
MDA baseline VS MDA PME 250 ng	0.000

$p<0.05$ significant difference (paired T test)

From Figure 1, it is seen that the MDA level decreases on a fairly large scale compared to the baseline at the PME dose of 62.5 mg, while the PME dose of 125 mg and 250 mg shows a decrease in MDA levels but not too large compared to the 62.5 mg dose.

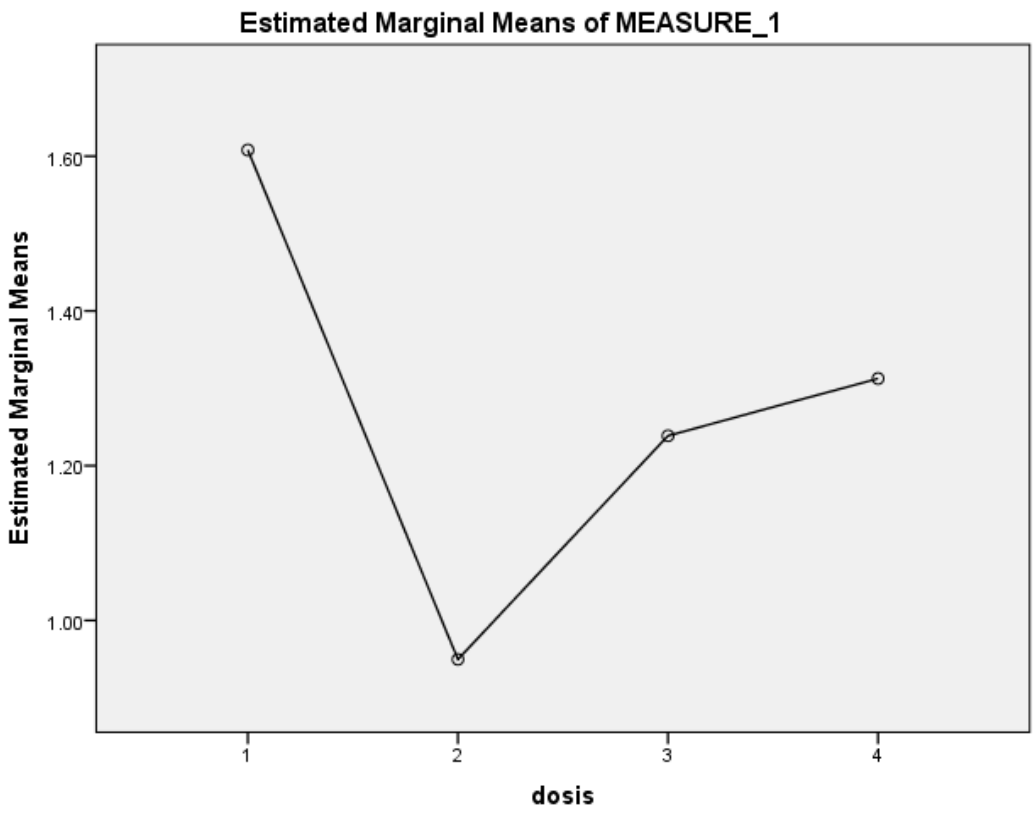

Figure 1 The Effect of PME on Level MDA (nmol/mL) of Subject Baseline (1), and PME dose 62.5 mg (2), 125 mg (3) dan 250 mg (4)

Discussion

In this study using 30 volunteer subjects with an average age of 41.4 years, this was to show the anti-oxidant effect of PME . As it is known that with age or aging was associated with free radicals. Aging is a biological process that cannot be avoided and is related to biochemical and physiological changes that are gradual and spontaneous and increase the body's susceptibility to disease. Scientific studies have shown that the aging process causes a decrease in the body's ability to use calories from food, decrease hormone function, suppress enzyme function, and decrease the body's resistance to fight disease. Aging results from an accumulation of changes caused by reactions in the body that are started by high reactive molecules known as 'free radicals'. These changes that produce free radicals are believed to be a major cause of the aging process. [1,3]

Free radicals are defined as an atom, molecule, or component that contains unpaired electrons, so that it is generally unstable, has a short life, and is very reactive, which is produced due to the use of oxygen in the metabolic process. These free radicals are produced by normal body cells through metabolic processes, and also by external sources such as carcinogenic compounds and ionizing radiation.[3,9]

Free radicals can be produced from the results of the body's metabolism and external factors such as cigarette smoke, the results of ultra violet irradiation, radical trigger substances in foods and other pollutants. Disease caused by free radicals is chronic, ie it takes years for the disease to become apparent. Examples of diseases that are often associated with free radicals

are heart attacks, cancer, cataracts and decreased kidney function. To prevent or reduce chronic disease because free radicals are needed antioxidants. The human body can neutralize these free radicals, only if the amount is excessive, the ability to neutralize it will decrease .[10,11]

Research on the chemical content of eggshell seeds and fruit flesh PM showed that in hexane, ethyl acetate and methanol extract flavonoids, phenols, tannins, saponins and sterols/terpenes were obtained. The highest content is saponins. [12-14]

The four main parts of MD plants that are often used in society are the stems, leaves, seed shells and fruit flesh.[8,15] Research on the chemical content of seeds and fruit flesh obtained alkaloid compounds, saponins, flavonoids and polyphenols. Besides having the antioxidant effect of flavonoid content, the use of PME in DM cases can increase insulin expenditure by changing the metabolism of Ca^{2+} and can regenerate the island of Langerhans, especially β cells. Flavonoid contained in MD as an antioxidant that will protect pancreatic cell damage from free radicals.[16,17] Flavanoids are a natural antioxidant and have biological activities, including antioxidants that can inhibit various oxidation reactions and can act as reducing hydroxyl radicals, superoxide and peroxyl radicals. Extract the ethanol of young PM fruits has an inhibitory power of 78.48% and the old fruit has an 83.08% inhibitory effect, meaning that the old fruit PM has a greater antioxidant effect.[11-14]

This study used an oral glucose tolerance test procedure. In a hyperglycemic state it will produce free radicals. From Table 3, the highest

MDA levels were seen in 150 minutes after 45 gram glucose loading such as glucose tolerance test. MDA levels in the 150th minute will be taken as a baseline (without the administration of PME) and MDA levels will be measured again at the same time (150 minutes) with extracts of 62.5, 125 mg and 250 mg.

This research is an advanced study of the utilization of PME as an antihypoglycemic and antioxidant drug in patients with DM. This diabetes is characterized by relative or absolute deficiency of insulin secretion and/or resistance which causes chronic hyperglimeia and impaired carbohydrate, lipid and protein metabolism. DM is known as an oxidative stress disorder that occurs due to an imbalance between the formation of free radicals and the natural antioxidant abilities of the body. Many studies have reported that oxidative stress plays a role in systemic inflammation, endothelial dysfunction, impaired pancreatic β cell secretion and impaired glucose utilization in peripheral tissues. [3,4,6]

This oxidative stress also plays an important role with complications that occur in diabetic patients. Sources of oxidative stress in diabetes include enzymatic, non-enzymatic and mitochontrial pathways. The increased oxidative stress in DM is influenced by many factors. The dominant factor is auto oxidation of glucose which causes an increase in free radicals. Increased extracellular glucose levels will induce dysregulation of reactive oxygen and nitrogen pathways. This situation will cause disruption of the vascular endothelium and the production of nitric oxid (NO). Superoxide,

when joining NO on endothelial cells will produce perocynitrite which is a cytotoxic antioxidant. [18,19]

This research can be done in humans because PM is relatively safe and has been used for generations in Indonesian society. PM is extracted with water and ethanol in order to obtain active ingredients which are thought to be antioxidants.[20,21] The volume of medicinal ingredients is less than that of PM meat powder, with a ratio of 1 gram of PM powder equal to 250 mg of dried extract of the god's crown (PME).

