THE EFFECT OF KARAMUNTING (*Rhodomyrtus tomentosa*) LEAVES ETHANOLIC EXTRACT ON SPERMATOZOA CONCENTRATION OF RATS INDUCED HIGH-FAT DIET

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ABSTRACT

Background: The side effects of medication, especially cholesterol-lowering drugs, make people choose to use traditional medicine. The active compounds in karamunting (*Rhodomyrtus tomentosa*) leaves are believed to have the potential as antioxidants to overcome hypercholesterolemia and the effects of diseases related to male fertility. This study aimed to determine the effect of ethanol extract of karamunting leaves on the spermatozoa concentration of male Wistar rats (*Rattus norvegicus*) given different extract doses compared to controls induced by a high-fat diet.

Method: This research is an observational analytic study. The karamunting leaves extract was obtained from maceration with 70% ethanol and evaporated with a rotary evaporator. There were 15 videos of male rats spermatozoa divided into three groups: the control group received 5% Na-CMC, treatment group 1 (P1) received karamunting leaves extract 400 mg/kgBW, and treatment group 2 (P2) received karamunting leaves extract 800 mg/kgBW administered orally for 30 days. Previously, rats were induced with a high-fat diet for 30 days. Spermatozoa concentration was calculated using the counting chamber method, observed on the recorded video media.

Result: There was no significant difference in the concentration of spermatozoa ($p>0.05$) and relative testicular weight ($p>0.05$) in the P1 and P2 extract dose groups compared to the control group.

Conclusion The ethanol extract of karamunting leaves did not affect the concentration of rat spermatozoa induced by a high-fat diet.

Keywords: Karamunting Leaves Extract, Spermatozoa Concentration, High-Fat Diet, Male Fertility

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INTRODUCTION

People's diet has changed in the last few decades towards a "Western diet." This eating pattern is generally characterized by a high intake of industrially processed foods, simple carbohydrates, low fiber, high animal protein, high sugar, and high fat.[1] Intake of high-fat foods is a cause of hypercholesterolemia.[2,3]

Riset Kesehatan Dasar (Riskesdas) 2018 data on Indonesian residents aged >15 revealed that as many as 28.8% had abnormal total cholesterol levels.[4] Thus, cholesterol must be strictly regulated because any disruption of cholesterol

homeostasis can affect male reproductive fitness and fertility.[5]

In recent decades infertility has become a global public health problem affecting 15% of all reproductive couples. The male factor is responsible for 25% of infertility cases.[6] The most significant cause of male infertility is low sperm concentration. As much as 90% of male infertility problems are related to the number, and there is a positive relationship between abnormal semen parameters and sperm count.[7]

Studies conducted on rats have shown that excess consumption of dietary cholesterol causes hypercholesterolemia and negatively impacts male fertility.[1] This condition is a threatening problem for men because it relates to their well-being. Therefore, to overcome hypercholesterolemia and the impact of hypercholesterolemia itself, various forms of treatment are needed.

It was reported that karamunting plants in various Asian countries, such as China, Vietnam, and Malaysia, have been used as traditional medicine. Karamunting plants contain various active compounds, including phloroglucinol, anthracene glycosides, stilbenes, tannins, terpenoids, flavonoids, and saponins.[8] Flavonoids act as antioxidants and protect the body against Reactive Oxygen Species (ROS).[9] Flavonoids which also work to inhibit HMG CoA reductase, will be useful in lowering total cholesterol levels.[10] But in contrast to flavonoids, active saponin compounds interfere with spermatogenesis activity.

There are active compounds that play a medicinal role, and there are no reports on the quality of the spermatozoa of rats fed a high-fat diet after administration of karamunting leaves extract, so this study was conducted to determine the effect of ethanol extract of karamunting leaves on the concentration of rat spermatozoa induced by a high-fat diet.

METHODS

This research is an observational analytic study with a cross-sectional study design on rat spermatozoa concentrations from previous experimental studies. The sample in this study was video documentation of spermatozoa preparations of male Wistar rats (*Rattus norvegicus*) in the Improved Neubauer counting chamber of as many as 15 pieces which were divided into three groups, namely the control group (K) with a high-fat diet and no extracts, the first treatment group (P1). The second treatment (P2) was given a high-fat diet, and then each was given an ethanol extract of karamunting (*Rhodomyrtus tomentosa*) leaves at a dose of 400 mg/kg BW and 800 mg/kg BW.

