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EFFECTIVENESS OF ETHANOL EXTRACT MEAT FRUIT MAHKOTA DEWA (*Phaleria macrocarpa (Scheff) Boerl*) ON REDUCE URIC ACID LEVELS IN MALE MICE *(Mus musculus)*

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ABSTRACT

Mahkota Dewa *(Phaleria macrocarpa (Sceff.) Boerl.)* is a medicinal plant that is used empirically as a medicine for various diseases, one of which is gout. This study aims to determine the ethanol extract of the crown of the gods in reducing uric acid levels in mice. This study used an experimental method with the research subject being male mice *(mus musculus)* and the object of research was the decrease in uric acid levels in mice when given treatment. The independent variable was the ethanol extract of the crown of the god flesh and the dependent variable was uric acid levels in male mice *(Mus musculus).* Data on the percentage decrease in uric acid levels was tested using one-way ANOVA (5% confidence level) followed by the Bonferroni test. The results showed that the dosage of ethanol extract of Mahkota Dewa fruit was 1.25; 2,5 and 5g/kgbw were able to reduce uric acid levels in the blood serum of male mice induced by potassium oxonate. of the five treatment groups, the average uric acid level with the negative control was 0.48 ± 0.04 mg/dl. Positive control was 0.62 ± 0.08 mg/dl. The ethanol extract dose of 1.25g/kgBB was 1.68 ± 0.13. The ethanol extract dose of 2.5g/kgbw was 1.62 ± 0.08 and the ethanol extract dose of 5g/kgBW was 1 ± 0.5.

Keywords: *phaleria macrocarpa (Sceff.), uric acid.*

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INTRODUCTION

Herbal medicine is one of the mainstays for health care for 75-80% of the world's population, especially in developing countries. According to the World Health Organization (WHO), traditional herbal medicines are natural medicines, which have derivative substances derived from plants with minimal or no industrial processing and are used as local or regional medicine. The development of traditional herbal

medicine in Indonesia has long been known to the public. Apart from that, traditional medicine has several advantages, including relatively low side effects, in a mixture with different components they have mutually supporting effects, in one plant it has more than one pharmacological effect and is more suitable for metabolic and degenerative diseases (Katno, et al. al. 2002). Gout is a metabolic disorder caused by the accumulation of uric acid in body tissues (Anonymous, 2009). Uric acid is the end product of purine metabolism which can deposit in tissues and cause inflammation known as gout or gout. Gout or gout is a metabolic disease that occurs due to high levels of uric acid in the blood (Simon et al., 2001). Gout usually attacks men over 40 years of age and postmenopausal women. One of the main triggers for gout is a change in a person's lifestyle accompanied by an incorrect diet (Sudewo, 2004). Further consequences of increased uric acid are the formation of tophi (chalk) around the joints, kidney disorders and the formation of urate stones (Winarto, 2003). This research is used to look for plants that have potential as medicinal plants. One of the plants being developed is the crown of god plant. The dewa crown plant or Phaleria macrocarpa is a native herbal plant originating from the island of Papua in Indonesia and can grow in tropical areas (Alara et al., 2016; Parhizkar, 3013). The antioxidant content such as alkaloids, saponins and phalerin in this plant have their respective functions. This antioxidant substance plays a role in suppressing the amount of Reactive Oxygen Species (ROS), which are free radicals. This fruit is believed to have various compounds for treatment such as cancer, diabetes, hypercholesterolemia, hypertension, and so on (Parhizkae et al., 2014).