It is seen that the MDA level decreases on a fairly large scale compared to the baseline at the PME dose of 62.5 mg, while the PME dose of 125 mg and 250 mg shows a decrease in MDA levels but not too large compared to the 62.5 mg dose. MDA levels were assessed at 150 minutes after glucose induction. The mean MDA baseline level was 1,608, and with a dose of 62.5 mg PME we found a decrease in MDA levels of 40.9% compared to the baseline. Giving an PME dose of 125 mg and 250 mg obtained a decrease in MDA levels of 22.9% and 18.3% compared to MDA baseline levels.

PME antioxidant activity was obtained from fenolic components and flavanoids contained in PM fruit.[22] Karimi et al[23] reported that the content of the fruit consisted mainly of flavonoids and kaempferol, myricetin, naringin, quercetin. Correlation of flavanoid content in PM fruit with antioxidant activity may be caused by the presence of 3-hydroxyl groups in heterocyclic rings while additional hydroxyl or methoxyl groups at position 3.5, and 7 rings A and C appear to be of less importance. Highly active

favonoids have ring B which is occupied by 3'4'-dihydroxy and /or 3-OH groups. [24-25]

Table 5 shows the results of statistical analysis of baseline MDA levels, PME doses of 62.5 mg, 125 mg and 250 mg. From the results of statistical analysis using paired t test between baseline and MDA administration level after PME was given a significant difference ($p < 0.05$).

Complaints found in the subject are nausea and fullness on 250 mg PME, this is probably due to the high saponin content in the PM fruit which can cause gastrointestinal irritation. In addition, it was also seen the effect of reducing blood pressure in subjects with 125 mg or 250 mg PME doses, this effect was caused by the presence of PM fruit action mechanism such as ACE receptor inhibitors.[25]

The main limitation of this study is design as pre-post test experimental study without controls, so that further studies should be conducted with the standards of a good clinical trial.

Conclusion

Phaleria macrocarpa extract has antioxidant effects. The PME dose of 62.5 mg has better antioxidant efficacy than the dose 125 mg or 250 mg.

Conflict of Interest

The authors do not have any conflict of interest to declare.

Acknowledgement

The investigators thank the Faculty of Medicine Trisakti University and Trisakti University Research Institute (Lembaga Penelitian Universitas Trisakti) for the funding this study. Thanks are also due to the Dhammasavana Foundation and all subjects are agreed to participate in this study.

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This study used 30 healthy adult volunteers who fulfilled the following inclusion criteria: i) men and women aged 30-55 years, ii) having a normal body weight (body mass index: 18.5-25 kg/m^2), iii) not having chronic diseases and no history of chronic diseases in the family, especially diabetes mellitus, hypertension, heart disease, supported by laboratory tests showing routine blood tests, liver function (SGPT, alkaline phosphatase) and normal

kidney function (creatinine levels), iv) no consume certain herbs or vitamin supplements a week before the study takes place/during the study takes place, v) in the previous 24 hours do not consume drinks containing caffeine, fruit juice or smoking, vi) willing to take part in research and sign an informed consent. Exclusion criteria are consisted of : i) being pregnant or breastfeeding, ii) taking other drugs for the previous a week or during research that can affect blood glucose levels such as corticosteroid drugs, iii) participating in other studies within 3 months before this study.

The PM pericarp (without seed coat and seed) was obtained from plant nurseries in the region of Semarang, Central Java. At each visit given a glucose load of 400 calories (or 75 grams of glucose). The first visit was only given glucose load, whereas in the second visit 62,5 mg PME was given, wash out one week at the next visit was given 125 mg and the following week was given 250 mg PME parameters used to measure malondialdehyde (MDA) levels.

Antioxidant effects were assessed by measuring MDA levels by determining MDA levels in serum after reacting with TBA at hot temperatures in an acidic atmosphere. This reaction produces a solution that is red, then measured using a spectrophotometer. The reagents used are the MDA standard, TCA (trichloroacetic acid) and TBA (thiobarbituric acid). Before the research took place an accuracy and precision test of MDA examination was carried out. This research has passed the ethical review of the Triskati University Faculty of Medicine with no: 137/KER/FK / I /2019.

Results

A total of 40 subjects were examined for anthropometry (body weight, height), body mass index examination. In addition, laboratory tests (hematology, blood sugar, SGPT and creatinine) were also carried out. Of the 40 subjects examined, only 30 people met the inclusion and exclusion criteria. Of the 30 volunteer subjects, 16 were women and 14 were men. Characteristic data from 30 subjects can be seen in Table 1.

Table 1 Distribution of Features Characteristics of the Subject (n=30)

Characteristic	**X± SD** **n= 30**
Age (years)	41.40 ± 6.41
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In this study the average age of 41.4 years was obtained. For the age range of healthy volunteer subjects aged years, with a range of 40-70 kilograms of weight, the range of body height was 143 to 174 cm, and the range of body mass index was 18.66 to 24.84 kg /m^2.

In this study, autocontrol was carried out. At the beginning of the study all subjects were tested for oral glucose tolerance (TTGO) with 75 grams of

glucose given and samples taken in 150 minutes after glucose induction as baseline. One week then washout was carried out on the same subject with glucose induction and PME at a dose of 62.5 mg, 125 mg and 250 mg with wash out one week before treatment with different doses. MDA was at 150 minutes after glucose induction. The results of the average examination of MDA levels in the 150th minute can be seen in Table 2.

Table 2 Level MDA Baseline and PME

	Level MDA (average ± SD)
Baseline	1.608 ± 0.509
PME 62.5 mg	0.950 ± 0.215
PME 125 mg	1.239 ± 0.230
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Table 3 shows the results of statistical analysis of baseline MDA levels, PME doses of 62.5 mg, 125 mg and 250 mg. From the results of statistical analysis using paired t test between PME baseline dose and MDA administration level after PME was given a significant difference ($p < 0.05$).

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MDA baseline VS MDA PME 62.5 mg	0.000
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$p<0.05$ significant difference (paired T test)

From Figure 1, it is seen that the MDA level decreases on a fairly large scale compared to the baseline at the PME dose of 62.5 mg, while the PME dose of 125 mg and 250 mg shows a decrease in MDA levels but not too large compared to the 62.5 mg dose.

