SUBCRONIC TOXICITY TEST OF EXTRACT PERIA FRUIT (*Momordica charantia L*) ON WISTAR RATS TOTAL CHOLESTEROL

(UJI TOKSISITAS SUBKRONIK EKSTRAK PERIA (Momordica charantia L) TERHADAP KADAR KOLESTEROL TOTAL TIKUS WISTAR)

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ABSTRACT

Bitter melon had been widely used by the community as traditional medicine and proven to have effects including antidiabetic, anti-inflammatory, antipyretic, antioxidant, antitumor. Further testing is needed to determine the toxic effects that have not been detected in the acute toxicity test, the subchronic toxicity test. This study aims to assess the toxicity seen from the number of deaths, changes in body weight, changes in the weight of rat liver, and cholesterol levels in rat blood. This research is an experimental study using the Posttest Only Control Group Design method. A total of 40 male and female rats wistar strains were divided into a control group (K) and 3 treatment groups (P). Each group consisted of 5 male rats and 5 female rats. Group K is only given food (pellets) and drinking water. P1, P2, and P3 groups were given feed (pellets), drinking water and ethanol extract of peria fruit (*Momordica charantia L.*) at levels of 250 mg/kg, 500 mg/kg, and 1000 mg/kg. After the first 24 hours of administration the number of

rats that died was counted, the change in body weight was weighed for 29 days and on the 29th day a rat's blood was taken to calculate the cholesterol level of the rat, surgery was also performed to weigh the relative organ weigh of the rat liver. There were no rat deaths in the entire group, there were no weight loss, relative liver organ weight, and cholesterol levels were significant in all group(p>0,05). The ethanol extract of peria fruits in this study showed no toxic. The dose has reached the maximum dose of 1.000 mg/kg but the results have no effect on death, body weight, relative organ weight of the liver, and cholesterol levels.
Keywords: *Momordica charantia L*; subchronic toxicity

ABSTRAK

Buah peria telah banyak. dimanfaatkan masyarakat sebagai obat tradisional dan terbukti memiliki efek diantaranya antidiabetes, antiinflamasi, antipiretik, antioksidan, antitumor. Diperlukan pengujian lebih lanjut untuk mengetahui efek toksik yang belum terdeteksi pada uji toksisitas akut, yaitu dengan uji toksisitas subkronis. Penelitian ini bertujuan 'untuk menilai toksisitas dilihat dari jumlah kematian, perubahan berat badan, perubahan berat organ hepar tikus, dan kadar kolesterol dalam darah tikus. Penelitian ini adalah penelitian eksperimental menggunakan metode Posttest Only Control Group Design. Sebanyak 40 ekor tikus jantan dan betina galur wistar dibagi menjadi kelompok kontrol (K) dan 3 kelompok perlakuan (P). Tiap kelompok terdiri dari 5 ekor tikus jantan dan 5 ekor tikus betina. Kelompok K hanya diberi pakan (pelet) dan air minum. Kelompok PI, P2, dan P3 diberi pakan (pelet), air minum dan ekstrak etanol daging buah peria (Momordica charantia L.) dengan dosis bertingkat 250 mg/kgbb, 500 mg/kgbb, dan 1000 mg/kgbb. Setelah 24 jam pertama pemberian dihitung'jum1ah tikus yang mati, perubahan berat badan ditimbang selama 29 hari, kemudian pada hari ke-29 dilakukan pengambilan darah tikus untuk menghitung kadar kolesterol tikus. Dilakukan juga pembedahan untuk penimbangan berat organ relatif

hepar tikus. Tidak ada kematian tikus pada seluruh kelompok, tidak terjadi penurunan berat badan, berat organ relatif hepar dan kadar kolesterol tikus dengan signifikan pada seluruh kelompok (p>0,05). Pemberian ekstrak etanol buah peria pada penelitian ini menunjukkan tidak toksik. Hal ini dikarenakan pemberian dosis telah mencapai dosis maksimal yaitu 1.000 mg/kgbb tetapi hasilnya tidak berpengaruh pada kematian, berat badan, berat organ relatif hepar, dan kadar kolesterol.
Kata kunci: *Momordica charantia L; toksisitas subkronis*

INTRODUCTION

Indonesia is a country known to have very abundant biodiversity. One of the biodiversity is plants that can be used as traditional medicines.[1] One of the traditional medicines used by the community is bitter melon. Peria fruits a plant that belongs to the *Curcubitacea* family.[2,3] Many studies using bitter melon have been carried out. This is because bitter melon has a chemical content contained in it. Peria fruit contains triterpenoid compounds, carotenoids, flavonoids, alkaloids, steroids and essential oils.[4] Not only the flesh, peria leaves are also an antioxidant, antimicrobial, and antidiabetic because they contain chemical compounds, namely alkaloids, flavonoids, and saponins. In addition, flavonoids can also change cell membranes and inhibit fat peroxidase.[5,6]