Several research results that have been carried out show that the Mahkota dewa plant has the potential to be anti-inflammatory (Siswanto, 2005), antihistamine (Siswono, 2001) and has an inhibitory effect on THP-1 leukemia cancer cells (Kurnia, et al., 2005). The flesh of the dewa crown fruit also has a hypoglycemic effect (Primsa, 2002). Safety test results showed that fresh squeezed flesh of Mahkota dewa fruit did not affect kidney function in male and female white rats during long-term use (Hendra, 2003). Juice from the flesh of Mahkota dewa fruit has been proven to be effective in reducing uric acid levels in Lohman Brown roosters at a dose of 13.16g/kgBB (Hasturani, 2003). Based on research by Arini (2003), it is known that the flesh of Mahkota dewa fruit contains flavonoids. The 70% ethanol extract of Mahkota dewa fruit flesh had the largest relative content of flavonoids (45,734 µg/mg). The effectiveness of the flesh of Mahkota dewa fruit for treating gout is thought to be based on its flavonoid content. The ability of this compound to reduce uric acid is by inhibiting the activity of xanthine oxidase on purine bases, thereby reducing uric acid production. The IC50 value of flavonoids states that 50% inhibition of xanthine oxidase is equivalent to a 50% reduction in uric acid production. The types of flavonoids that play a role in the mechanism of inhibiting the xanthine oxidase enzyme are flavones and flavonols (Cos et.al., 1998). The flavonoids in the Mahkota dewa plant can be in the form of aglycones or glycosides. The polarity of flavonoid compounds is from non-polar to polar so that they can be extracted in non-polar to polar filters (Markham, 1988).

Allopurinol has been used to treat gout for many years, however allopurinol can cause side effects, namely gastrointestinal damage, allergic reactions and liver toxicity (Mo et.al, 2007). So far, the use of the god's crown as a gout medicine has only been based on empirical evidence and user experience. Based on empirical use and the results of previous research, the ethanol extract of the flesh of Mahkota dewa fruit has the potential to be developed into a phytopharmaceutical. For this reason, it is necessary to carry out research on the ethanol extract of the flesh of the crown of god fruit to prove its effectiveness in reducing uric acid levels. It is hoped that the results of this research can be used as scientific evidence that the Mahkota dewa plant can be used as a medicine to reduce uric acid levels.

METHOD

This type of research is experimental research, samples were taken from Hatu Village, Central Maluku.

Preparation of ethanol extract

1. Filtering I: 300 grams of Mahkota dewa powder is put into a closed vessel then soaked in 1250 ml of 70% ethanol until all parts of the powder are submerged for 5 days with several shaking. After 5 days squeezed. Then the juice is allowed to settle for 1 night, then filtered and the filtrate is stored.

2. Filtration II: Next, 850 ml of 70% ethanol is added to the dregs until all parts of the dregs are submerged, then covered, left for 2 days protected from light, stirring three times a day. Then squeezed. The ethanol juice is transferred to a closed vessel, left in a cool place protected from light for 2 days to settle and then filtered.
3. Filter I and Filter II are combined and evaporated using an evaporator. Then it is concentrated using a porcelain cup over a water bath until all the solvent has evaporated so that a thick extract is obtained.

Maintenance and adaptation of test animals

Test animals used for research were male white mice with a body weight of between 20-30 grams, aged 2-3 months. Adaptation of mice was carried out in the Mathematics and Natural Sciences Laboratory at Pattimura University Ambon for 1 week and they were given standard food and distilled water ad libitum.

Determination of the dose of ethanol extract of Mahkota dewa fruit flesh

1. The maximum volume that can be given to mice weighing between 20-30 grams is 1 ml, but the volume gived is ½ of the maximum volume.
2. The dose of the ethanol extract from the flesh of the crown of god fruit is obtained by conducting a dose orientation first. Dosage orientation was carried out using 5 test animals which were given a middle dose of ethanol extract, namely 2.5g/kgBW. After that, the dose will be applied next in the treatment test.

Determination of Allopurinol and Potassium oxonate dose ranking

The dose of allopurinol used is 10mg/kgbw or 0.2mg/20gbw, while the dose of potassium oxonate used intraperitoneally in mice is 250mg/kgBW or 5mg/20gBW. This dose refers to previous research (Zhao et al., 2005).

Creation of Hyperuricemia

High uric acid levels (hyperuricemia) are created by inducing potassium oxonate intraperitoneally at a dose of 250 mg/kgBW or 5mg/20gBW in mice 1 hour after administering the test preparation (Mo et al, 2007). However, before the test animals are used for research, they are given additional food in the form of chicken liver juice with a concentration of 10% 3 times a day for 2 days.