After obtaining data on the number of spermatozoa in the counting chamber, then the data is calculated using the formula:

$$Spermatozoa\ concentration = n \times \frac{25}{s} \times 10^4 \times \mathrm{Df}$$

n: Total number of spermatozoa counted; s: The number of squares counted; Df: Dilution factor; and 10^4 is 1/volume (ml) of the central box of the Improved Neubauer counting chamber.

Research data were analyzed using SPSS 27 version. Data normality was tested by Shapiro-Wilk (sample size less than 50). Homogeneity of variance was tested with Levene. The normally distributed data were tested using a one-way ANOVA parametric bivariate statistical test, while the non-normally distributed data were tested using the Kruskal-Wallis non-parametric bivariate statistical test.

This research has received approval for ethical clearance from the health research ethics committee of FKIK Universitas Jambi No. 3485/UN21.8/PT.01.04/2022.

RESULTS

The results of calculating the concentration of rat spermatozoa in the control group and the treatment group of karamunting leaves ethanolic extract can be seen in Table 1.

Table 1. Rat spermatozoa concentration

Group	*n*	*Spermatozoa concentration (million/ml)*	*p-value*
K (Without extract)	*5*	*17.86 ± 15.72*	
P1 (400 mg/kg BW)	*5*	*16.38 ± 4.19*	*0.858*
P2 (800 mg/kg BW)	*5*	*13.86 ± 7.59*	

Based on Table 1, the highest mean concentration of spermatozoa is at K (0.5% Na-CMC) of 17.86 million/ml, while the lowest mean concentration of spermatozoa is at P2 (extract dose of 800 mg/kg BW) of 13.48 million/ml. The results of the Kruskal-Wallis test show no significant difference in the concentration of spermatozoa in each group (p>0.05).

Table 2. Rat relative testis weight

Group	*n*	*Relative testis weight (gr)*	*p-value*
K (Without extract)	*5*	*0.452 ± 0.019*	
P1 (400 mg/kg BB)	*5*	*0.547 ± 0.088*	*0.114*
P2 (800 mg/kg BB)	*5*	*0.573 ± 0.085*	

Table 2 shows the highest relative testes weight at P2 (dose extract of 800 mg/kgBB) of 0.573 grams, and the lowest testicular weight at K (Na-CMC 0.5%) is 0.452 grams. In the One Way Anova test on the relative testes weight, there is no

significant difference in the relative testicular weight of each group (P> 0.05).

DISCUSSION

Spermatozoa concentration is an indicator of spermatozoa quality. According to Blandau & Odor, the spermatozoa male Wistar mouse concentration is $58x10^6$ /ml.[11] In table 1, the highest average concentration of spermatozoa concentrations is found in the control group, which is 17.86 million/ml and then sequentially followed by the group P1 (16.38 million/ml) and P2 (13.86 million/ml). Based on this description, the concentration of spermatozoa in treatment groups P1 and P2 have a lower average than in the control group.

The results obtained from this study descriptively have similarities with the research of Nurlely et al., which reported that the administration of ethanol extract of pakan banyu bark (*Croton argyratus* lume) containing active compounds of saponins, alkaloids, tannins, and steroids show differences with normal control groups. The number of spermatozoa decreased in the treatment group compared to the control caused of a compound in the form of saponins which can trigger inhibition of spermatogenesis. This inhibition process occurs at a stage of differentiation of spermatid morphological transformation to form spermatozoa through the supply of compounds needed during the process[12]

This study showed different things compared to the Febrianty et al. report that the administration of kebar grass extract was proven to increase the concentration of spermatozoa. Kebar grass contains chemical compounds of flavonoids, tannins, and saponins that have the potential to have a positive influence on spermatogenesis.[13] In addition, Khatimah et al. also stated that the administration of karamunting leaves ethanolic extract could increase testosterone levels in the diabetes mellitus mouse model.[14]