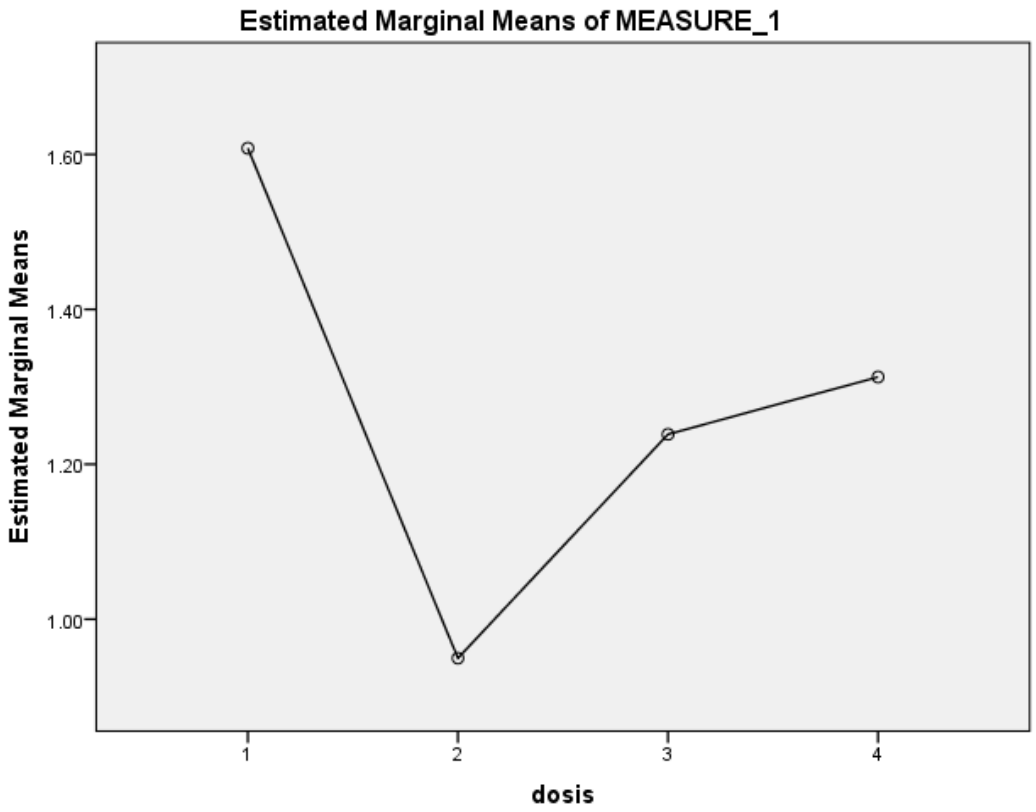

Figure 1 The Effect of PME on Level MDA (nmol/mL) of Subject Baseline (1), and PME dose 62.5 mg (2), 125 mg (3) dan 250 mg (4)

Discussion

In this study using 30 volunteer subjects with an average age of 41.4 years, this was to show the anti-oxidant effect of PME . As it is known that with age or aging was associated with free radicals. Aging is a biological process

that cannot be avoided and is related to biochemical and physiological changes that are gradual and spontaneous and increase the body's susceptibility to disease. Scientific studies have shown that the aging process causes a decrease in the body's ability to use calories from food, decrease hormone function, suppress enzyme function, and decrease the body's resistance to fight disease. Aging results from an accumulation of changes caused by reactions in the body that are started by high reactive molecules known as 'free radicals'. These changes that produce free radicals are believed to be a major cause of the aging process. [1,3]

Free radicals are defined as an atom, molecule, or component that contains unpaired electrons, so that it is generally unstable, has a short life, and is very reactive, which is produced due to the use of oxygen in the metabolic process. These free radicals are produced by normal body cells through metabolic processes, and also by external sources such as carcinogenic compounds and ionizing radiation.[3,9]

Free radicals can be produced from the results of the body's metabolism and external factors such as cigarette smoke, the results of ultra violet irradiation, radical trigger substances in foods and other pollutants. Disease caused by free radicals is chronic, ie it takes years for the disease to become apparent. Examples of diseases that are often associated with free radicals are heart attacks, cancer, cataracts and decreased kidney function. To prevent or reduce chronic disease because free radicals are needed antioxidants. The human body can neutralize these free radicals, only if the amount is excessive, the ability to neutralize it will decrease .[10,11]

Research on the chemical content of eggshell seeds and fruit flesh PM showed that in hexane, ethyl acetate and methanol extract flavonoids, phenols, tannins, saponins and sterols/terpenes were obtained. The highest content is saponins. [12-14]

The four main parts of MD plants that are often used in society are the stems, leaves, seed shells and fruit flesh.[8,15] Research on the chemical content of seeds and fruit flesh obtained alkaloid compounds, saponins, flavonoids and polyphenols. Besides having the antioxidant effect of flavonoid content, the use of PME in DM cases can increase insulin expenditure by changing the metabolism of Ca^{2+} and can regenerate the island of Langerhans, especially β cells. Flavonoid contained in MD as an antioxidant that will protect pancreatic cell damage from free radicals.[16,17] Flavanoids are a natural antioxidant and have biological activities, including antioxidants that can inhibit various oxidation reactions and can act as reducing hydroxyl radicals, superoxide and peroxyl radicals. Extract the ethanol of young PM fruits has an inhibitory power of 78.48% and the old fruit has an 83.08% inhibitory effect, meaning that the old fruit PM has a greater antioxidant effect.[11-14]

This study used an oral glucose tolerance test procedure. In a hyperglycemic state it will produce free radicals. From Table 3, the highest MDA levels were seen in 150 minutes after 45 gram glucose loading such as glucose tolerance test. MDA levels in the 150th minute will be taken as a baseline (without the administration of PME) and MDA levels will be

measured again at the same time (150 minutes) with extracts of 62.5, 125 mg and 250 mg.

This research is an advanced study of the utilization of PME as an antihypoglycemic and antioxidant drug in patients with DM. This diabetes is characterized by relative or absolute deficiency of insulin secretion and/or resistance which causes chronic hyperglimeia and impaired carbohydrate, lipid and protein metabolism. DM is known as an oxidative stress disorder that occurs due to an imbalance between the formation of free radicals and the natural antioxidant abilities of the body. Many studies have reported that oxidative stress plays a role in systemic inflammation, endothelial dysfunction, impaired pancreatic β cell secretion and impaired glucose utilization in peripheral tissues. [3,4,6]

This oxidative stress also plays an important role with complications that occur in diabetic patients. Sources of oxidative stress in diabetes include enzymatic, non-enzymatic and mitochontrial pathways. The increased oxidative stress in DM is influenced by many factors. The dominant factor is auto oxidation of glucose which causes an increase in free radicals. Increased extracellular glucose levels will induce dysregulation of reactive oxygen and nitrogen pathways. This situation will cause disruption of the vascular endothelium and the production of nitric oxid (NO). Superoxide, when joining NO on endothelial cells will produce perocynitrite which is a cytotoxic antioxidant. [18,19]

This research can be done in humans because PM is relatively safe and has been used for generations in Indonesian society. PM is extracted with

water and ethanol in order to obtain active ingredients which are thought to be antioxidants.[20] The volume of medicinal ingredients is less than that of PM meat powder, with a ratio of 1 gram of PM powder equal to 250 mg of dried extract of the god's crown (PME).

It is seen that the MDA level decreases on a fairly large scale compared to the baseline at the PME dose of 62.5 mg, while the PME dose of 125 mg and 250 mg shows a decrease in MDA levels but not too large compared to the 62.5 mg dose. MDA levels were assessed at 150 minutes after glucose induction. The mean MDA baseline level was 1,608, and with a dose of 62.5 mg PME we found a decrease in MDA levels of 40.9% compared to the baseline. Giving an PME dose of 125 mg and 250 mg obtained a decrease in MDA levels of 22.9% and 18.3% compared to MDA baseline levels.