Determination of the safety of a drug product must go through a standardization test. Toxicity tests are carried out to determine the presence of toxic effects or safety thresholds from the use of a drug product. Toxicity tests can be carried out by means of acute, subchronic, chronic and special toxicity.[7,8,9]

Sub-chronic toxicity test is a test performed by giving an oral dose which is carried out every day for 28 or 90 days in test animals to determine the toxic effect. The dosing was carried out for part of the age of the experimental animals but did not exceed 10% of the entire age of the animals. If an animal dies before the rigor mortis period occurs, surgery must be performed to see the organs of the animal. Observations on organs were also carried out at the end of dosing.[10] Subchronic toxicity test aims to see a complete blood picture such as hematocrit, leukocytes, erythrocytes, platelets and hemoglobin. In addition, it can also assess the aspects of biochemistry and histopathology. The biochemical aspects that can be assessed are changes in kidney function, namely urea and creatinine levels, besides that it can also be seen from changes

in liver function, namely assessing changes in levels of Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvate Transaminase (SGPT) and can assess total cholesterol and triglycerides. Meanwhile, from the histopathological aspect, you can see an overview of organs from the liver, kidneys, ovaries or testes, stomach and heart.[11]

This research is a follow-up study that has been carried out by Safitri (2018) using experimental animals of DDY strain male mice for acute toxicity tests. The administration of ethanol extract of bitter melon flesh was carried out for 14 days using various doses, namely 625 mg/kg body weight, 1250 mg/kg body weight, 2500 mg/kg body weight, and 5000 mg/kg body weight. This dose did not give a toxic effect because there was no death in experimental animals and there was no decrease in body weight and relative organ weight of the heart, lungs, liver, kidneys and testes.[12] The liver is one of the organs that functions to evaluate the toxicity of medicinal products. In addition, the liver also has a function as a place for metabolism. One of the substances that can be metabolized by the liver is cholesterol. Liver damage due to continuous exposure to substances will cause disturbance cholesterol metabolism so that cholesterol levels in the body will decrease.[13,14]

Based on these data, researchers will conduct a study on subchronic toxicity tests on blood cholesterol levels by giving oral bitter melon extract ethanol extract with varying doses, namely 250 mg/kg, 500 mg/kg and 1000 mg/kg for 28 days and observations regarding changes in body weight, organ weight and number of deaths. In addition, biochemical tests such as cholesterol levels in the blood will also be carried out.

METHOD

This research is an experimental study with a research design using Posttest Only Control Group Design. In this study, the grouping and treatment of experimental animals was carried out using a Completely Randomized Design (CRD). This research was conducted after being declared free from ethical review by the Medical and Health Research Ethics Committee (KEPK) Faculty of Medicine, Universitas Padjadjaran with No 03/UN6.KEP/EC/2020.

The object of this research was peria fruits/bitter melon fruit with an age of 1-2 months and the outer surface of the material is not damaged. The subjects in this study were male and female white rats of the Wistar strain, healthy or without defects and actively moving, 3-4 months of age, 120 grams of body weight, and had never been

tested. The number of experimental animals used was 40 rats.

This study used various doses, namely 250 mg/kg body weight, 500 mg/kg body weight, and 1000 mg/kg body weight. Before conducting the research, one must prepare the research object, tools, materials and research subjects or experimental animals used. Rats were acclimatized for 7 days in the Pharmacology Laboratory of the Faculty of Medicine, Padjadjaran University. Experimental animals were put into cages measuring 30x50 cm with a height of 15 cm. One cage contains 5 experimental animals, this is so that the experimental animals can still move and carry out their activities. The base of the cage used was husk as high as 3 cm which was replaced 1-2 times per week. While in the experimental animal cages, they were given food in the form of pellets as much as 20-25 grams/head/day and given drinking water as needed.

The acclimatized mice were then divided into 4 groups, group 1 as a negative control (K) which was only given pellets and drinking water, group 2 (P1) was given pellets, drinking water, EEDBP dose of 250mg/kgbw, group 3 (P2) given pellets, drinking water, EEDBP at a dose of 500 mg/kgbw, group 4 (P3) was given pellets, drinking water, EEDBP at a dose of 1.000 mg/kgbw. Each group consisted of 10 experimental animals of the Wistar strain consisting of 5 male experimental animals and 5 female experimental animals. The experimental animals will be given an extract using an oral probe and then 24 hours after being given the treatment, the death of the experimental animal will be observed, and the body weight will be weighed every day for 29 days, on the 29th day the blood cholesterol levels will be seen in the experimental animal. The next day the experimental animal will be dissected to weigh organs such as the liver.