Test treatment on test animals

The next test was a treatment test, using 25 mice which had been weighed and then divided into 5 groups of 5 mice each, namely:

1) Negative control: given CMC Na 0.5% p.o 0.5ml/20gBW.
2) Positive control: given allopurinol p.o 10mg/kgBW or 0.2mg/20gBW.
3) Ethanol extract 1: given ethanol extract of the flesh of the crown of god fruit at a dose of 1.25g/kgbw.
4) Ethanol extract 2: given ethanol extract of the flesh of the crown of god fruit at a dose of 2.5g/kgbw.
5) Ethanol extract 3: given ethanol extract from the flesh of the crown of god fruit at a dose of 5g/kgbw.

One hour after treatment, intraperitoneally induced with potassium oxonate 250mg/kgBW. 2 hours later blood was taken via the ophthalmic vein from the mice's eyes using a capillary tube and then the blood was collected (± 0.5 ml) in an Ependorf tube. Blood flows through the walls of the ependorf tube to avoid hemolysis. The blood was centrifuged at 5000 rpm for 5 minutes to obtain the serum. The separated serum was taken with a micropipette and placed in a new Ependorf tube. Serum levels were read for uric acid at a wavelength of 546 nm.

Determination of uric acid levels

1. Uric acid levels are determined based on an enzymatic reaction using FS* TBHBA uric acid reagent. The sample solution was made by taking 20 µl of serum plus 1000 µl of monoreagent (4 parts of Reagent 1 plus 1 part of Reagent 2). Serum that has been mixed homogeneously with Uric Acid FS* TBHBA reagent is incubated for 6-8 minutes at 37ºC. Next, the levels of the sample solution, standard and blank were read using a StartDust FC* 15 spectrophotometer at a wavelength of 546 nm.
2 Normal uric acid levels in mice are 0.5-1.4 mg/dl, and mice are said to have hyperuricemia if their uric acid levels are 1.7-3.0 mg/dl. (Erawan, 2016).

DISCUSSION RESULT

The effectiveness of the flesh of the dewa crown fruit (Phaleria macrocarpa (Scheff) Boerl) used 25 mice which had been weighed and then divided into 5 groups of 5 each consisting of a negative control group given CMC Na 0.5% p.o 0.5ml/20gbw. The positive control group was given allopurinol p.o 10mg/kgBW or 0.2mg/20gBW. Treatment group I was given ethanol extract of Mahkota dewa fruit flesh at a dose of 1.25g/kgbw. Treatment group II was given ethanol extract of Mahkota dewa fruit flesh at a dose of 2.5g/kgbw. And treatment group III was given ethanol extract of the flesh of the crown of god fruit at a dose of 5g/kgbw

Table 1. Uric acid levels after administration of potassium oxonate and levels after treatment in serum of male mice

group	Levels after administration of Potassium Oxonate (mg/dl)	Levels After Treatment (mg/dL)
Control (-) CMC Na 0,5%	0,5 0,5 0,5 0,4 mean ± SD = 0,48 ± 0,04	0,5 0,4 0,5 0,5 0,5 mean ± SD = 0,48 ± 0,04
Control (+) Allopurinol	1,8 1,7 1,8 1,9 1,9 mean ± SD = 1,82 ± 0,08	0,7 0,6 0,5 0,7 0,6 mean ± SD = 0,62 ± 0,08
Extract mahkota dewa 1,25g/kgbw	1,8 1,9 1,9 1,8 1,7 mean ± SD = 1,82 ± 0,08	1,5 1,6 1,8 1,8 1,7 mean ± SD = 1,68 ± 0,13
Extract mahkota dewa 2,5g/kgbw	1,8 1,8 1,8 1,9 1,9 mean ± SD = 1,84 ± 0,05	1,7 1,6 1,6 1,7 1,5 mean ± SD = 1,62 ± 0,08
Extract mahkota dewa 5g/kgbw	1,8 1,8 1,8 1,9 1,7 mean ± SD =	0,6 0,7 0,6 1,5 0,6 mean ± SD =

1,8 ± 0,07	1,00 ± 0,5

Determine the differences between the 5 groups, a statistical analysis of variance test was carried out using SPSS 20. The results of the analysis of variance obtained a significance value of 0.000 ($p<0.05$). And then a Post Hoc Bonferroni test was carried out to find out which groups had significant differences

Table 2. Average uric acid levels in male mice. After administering Potassium Oxonate and after treatment with Mahkota Dewa Fruit Extract.