PME antioxidant activity was obtained from fenolic components and flavanoids contained in PM fruit. Karimi et al[21] reported that the content of the fruit consisted mainly of flavonoids and kaempferol, myricetin, naringin, quercetin. Correlation of flavanoid content in PM fruit with antioxidant activity may be caused by the presence of 3-hydroxyl groups in heterocyclic rings while additional hydroxyl or methoxyl groups at position 3.5, and 7 rings A and C appear to be of less importance. Highly active favonoids have ring B which is occupied by 3'4'-dihydroxy and /or 3-OH groups. [22-23]

Table 5 shows the results of statistical analysis of baseline MDA levels, PME doses of 62.5 mg, 125 mg and 250 mg. From the results of statistical

analysis using paired t test between baseline and MDA administration level after PME was given a significant difference ($p < 0.05$).

Complaints found in the subject are nausea and fullness on 250 mg PME, this is probably due to the high saponin content in the PM fruit which can cause gastrointestinal irritation. In addition, it was also seen the effect of reducing blood pressure in subjects with 125 mg or 250 mg PME doses, this effect was caused by the presence of PM fruit action mechanism such as ACE receptor inhibitors.

The main limitation of this study is design as pre-post test experimental study without controls, so that further studies should be conducted with the standards of a good clinical trial.

Conclusion

Phaleria macrocarpa extract has antioxidant effects. The PME dose of 62.5 mg has better antioxidant efficacy than the dose 125 mg or 250 mg.

Conflict of Interest

The authors do not have any conflict of interest to declare.

Acknowledgement

The investigators thank the Faculty of Medicine Trisakti University and Trisakti University Research Institute (Lembaga Penelitian Universitas Trisakti) for the funding this study. Thanks are also due to the

Dhammasavana Foundation and all subjects are agreed to participate in this study.

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6. Bukti konfirmasi review dan permintaan revisi dari jurnal (review ketiga)

(21 April 2020)

UNIVERSITAS TRISAKTI

Meiyanti Meiyanti <meiyanti@trisakti.ac.id>

[GMHC] Revisions Required for Manuscript No.#5415 [3rd]

2 messages

gmhc. unisba <gmhc.unisba@gmail.com> Tue, Apr 21, 2020 at 7:45 PM
To: dr Meiyanti Meiyanti <meiyanti@trisakti.ac.id>

Dear
Mrs. dr Meiyanti

We have received your revision file to Global Medical & Health Communication (GMHC) No. #5415, "Antioxidant Effect of Phaleria macrocarpa (Scheff.) Boerl Dry Extract to the Level of Malondialdehyde".

Under the author's guidelines on the GMHC website, the number of citations is at least 25. We decide that your article needs to add 2 (two) citations so we can receive your manuscript and publish it in Vol.8 No.1, April 30, 2020.

Regards,
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Thank you, I will do that.
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[Quoted text hidden]

7. Bukti konfirmasi submit revisi ketiga, respon kepada reviewer, dan artikel yang diresubmit

(23 April 2020)

Antioxidant Effect of *Phaleria macrocarpa* (Scheff.) Boerl Dry Extract to The Level of Malondialdehyde

Meiyanti [1], Eveline Margo, Juni Chudri [2]
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Abstract
Increased age causes an increase in degenerative diseases. This disease is caused by antioxidants in the body unable to neutralize the increased concentration of free radicals. The flesh of the *Phaleria macrocarpa (Scheff.) Boerl* contains flavonoids which have antioxidant effects. At present there are still very limited clinical trials of *Phaleria macrocarpa (Scheff.) Boerl*. This research was an experimental pre- and post-test involving 30 healthy volunteer receiving glucose loads in November 2018-February 2019 in Jakarta. The aim of this study was to assess the antioxidant effect of *Phaleria macrocarpa (Scheff.) Boerl* dry fruit extract in various dosage ranges. Research subjects aged 30-55 years The data collection included anthropometric examination, baseline malondialdehyde levels and administration of dry fruit extract doses of 62.5 mg, 125 mg and 250 mg at 150 minutes after oral glucose induction. Data analysis using paired T test with p <0.05. Decreased levels of malondialdehyde in the administration of *Phaleria macrocarpa (Scheff.) Boerl* with a dose of 62.5 mg, 125 mg and 250 mg by 40.9%, 22.9% and 18.3% compared to the baseline malondialdehyde level (1,608 nmol / mL). Statistical analysis using paired T test showed p = 0,000 for all three doses compared with baseline levels. Dry fruit extract of *Phaleria macrocarpa (Scheff.) Boerl* has an anti-oxidant effect, the extract dose of 62.5 mg has an antioxidant effect better than other doses. The antioxidant effect of the extract is not directly proportional to the dose.

Keywords: : Extract, malondialdehyde , *Phaleria macrocarpa*.

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Efek Antioksidan Ekstrak Kering *Phaleria macrocarpa* (Scheff.) Boerl Terhadap Kadar Malondialdehid

Abstrak

Peningkatan usia menyebabkan peningkatan penyakit degeneratif. Penyakit ini disebabkan oleh antioksidan yang ada di dalam tubuh tidak mampu menetralisir peningkatan konsentrasi radikal bebas. Daging buah *Phaleria macrocarpa (Scheff.) Boerl* mengandung flavonoid mempunyai efek antioksidan. Saat ini masih sangat terbatas uji klinis *Phaleria macrocarpa (Scheff.) Boerl*. Penelitian ini merupakan uji eksperimental sebelum dan sesudah perlakuan pada 30 sukarelawan sehat yang diinduksi dengan glukosa dilaksanakan pada bulan November 2018-Februari 2019 di Jakarta. Penelitian ini bertujuan menilai efek antioksidan ekstrak kering *Phaleria macrocarpa (Scheff.) Boerl* dalam beberapa kisaran dosis yang diberikan. Subjek penelitian berusia 30-55 tahun Pengumpulan data meliputi pemeriksaan antropometri, kadar malondialdehid sebelum dan pemberian ekstrak kering dosis 62,5 mg, 125 mg dan 250 mg pada menit 150 setelah dilakukan induksi glukosa oral. Analisis data menggunakan uji T berpasangan dengan p <0,05. Penurunan kadar malondialdehid pada pemberian ekstrak *Phaleria macrocarpa (Scheff.) Boerl* dosis 62,5 mg, 125 mg dan 250 mg sebesar 40,9%, 22,9% dan 18,3% dibandingkan kadar malondialdehid sebelum pemberian (1.608 nmol/mL). Hasil analisis statistik menggunakan uji T berpasangan didapatkan p=0.000 untuk ketiga dosis dibandingkan dengan kadar awal. Ekstrak kering buah *Phaleria macrocarpa (Scheff.) Boerl* mempunyai efek antiooksidan, dosis ekstrak 62,5 mg mempunyai efek antioksidan lebih baik dibandingkan dengan dosis lainnya. Efek antioksidan ekstrak tidak berbanding lurus dengan dosis.