Flavonoids are antioxidants that donate one electron to unpaired electrons to free radical compounds to stabilize free radicals. Stable free radicals will prevent oxidative stress conditions.[15] Hypercholesterolemia can increase ROS production in the body. Reactive oxygen compounds that increase concentration in the body can trigger oxidative cell stress if not balanced by antioxidant activity.[16]

Spermatozoa are more vulnerable to oxidative stress than other cells due to the limited number of cytoplasm in mature spermatozoa and the lack of antioxidant concentrations in sperm. In addition, the high levels of unsaturated fatty acids in the structure of spermatozoa exacerbated the situation.[17] Flavonoids are also able to suppress the activity of the HMG-CoA reductase enzyme to convert HMG-COA to mevalonate and reduce cholesterol esterification in the intestine and liver so

that it is likely to cause a decrease in cholesterol levels.[18,19] Decreased cholesterol levels have an impact on spermatogenesis.

This difference in yield may occur because the number and content of each active compound of Karamunting leaves in this study are different from other studies. Based on various studies that use karamunting leaves, the content of different compounds is obtained between studies after the screening of metabolic compounds. The Hasibuan et al. research shows the presence of alkaloids, flavonoids, and tannin/phenolic compounds. Tannin compounds have the highest content in karamunting leaves; the lowest is a terpenoid compound.[20] Whereas research conducted by Suryadinata et al. reported the compounds contained in karamunting leaves extract with ethyl acetate and methanol extracts, including tannins, flavonoids, quinones, saponins, monoterpenes, steroids, and polyphenolic.[21] Other factors that are thought to affect the difference in the content of compounds are leaves aging, extraction methods, and type of solvent.[22–24]

The non-parametric statistical results of the spermatozoa concentration obtained a P-value of 0.858. This shows the value of P> 0.05, meaning there is no significant difference in the average concentration of spermatozoa between the control group and the P1 and P2 treatment group. This is likely to occur due to the dilution factor, and the calculation process in the calculation chamber experienced liquefaction of liquefaction because the long liquefaction process makes the homogenization process more difficult to do. The poor homogenization process results in the uneven distribution of spermatozoa so that the reading and assessment of spermatozoa become not constant.[25]

The selection of extraction methods can affect the activity of an extract. Extraction methods can increase or even eliminate extract activity because simplicia have relatively unstable and easily decomposed properties. In this study, the extraction method used was maceration. The maceration method is used to prevent damage to compounds due to heating. Based on research conducted by Marwati et al., who conducted research on Karamunting leaves extract by maceration, sonication, and reflux methods to assess antioxidant activity, it was found that the sonication method is the most effective method of obtaining active antioxidant substances compared to other extraction methods.[23] In addition, the duration of extract administration may also influence the study results.

Spermatogenesis cycle process lasts around 52-53 days.[26] The duration of extracts in this study is 28 days, shorter than spermatogenesis. However, this also cannot be used as a strong reason why the extract is not significantly influenced. Because when the extract administration begins, there is also spermatogenesis in

the middle phase, so spermatozoa have also been formed after giving extracts for four weeks. Based on statistical results, it can be said that the administration of Karamunting leaves ethanolic extract in this study has not been able to have an effect, either to decrease or increase spermatozoa concentration.

The amount of spermatozoa depends on the process of spermatogenesis that occurs in seminiferous tubules. In the event of damage or atrophy of seminiferous tubules, the testis' weight loss will occur.[27] In Table 2, the average weight of the largest rat testes is found in treatment group two, which is 0.573 grams. Then sequentially followed by the treatment group one (P1) and control (K), which is 0.547 grams and 0.452 grams. The average heavy testes in this study showed results that were inversely proportional to the concentration of spermatozoa.

Then, the One Way Anova test obtained the P-value > 0.05, which showed statistically there was no significant difference in the weight of testicular weight between the control group (K) and treatment group one (P1), and treatment two (P2). In this case, it can be said that the ethanol extract of Karamunting leaves in this study has not been able to affect the weight of the testicles even though the treatment dose is higher.

CONCLUSION

There is no effect on the administration of karamunting leaves ethanolic extract (*Rhodomyrtus tomentosa*) on spermatozoa concentration of male rats induced high-fat diet.

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