Treatment	After administrated Potassium Oxonate	After treatment	Difference
+	0,48	0,48	0
-	1,82	0,62	-1,2
1,25gr	1,82	1,68	-0,14
2,5gr	1,84	1,62	-0,22
5gr	1,8	1,00	-0,8

The average uric acid level after being induced by administering potassium oxonate (p.o) was between 0.48 mg/dL to 1.8 mg/dL, and the uric acid level after administering Mahkota dewa extract for 1 week ranged from 1.00 mg/dL up to 0.48 mg/dL. From the test results, the average uric acid levels showed that group I (negative control CMC Na o.5%) was not significantly different from group II (positive control Allopurinol) and groups III, IV and V (various doses of ethanol extract of Mahkota dewa fruit flesh) with a P.Sig value of 0.790<0.05, namely. This means that allopurinol and variations in the dose of ethanol extract of the flesh of the crown of god fruit, namely a dose of 1.25; 2.5 and 5g/kgBB can have the effect of reducing uric acid levels. Meanwhile, data on the percentage reduction in uric acid levels was then carried out with statistical tests. The statistical test results show that the percentage reduction data is normally distributed and the variance is homogeneous because it has a Sig value of 0.984<0.05.

The highest uric acid occurs at 09.00 with an average of 0.62 mg/dL to 1.82 mg/dL, while at 09.30 the average uric acid is 1.68 mg/dL to 1.82 mg/dL, at At 10.00 the average uric acid level was 1.62 mg/dL to 1.84 mg/dL and at 10.30 the average uric acid level in male mice increased to 1.00 mg/dL to 1.8 mg/dL. The increase that occurred was influenced by the water consumed and the metabolism of the mice themselves. This is supported by the opinion of Salsabilah et al (2015) who stated that the uric acid excretion process is not only influenced by water, but is also influenced by the metabolism of the test animals and the excretion of allantoin as the final product of uric acid. Based on the results of research that has been carried out, it is known that the uric acid levels of male mice (Mus musculus) after being given treatment with Mahkota dewa fruit extract (Phaleria macrocarpa (Scheff.) Boerl.) In (negative control), the average uric acid levels of mice after induction potassium oxonate was 0.48 mg/dL and after administration of Mahkota dewa fruit extract it was 0.48 mg/dL. In (positive control), the average uric acid level before potassium oxonate induction was 1.82 mg/dL and administration of Mahkota dewa fruit extract was 0.62 mg/dL. In the ethanol extract dose of 1.25 gr/kgBW, the average uric acid level before potassium oxonate induction was 1.82 mg/dL and after administration the level was 1.68 mg/dL. In the ethanol extract dose of 2.5 gr/kgBW, the average uric acid level before potassium oxonate induction was 1.84 mg/dL and after administration the level was 1.62 mg/dL. In the ethanol extract dose of 5g/kgbw, the average uric acid level before potassium oxonate induction was 1.8mg/dL and after administration the level was 1.00 mg/dL. However, based on the data, it can be seen that the uric acid levels of mice after treatment tended to decrease at a dose of 5g/kgbw of ethanol extract and in negative controls there was a decrease in uric acid levels of 1.00 mg/dL and 0.48mg/dL. and in the negative control there was a decrease of 0.48 mg/dL. The highest decrease in uric acid levels was found in the ethanol extract dose of 2.5 gr/kgBW at 1.84 mg/dL, and the lowest average uric acid levels were also found in the negative control and positive control at 0.48. The increase that occurred was probably due to the process. The metabolism of test animals in negative control, positive control and ethanol extract at a dose of 5g/kgBW were less able to excrete allantoin properly.