Kata kunci: Ekstrak, malondialdehid, *Phaleria macrocarpa*

Introduction

At present the development of medical science is so rapid that it raises an increase in one's life expectancy. Life expectancy is the leading indicator of a person's health quality.[1] In 1999 life expectancy in Indonesia was 66.2 years, in 2007 it increased to 70 years and in 2014 it became 71 years[1,2] causing an increase in risk for various degenerative diseases. Degenerative diseases found in many communities such as cancer, heart disease, diabetes, arthritis, liver disease and others.

This degenerative disease is caused by inability of antioxidants in the body to neutralize the increase in free radical concentration. Free radicals are molecules which in their outer orbit have one or more unpaired electrons, they are very labile and very reactive so that they can cause damage to the cell components. Superoxide formed from hydroxyl radicals will initiate lipid peroxidation, which causes damage to the endothelial plasma membrane, lipoproteins and cell genetic carrier DNA. Hydrogen peroxide causes oxidative stress, one of which can be measured by using MDA. Lipid peroxidation can be detected indirectly by measuring plasma hydrolysis of lipoperoxidase to MDA form.[3,4]

Diabetes mellitus (DM) is currently a worldwide health problem. In 2011 around 366 million adult suffered from DM, and it was estimated in year 2030 there would a very large increase of up to 551 millions patients with DM.[5] The condition of hyperglycemia results in an increase in oxidative stress, where it results from an imbalance between the formation of free radicals and antioxidants which is an important factor in the occurrence of

blood vessel disorders. Previous clinical studies report that oxidative stress play a major role in the pathogenesis and development of complications of DM.[3,4,6]

One of the medicinal plants that is known to have antioxidant effects and is widely used in the community is *Phaleria macrocarpa (Scheff.) Boerl* (PM). Some laboratory analysis studies show that PM showed an antioxidant effect, especially in young fruit and leaves.[7,8] Currently clinical trial are conducted very rare to prove the antioxidant effects of PM, so this study aims to assess the antioxidant effects of dried fruit pulp extract PM in a range of doses given. Based on the description above, it is necessary to conduct research that aims to determine the anti-oxidant effects of dried extract and the range of doses that provide the best antioxidant effect.

Methods

This study is a test of the dose range of antioxidant effects of dry extract of PM pericarp (PME) in healthy volunteers. This study used an open clinical trial design (without comparison) with an increased dose. This research was conducted in the area of Angke Village, Tambora District, Jembatan Dua, West Jakarta in November 2018-February 2019.

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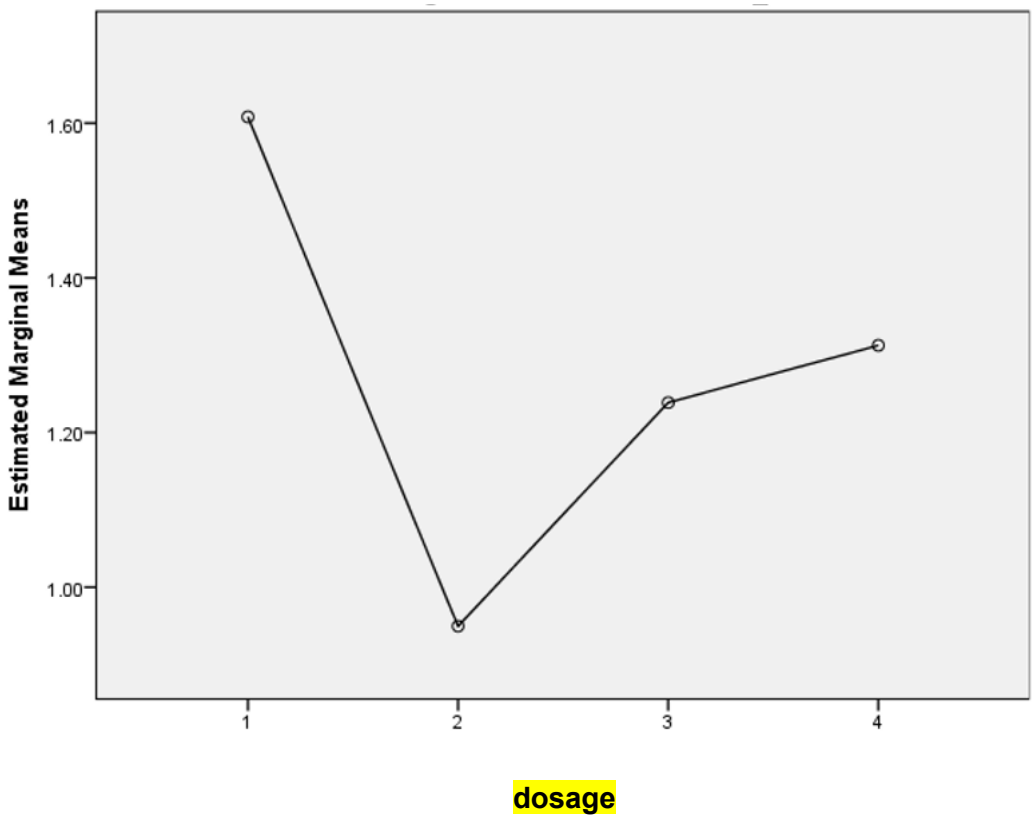

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Free radicals are defined as an atom, molecule, or component that contains unpaired electrons, so that it is generally unstable, has a short life, and is very reactive, which is produced due to the use of oxygen in the metabolic process. These free radicals are produced by normal body cells through metabolic processes, and also by external sources such as carcinogenic compounds and ionizing radiation.[3,9]

Free radicals can be produced from the results of the body's metabolism and external factors such as cigarette smoke, the results of ultra violet irradiation, radical trigger substances in foods and other pollutants. Disease caused by free radicals is chronic, ie it takes years for the disease to become apparent. Examples of diseases that are often associated with free radicals

are heart attacks, cancer, cataracts and decreased kidney function. To prevent or reduce chronic disease because free radicals are needed antioxidants. The human body can neutralize these free radicals, only if the amount is excessive, the ability to neutralize it will decrease .[10,11]

Research on the chemical content of eggshell seeds and fruit flesh PM showed that in hexane, ethyl acetate and methanol extract flavonoids, phenols, tannins, saponins and sterols/terpenes were obtained. The highest content is saponins. [12-14]

The four main parts of MD plants that are often used in society are the stems, leaves, seed shells and fruit flesh.[8,15] Research on the chemical content of seeds and fruit flesh obtained alkaloid compounds, saponins, flavonoids and polyphenols. Besides having the antioxidant effect of flavonoid content, the use of PME in DM cases can increase insulin expenditure by changing the metabolism of Ca^{2+} and can regenerate the island of Langerhans, especially β cells. Flavonoid contained in MD as an antioxidant that will protect pancreatic cell damage from free radicals.[16,17] Flavanoids are a natural antioxidant and have biological activities, including antioxidants that can inhibit various oxidation reactions and can act as reducing hydroxyl radicals, superoxide and peroxyl radicals. Extract the ethanol of young PM fruits has an inhibitory power of 78.48% and the old fruit has an 83.08% inhibitory effect, meaning that the old fruit PM has a greater antioxidant effect.[11-14]

This study used an oral glucose tolerance test procedure. In a hyperglycemic state it will produce free radicals. From Table 3, the highest

MDA levels were seen in 150 minutes after 45 gram glucose loading such as glucose tolerance test. MDA levels in the 150 minute will be taken as a baseline (without the administration of PME) and MDA levels will be measured again at the same time (150 minutes) with extracts of 62.5, 125 mg and 250 mg.