The tools used in this study were rat cage measuring 30x50 cm with a height of 15 cm, beaker glass, measuring cup, gloves, digital scale, oral sonde, disposable syringe, minor surgical instrument, micropipette, maceration container, filter paper, rotary evaporator, cotton swabs, anesthetic chamber, syringe, EDTA tube, milligram scale, Semi-automatic Chemistry Analyzer were used to measure cholesterol levels along with reagents for total cholesterol, tissue, and trash bags, and CO_2 tubes.

The process of making ethanol extract of bitter melon flesh which will be used in this study is the result of processing at the School of Biological Science and Technology, Bandung Institute of Technology. Bitter melon used to have green skin comes from the Manoko plantation area, West Bandung, West Java

Indonesia.

The data obtained were analyzed by normality test using the Shapiro-Wilk test because the samples used in the study were less than 50 samples. In this study the data were normally distributed so that the analysis used the one-way ANOVA test.

RESULT

Effect of Ethanol Extract of Peria fruit(*Momordica Charantia L*) on Death of Rats

The results of the research carried out that there were no experimental animal deaths in the first 24 hours after treatment or more for up to 28 days even though ethanol extract of bitter melon flesh was given with the highest dose of 1000 mg/kgbw.

Effect of Ethanol Extract of Peria fruit(Momordica Charantia L) on Rat Body Weight

Average body weight of rats before and after administration of ethanol extract of peria fruit can be seen in Table 1.

Table 1. Average body weight of rats before and after administration of ethanol extract of peria fruit

*Anova test, p-value≤0.05 indicates a difference in meaning.
Description: K: negative control; P1: EEDBP 250 mg/kgbw; P2: EEDBP 500 mg/kgbw; P3: EEDBP 1000 mg/kgbw.

The results of changes in rat body weight between groups can be seen in Table 2.

Table 2. Changes in rat body weight between groups

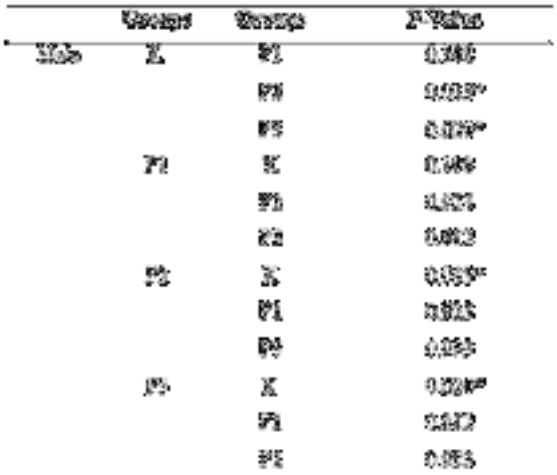
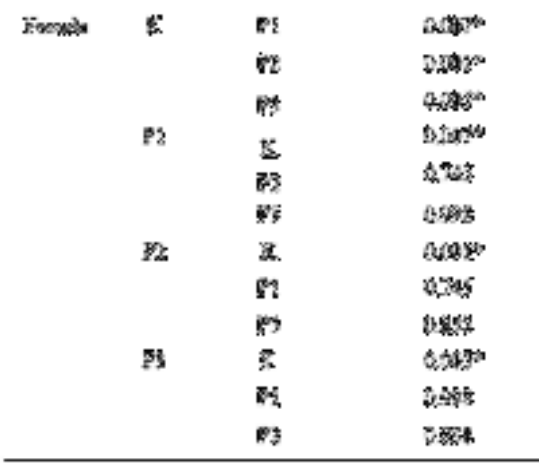


Table 2. Changes in rat body weight between groups

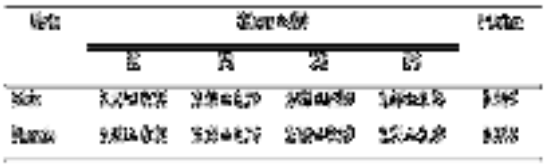


Post Hoc Tukey test *: p-value≤0.05 indicates a significant difference.
Description: K: negative control; P1: EEDBP 250 mg/kgbw; P2: EEDBP 500 mg/kgbw; P3: EEDBP 1000 mg/kgbw

Effect of Ethanol Extract of Peria Fruit(*Momordica charantia L*) on Liver Relative Weight

Effect of ethanol extract of peria fruit(*Momordica charantia l*) on the relative weight of the liver can be seen in Table 3.