group II (negative control) was not significantly different from groups III, IV and V (various doses of ethanol extract of Mahkota dewa fruit flesh). This means that the ethanol extract of the crown of god fruit flesh is dosed at 1.25; 2.5 and 5 g/kgbw have the potential to reduce uric acid levels in male mice that are hyperuricemic. Group II (negative control) showed no significant difference to groups III, IV and V (ethanol extract of Mahkota Dewa fruit flesh at a dose of 1.2'; 2.5 and 5 gr/kgBW). Likewise, group III was not significantly different from group III. IV. This shows that groups III, IV and V were able to reduce uric acid levels in male mice that were hyperuricemic. The ethanol extract of the flesh of the crown of god fruit has the same potential as allopurinol in reducing uric acid levels, but its ability is not as great as that of allopurinol. One of the compounds that plays a role in reducing uric acid levels is flavonoids (Cos et.al., 1998). The flesh of Mahkota dewa fruit is known to contain flavonoids. The ethanol extract of Mahkota dewa fruit flesh has the highest flavonoid content (45,734 µg/mg) (Arini et al., 2003). The flavonoids in the Mahkota dewa plant can be in the form of aglycones or glycosides. The polarity of flavonoid compounds is from non-polar to polar so that they can be extracted in non-polar to polar filters (Markham, 1988).

Markham's (1988) statement was strengthened by Setiani's (2010) research that an infusion of the flesh of the crown of god fruit at a dose of 2.5 g/kgBW was effective in reducing blood uric acid levels in male white mice made hyperuricemic with potassium oxonate at a dose of 250 mg/kgBW by around 73.77%. . The infusion preparation of Mahkota dewa fruit flesh requires a smaller dose than the ethanol extract preparation to reduce uric acid levels. This is because the active flavonoid compounds that are bound to the solvent are glycosides which are polar so they are more soluble in polar solvents, namely water. The effectiveness of Mahkota Dewa fruit in non-polar solvents was stated by Habsari (2010) that a dose of 2.5 g/kgBW of Mahkota Dewa fruit extract was able to reduce the uric acid levels of male mice that were hyperuricemic by 71.88%. It is possible that the active flavonoid compounds that are extracted from non-polar solvents such as n-hexane are flavonoids in the form of aglycones. The effectiveness of Mahkota Dewa fruit was also proven in Pramita's research (2010) which stated that the juice of the flesh of Mahkota Dewa fruit at a dose of 15g/kgBB was effective in reducing uric acid levels by 60.16%. Research on infusion preparations, ethanol extracts and hexane extracts from the flesh of the crown of god fruit shows that the flavonoids contained in the flesh of the crown of god fruit can be absorbed in polar and non-polar solvents.

Ethanol extract and hexane extract of Mahkota Dewa fruit flesh had a large percentage reduction in uric acid levels. This may occur because there are compounds other than flavonoids that are absorbed by the solvent in each preparation which can be responsible for reducing uric acid levels. The mechanism for reducing uric acid levels in this study was based on the flavonoid compounds contained in the flesh of Mahkota dewa fruit. Flavonoids are able to reduce uric acid levels by inhibiting the activity of the xanthine oxidase enzyme, thereby reducing uric acid production. The IC50 value of flavonoids states that 50% inhibition of xanthine oxidase is equivalent to a 50% reduction in uric acid production. The types of flavonoids that play a role in the mechanism of inhibiting the xanthine oxidase enzyme are flavones and flavonols (Cos et.al, 1998). The presence of other compounds besides flavonoids contained in Mahkota dewa may also play a role in reducing uric acid levels. Therefore, further research needs to be done to find out these compounds.

CONCLUSION

The effectiveness of the Ethanol Extract of Mahkota Dewa Fruit Flesh *(Phaleria macrocarpa (Scheff) Boerl)* on Reducing Urate Asan Levels in male mice *(Mus musculus)* has been carried out, the conclusions that can be drawn are as follows gived ethanol extract of mahkota dewa fruit flesh *(Phaleria macrocarpa (Scheff) Boerl)* can effectively reduce uric acid levels in mice *(Mus musculus)* test animals.

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