This research is an advanced study of the utilization of PME as an antihypoglycemic and antioxidant drug in patients with DM. This diabetes is characterized by relative or absolute deficiency of insulin secretion and/or resistance which causes chronic hyperglimeia and impaired carbohydrate, lipid and protein metabolism. DM is known as an oxidative stress disorder that occurs due to an imbalance between the formation of free radicals and the natural antioxidant abilities of the body. Many studies have reported that oxidative stress plays a role in systemic inflammation, endothelial dysfunction, impaired pancreatic β cell secretion and impaired glucose utilization in peripheral tissues. [3,4,6]

This oxidative stress also plays an important role with complications that occur in diabetic patients. Sources of oxidative stress in diabetes include enzymatic, non-enzymatic and mitochontrial pathways. The increased oxidative stress in DM is influenced by many factors. The dominant factor is auto oxidation of glucose which causes an increase in free radicals. Increased extracellular glucose levels will induce dysregulation of reactive oxygen and nitrogen pathways. This situation will cause disruption of the vascular endothelium and the production of nitric oxid (NO). Superoxide,

when joining NO on endothelial cells will produce perocynitrite which is a cytotoxic antioxidant. [18,19]

This research can be done in humans because PM is relatively safe and has been used for generations in Indonesian society. PM is extracted with water and ethanol in order to obtain active ingredients which are thought to be antioxidants.[20,21] The volume of medicinal ingredients is less than that of PM meat powder, with a ratio of 1 gram of PM powder equal to 250 mg of dried extract of the god's crown (PME).

It is seen that the MDA level decreases on a fairly large scale compared to the baseline at the PME dose of 62.5 mg, while the PME dose of 125 mg and 250 mg shows a decrease in MDA levels but not too large compared to the 62.5 mg dose. MDA levels were assessed at 150 minutes after glucose induction. The mean MDA baseline level was 1,608, and with a dose of 62.5 mg PME we found a decrease in MDA levels of 40.9% compared to the baseline. Giving an PME dose of 125 mg and 250 mg obtained a decrease in MDA levels of 22.9% and 18.3% compared to MDA baseline levels.

PME antioxidant activity was obtained from fenolic components and flavanoids contained in PM fruit.[22] Karimi et al[23] reported that the content of the fruit consisted mainly of flavonoids and kaempferol, myricetin, naringin, quercetin. Correlation of flavanoid content in PM fruit with antioxidant activity may be caused by the presence of 3-hydroxyl groups in heterocyclic rings while additional hydroxyl or methoxyl groups at position 3.5, and 7 rings A and C appear to be of less importance. Highly active

favonoids have ring B which is occupied by 3'4'-dihydroxy and /or 3-OH groups. [24-25]

Table 5 shows the results of statistical analysis of baseline MDA levels, PME doses of 62.5 mg, 125 mg and 250 mg. From the results of statistical analysis using paired t test between baseline and MDA administration level after PME was given a significant difference ($p < 0.05$).

Complaints found in the subject are nausea and fullness on 250 mg PME, this is probably due to the high saponin content in the PM fruit which can cause gastrointestinal irritation. In addition, it was also seen the effect of reducing blood pressure in subjects with 125 mg or 250 mg PME doses, this effect was caused by the presence of PM fruit action mechanism such as ACE receptor inhibitors.[25]

The main limitation of this study is design as pre-post test experimental study without controls, so that further studies should be conducted with the standards of a good clinical trial.

Conclusion

Phaleria macrocarpa extract has antioxidant effects. The PME dose of 62.5 mg has better antioxidant efficacy than the dose 125 mg or 250 mg.

Conflict of Interest

The authors do not have any conflict of interest to declare.

Acknowledgement

The investigators thank the Faculty of Medicine Trisakti University and Trisakti University Research Institute (Lembaga Penelitian Universitas Trisakti) for the funding this study. Thanks are also due to the Dhammasavana Foundation and all subjects are agreed to participate in this study.

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8. Bukti Accept submission of manuscript

(23 April 2020)

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[GMHC] Accept Submission for Manuscript No.#5415

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9. Publikasi artikel di GMHC

(30 April 2020)

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#5415 REVIEW

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RESEARCH ARTICLE

Effect of *Phaleria macrocarpa* (Scheff.) Boerl Dry Extract to the Level of Malondialdehyde

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Abstract

Increased age causes an increase in degenerative diseases. Antioxidants in the body unable to neutralize the increased concentration of free radicals. The flesh of the *Phaleria macrocarpa* (Scheff.) Boerl contains flavonoids which have antioxidant effects. At present, there are still very limited clinical trials of *Phaleria macrocarpa* (Scheff.) Boerl. This study was an experimental pretest and posttest involving 30 healthy volunteers receiving glucose loads in November 2018–February 2019 in Jakarta. This study aimed to assess the antioxidant effect of *Phaleria macrocarpa* (Scheff.) Boerl dry fruit extract in various dosage ranges. Subjects of this study aged 30–55 years. The data collection included anthropometric examination and malondialdehyde levels before and after administration of dry fruit extract doses of 62.5 mg, 125 mg, and 250 mg at 150 minutes after oral glucose induction. Data analysis using a paired t test with $p<0.05$. Decreased levels of malondialdehyde in the administration of *Phaleria macrocarpa* (Scheff.) Boerl with a dose of 62.5 mg, 125 mg, and 250 mg by 40.9%, 22.9%, and 18.3% compared to the baseline malondialdehyde level (1,608 nmol/mL). Statistical analysis using a paired t test showed $p=0.000$ for all three doses compared with baseline levels. Dry fruit extract of *Phaleria macrocarpa* (Scheff.) Boerl has an antioxidant effect; the antioxidant effect of the extract is not directly proportional to the dose.

Key words: Extract, malondialdehyde, *Phaleria macrocarpa*

Efek Antioksidan Ekstrak Kering *Phaleria macrocarpa* (Scheff.) Boerl terhadap Kadar Malondialdehid

Abstrak

Pertambahan usia menyebabkan peningkatan penyakit degeneratif. Antioksidan dalam tubuh tidak mampu menetralkan peningkatan konsentrasi radikal bebas. Daging buah *Phaleria macrocarpa* (Scheff.) Boerl mengandung flavonoid mempunyai efek antioksidan. Saat ini masih sangat terbatas uji klinis *Phaleria macrocarpa* (Scheff.) Boerl. Penelitian ini merupakan uji eksperimental sebelum dan sesudah perlakuan pada 30 sukarelawan sehat yang diinduksi dengan glukosa yang dilaksanakan pada bulan November 2018–Februari 2019 di Jakarta. Penelitian ini bertujuan menilai efek antioksidan ekstrak kering buah *Phaleria macrocarpa* (Scheff.) Boerl dalam beberapa kisaran dosis yang diberikan. Subjek penelitian berusia 30–55 tahun. Pengumpulan data meliputi pemeriksaan antropometri serta kadar malondialdehid sebelum dan sesudah pemberian ekstrak kering dosis 62,5 mg, 125 mg, dan 250 mg pada menit 150 setelah dilakukan induksi glukosa oral. Analisis data menggunakan uji t berpasangan dengan $p<0,05$. Penurunan kadar malondialdehid pada pemberian ekstrak *Phaleria macrocarpa* (Scheff.) Boerl dosis 62,5 mg, 125 mg, dan 250 mg sebesar 40,9%, 22,9%, dan 18,3% dibanding dengan kadar malondialdehid sebelum pemberian (1.608 nmol/mL). Analisis statistik menggunakan uji t berpasangan didapatkan $p=0,000$ untuk ketiga dosis dibanding dengan kadar awal. Ekstrak kering buah *Phaleria macrocarpa* (Scheff.) Boerl mempunyai efek antioksidan; efek antioksidan ekstrak tidak berbanding lurus dengan dosis.

Kata kunci: Ekstrak, malondialdehid, *Phaleria macrocarpa*

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Introduction

At present, the development of medical science is so rapid that it raises an increase in one's life expectancy. Life expectancy is the leading indicator of a person's health quality.[1] In 1999 life expectancy in Indonesia was 66.2 years. In 2007, it increased to 70 years, and in 2014, it became 71 years,[1,2] causing an increase in risk for various degenerative diseases. Degenerative diseases found in many communities are cancer, heart disease, diabetes, arthritis, liver disease, and others.

These degenerative diseases caused the inability of antioxidants in the body to neutralize the increase in free radical concentration. Free radicals are molecules that in their outer orbit have one or more unpaired electrons; they are very labile and very reactive so that they can cause damage to the cell components. An example is superoxide formed from hydroxyl radical, which initiates lipid peroxidation. The compound causes damage to the endothelial plasma membrane, lipoproteins, and deoxyribonucleic acid (DNA) genetic carrier. Hydrogen peroxide causes oxidative stress, one of which can be measured by using malondialdehyde (MDA). Lipid peroxidation can be detected indirectly by measuring plasma hydrolysis of lipoperoxidase to MDA form.[3,4]

One of the degenerative diseases, diabetes mellitus (DM), is currently a worldwide health problem. In 2011 around 366 million adults suffered from DM. It estimated that in 2030 there would a substantial increase of up to 551 millions patients with DM.[5] The condition of hyperglycemia results in an increase in oxidative stress, where it results from an imbalance between the formation of free radicals and antioxidants, which is an essential factor in the occurrence of blood vessel disorders. Previous clinical studies report that oxidative stress plays a significant role in the pathogenesis and development of complications of DM.[3,4,6]

One of the medicinal plants that are known to have antioxidant effects and widely used in the community is *Phaleria macrocarpa* (Scheff.) Boerl (PM). Some laboratory analysis studies show that PM showed an antioxidant effect, especially in young fruit and leaves.[7,8] Currently, scarce clinical trials are conducted to prove the antioxidant effects of PM. This study aims to assess the antioxidant effects of dried fruit pulp extract PM in a range of doses. It is necessary to determine the antioxidant effects of dried extract and the range of doses that provide the best antioxidant effect.

Methods

Respondents of this study were 30 healthy adult volunteers who fulfilled the following inclusion criteria. The criteria were men and women aged 30–55 years, having a healthy body weight (body mass index 18.5–25 kg/m^2). They are not having chronic diseases and no history of chronic diseases in the family, especially diabetes mellitus, hypertension, heart disease, supported by laboratory tests showing routine blood tests, liver function (SGPT, alkaline phosphatase), and normal kidney function (creatinine levels). They also have not consumed herbs or vitamin supplements a week before the study takes place/ during the study. Subjects in the previous 24 hours do not consume drinks containing caffeine, fruit juice, or smoking, and willing to take part in research and sign an informed consent. Exclusion criteria were pregnant or breastfeeding, consumed other drugs for the previous a week, or during the study that can affect blood glucose levels such as corticosteroid drugs, and participating in other studies within three months before this study.

The PM pericarp (without seed coat and seed) obtained from plant nurseries in the region of Semarang, Central Java. At each visit, subjects were given a glucose load of 400 calories (or 75 grams of glucose). The first visit only glucose load is given, whereas, in the second visit, 62.5 mg PME was given. They wash out for one week, and, at the next visit, subjects consumed 125 mg, and the following week consumed 250 mg PME. The parameters used to measure MDA levels.

Antioxidant effects were assessed by measuring MDA levels by determining MDA levels in serum after reacting with thiobarbituric acid (TBA) at hot temperatures in an acidic atmosphere. This reaction produces a red solution, which then measured using a spectrophotometer. The reagents used are the MDA standard, trichloroacetic acid (TCA), and TBA. Before the research took place, accuracy and precision test of the MDA examination carried out. This research has passed the ethical review of the Research Ethics Committee of Faculty of Medicine of Universitas Trisakti with letter number: 137/ KER/FK/I/2019.

Results

A total of 40 subjects examined for anthropometry (body weight and height) and body mass index examination. Besides, laboratory tests (hematology, blood sugar, SGPT, and creatinine) also carried out. Of the 40 subjects examined, only 30 people met the inclusion and exclusion criteria. Of the 30 volunteer subjects, 16 were women, and 14 were men. Personal data from 30 subjects are in Table 1.

In this study, the average age of subjects was 41.4 years, body weight in the range of 40–70 kilograms, height was 143–174 cm, and body mass index was 18.66–24.84 kg/m^2.

This study used auto control to monitor subjects. At the beginning of the study, all subjects tested for oral glucose tolerance with 75 grams of glucose and blood samples taken in 150 minutes after glucose induction as the baseline measure. After a week washout, subjects consumed glucose induction and PME at a dose of 62.5 mg, 125 mg, and 250 mg, respectively. The washout is performed one week before treatment with different doses. MDA was at 150 minutes after glucose induction. The results of the average examination of MDA levels in the 150th minute are in Table 2.

Table 3 shows the results of the statistical analysis of baseline MDA levels with PME doses of 62.5 mg, 125 mg, and 250 mg. From the results, there is a significant difference ($p<0.05$) between the two groups.

From Figure 1, it is seen that the MDA level decreases on a fairly large scale compared to the baseline at the PME dose of 62.5 mg, while the PME dose of 125 mg and 250 mg shows a decrease in MDA levels but not too large compared to the 62.5 mg dose.

Table 1 Characteristics of the Subject

Characteristics	Mean±SD n=30
Age (years)	41.40±6.41
Sex	
Female	16
Male	14
Weight (kg)	56.05±7.68
Height (m)	1.58±0.09
Body mass index (kg/m^2)	22.29±2.04

Table 2 Baseline MDA and PME Level

PME Doses	MDA Level (Average±SD)
Baseline	1.608±0.509
PME 62.5 mg	0.950±0.215
PME 125 mg	1.239±0.230
PME 250 mg	1.313±0.323

Table 3 Effect PME on Level MDA

Variables	p Value*
MDA baseline vs MDA PME 62.5 mg	0.000
MDA baseline vs MDA PME 125 mg	0.000
MDA baseline vs MDA PME 250 mg	0.000

Note: *Paired t test, $p<0.05$ significant difference

Discussion

In this study, 30 volunteered with an average age of 41.4 years to show the antioxidant effect of PME. Age or aging associated with free radicals. Aging is a biological process that cannot be avoided and is related to biochemical and physiological changes that are gradual and spontaneous and increase the body's susceptibility to disease. Scientific studies have shown that the aging process causes a decrease in the body's ability to use calories from food, decrease hormone function, suppress enzyme function, and decrease the body's

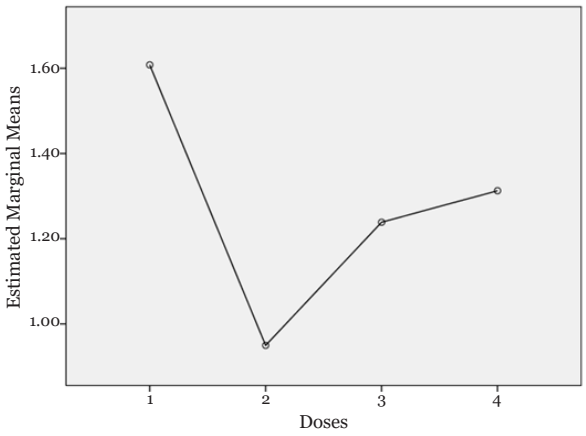

Figure The Effect of PME on MDA Level (nmol/mL) of Subject Baseline (1), and PME Dose 62.5 mg (2), 125 mg (3), and 250 mg (4)

resistance to fight disease. Aging results from an accumulation of changes caused by reactions in the body that are started by high reactive molecules known as 'free radicals.' These changes produce free radicals believed to be a significant cause of the aging process.[1,3]

Free radicals define as an atom, molecule, or component that contains unpaired electrons, so that it is generally unstable, has a short life, and is very reactive. The free radicals produced due to the use of oxygen in the metabolic process. These free radicals are produced by healthy body cells through metabolic processes, and also by external sources such as carcinogenic compounds and ionizing radiation.[3,9]

Free radicals produced from the results of the body's metabolism. They also caused by external factors such as cigarette smoke, the results of ultraviolet irradiation, organic trigger substances in foods, and other pollutants. Disease caused by free radicals is chronic. It will take years to become apparent or show any symptoms. Examples of diseases that are often associated with free radicals are heart attacks, cancer, cataracts, and decreased kidney function. To prevent or reduce chronic disease because free radicals are needed antioxidants. The human body can neutralize these free radicals; only if the amount is excessive, the ability to neutralize it will decrease.[10,11]

Research on the chemical content of eggshell seeds and fruit flesh PM showed that in hexane, ethyl acetate and methanol extract flavonoids, phenols, tannins, saponins, and sterols/terpenes with the highest content is saponins.[12–14]

The four main parts of MD plants that often used in society are the stems, leaves, seed shells, and fruit flesh.[8,15] Research on the chemical content of seeds and fruit flesh obtained alkaloid compounds, saponins, flavonoids, and polyphenols. Besides having the antioxidant effect of flavonoid content, the use of PME in DM cases can increase insulin expenditure by changing the metabolism of Ca^{2+} and can regenerate the island of Langerhans, especially β cells. Flavonoid contained in MD as an antioxidant that will protect pancreatic cell damage from free radicals.[16,17]

Flavanoids are a natural antioxidant and have biological activities, including antioxidants that can inhibit various oxidation reactions and can act as reducing hydroxyl radicals, superoxide, and peroxyl radicals. Extract the ethanol of young PM fruits has an inhibitory power of 78.48%, and the old fruit has an 83.08% inhibitory effect, meaning that the old fruit PM has a higher antioxidant effect.[11–14]

This study used an oral glucose tolerance test procedure. In a hyperglycemic state, it will produce free radicals. The highest MDA levels were seen in 150 minutes after 45-gram glucose loading, such as a glucose tolerance test. MDA levels in the 150th minute will be taken as a baseline (without the administration of PME), and MDA levels will be measured again at the same time (150 minutes) with extracts of 62.5 mg, 125 mg, and 250 mg.

This study is an advanced study of the utilization of PME as an anti-hypoglycemic and antioxidant drug in patients with DM. Diabetes characterized by a relative or absolute deficiency of insulin secretion and resistance. It causes chronic hyperglycemia and impaired carbohydrate, lipid, and protein metabolism. DM is known as an oxidative stress disorder that occurs due to an imbalance between the formation of free radicals and the natural antioxidant abilities of the body. Many studies have reported that oxidative stress plays a role in systemic inflammation, endothelial dysfunction, impaired pancreatic β cell secretion, and impaired glucose utilization in peripheral tissues.[3,4,6]

This oxidative stress also plays a vital role in complications that occur in diabetic patients. Sources of oxidative stress in diabetes include enzymatic, non-enzymatic, and mitochondrial pathways. Many factors influence the increased oxidative stress in DM. The dominant factor is the auto-oxidation of glucose, which causes an increase in free radicals. Increased extracellular glucose levels will induce the dysregulation of reactive oxygen and nitrogen pathways. This situation will cause disruption of the vascular endothelium and the production of nitric oxide (NO). Superoxide, when joining NO on endothelial cells, will produce peroxynitrite, which is a cytotoxic antioxidant.[18,19]

This research can be done in humans because PM is relatively safe and has been used for generations in Indonesian society. PM extracted with water and ethanol to obtain active ingredients that are antioxidants.[20,21] The volume of medicinal ingredients is less than that of PM meat powder, with a ratio of 1 gram of PM powder equal to 250 mg of dried extract of the god's crown (PME).

The MDA level decreases on a reasonably large

scale compared to the baseline at the PME dose of 62.5 mg, while the PME dose of 125 mg and 250 mg shows a decrease in MDA levels but not too large compared to the 62.5 mg dose. MDA levels assessed at 150 minutes after glucose induction. The mean MDA baseline level was 1,608, and with a dose of 62.5 mg PME, we found a decrease in MDA levels of 40.9% compared to the baseline. Giving a PME dose of 125 mg and 250 mg obtained a decrease in MDA levels of 22.9% and 18.3% compared to MDA baseline levels.

PME antioxidant activity from phenolic components and flavonoids contained in PM fruit.[22] Karimi et al.[23] reported that the content of the fruit consisted mainly of flavonoids and kaempferol, myricetin, naringin, quercetin. Correlation of flavonoid content in PM fruit with antioxidant activity caused by the presence of 3-hydroxyl groups in heterocyclic rings. In contrast, additional hydroxyl or methoxyl groups at position 3.5, and 7 rings A and C appear to be of less importance. Highly active flavonoids have ring B, which is occupied by 3'4'-dihydroxy and 3-OH groups.[24,25]

From the results of statistical analysis, using a paired t test between baseline and MDA administration level after PME was given a significant difference ($p<0.05$).

Complaints found in the subject are nausea and fullness on 250 mg PME; this is probably due to the high saponin content in the PM fruit, which can cause gastrointestinal irritation. Besides, the effect of reducing blood pressure in subjects with 125 mg or 250 mg PME doses were apparent. This effect caused by the presence of PM fruit action mechanism such as ACE receptor inhibitors.[25]

The main limitation of this study is the design as a pre-post test experimental study without controls—further studies needed with the standards of a good clinical trial.

Conclusion

Dry fruit extract of Phaleria macrocarpa (Scheff.) Boerl has an antioxidant effect; the extract dose of 62.5 mg has an antioxidant effect better than other doses. The antioxidant effect of the extract is not directly proportional to the dose.

Conflict of Interest

The authors do not have any conflict of interest to declare.

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