Table 3. Effect of ethanol extract of peria fruit(*Momordica charantia l*) on average relative weight of rat liver

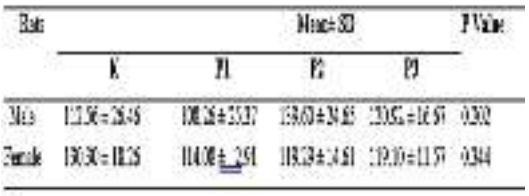


One-way ANOVA test, *: p-value≤0.05 indicates a significant difference
Description: K: negative control; P1: EEDBP 250 mg/kgbw; P2: EEDBP 500 mg/kgbw; P3: EEDBP 1000 mg/kgbw

Effect of Ethanol Extract of Peria Fruit(*Momordica charantia L*) on Cholesterol Levels

Effect of ethanol extract peria fruit(*Momordica charantia L)* on cholesterol levels can be seen in Table 4.

Table 4. Effect of ethanol extract of peria fruit(*Momordica charantia l*) on average cholesterol levels in rats



One-way ANOVA test, *: p-value≤0.05 indicates a significant difference
Description: K: negative control; P1: EEDBP 250 mg/kgbw; P2: EEDBP 500 mg/kgbw; P3: EEDBP 1000 mg/kgbw

DISCUSSION

Effect of Ethanol Extract of Peria Fruit(*Momordica charantia L*) on Rats Death

All groups of experimental animals

were measured for body weight before and after administration of ethanol extract of bitter melon flesh at a predetermined dose for 28 days to determine whether there was a decrease or increase in body weight in rats. The results of measuring the weight of the entire group can be seen in Table 1.

After administering the ethanol extract of bitter melon pulp to all rats according to the dose groups of 250 mg/kg, 500 mg/kg and 1000 mg/kg, there were no deaths observed until day 29. This is in line with Safitri's acute toxicity test research (2018) that administration of bitter melon fruit ethanol extract in acute toxicity tests with rats to the highest dose of 5000 mg/kgbw did not cause death. Sovia et al (2009) said that administration of bitter melon leaf ethanol extract in mice with the highest dose of 5000 mg/kgbw did not cause death. Bitter melon can be included in the practically non-toxic category according to the BPOM guidelines because up to a dose of 5000 mg/kg it has not caused death. However, one hour before blood collection, there was one female P1 rat found dead on day 29. This was not included in the toxic effect of bitter melon because the dose given to the group was included in the low dose. Toxic effect parameters such as rat body weight within normal limits, relative organ weight and cholesterol levels were not significantly different.[10,12, 15]

Based on BPOM guidelines, if it does not cause toxic effects at the highest dose of 1000 mg/kg, then the dose does not need to be increased again. From the results of the study, it can be concluded that there is no toxic effect and is said to be safe for drug use.[10]

Effect of Ethanol Extract of Peria Fruit(*Momordica charantia L*) on Rat Body Weight

Based on the normality test using the Shapiro Wilk Test and the homogeneity test, male and female rats were normally distributed and homogeneous ($p>0.05$). Furthermore, the results of the one-way ANOVA analysis in Table 4.1 of body weight before and after treatment there was a smaller increase in body weight at P1, P2, P3 when compared to K. Furthermore, it can be seen that the weight after treatment at P1, P2, and P3 experienced a greater increase. small compared to K and there is a significant difference seen from the p value = 0.016 in male rats and p value = 0.001 in female rats (p 0.05) or the effect of giving ethanol extract of bitter melon pulp with the above dose on changes in body weight. To find out more about the comparison between the control group and the three doses, further tests were carried out using the Post Hoc test.

Based on the results of the analysis

in Table 2 on male rats, there was a significant difference in body weight changes in groups P2 and P3 compared to group K with p=0.033 and p=0.020 values. Significant differences were also seen in female rats, namely in group P1 compared to group K (p=0.007), group P2 compared to group K (p=0.001), and group P3 compared to group K (p=0.005). The existence of this significant difference indicates the effect of giving the extract on changes in body weight of rats.

Toxic responses that affect changes in body weight can be influenced by the content in the plant, one of which is tannin compounds. Tannins have the potential as thermogenesis so that they can cause the burning of calories and fat in the body which causes weight loss. Tannins are also easy to bind to protein and cause protein on the surface of the intestine to settle, thereby reducing food absorption and causing weight loss. This is in line with the research of Hidayat et al (2015) that the combination of Detam soybean extract at a dose of 10 mg/kg and Dutch teak leaves (*Guazuma ulmifolia Lamk*) at a dose of 20 mg/kg containing tannin compounds such as bitter melon was effective in reducing body weight in mice. Peria fruit can lose weight because it can increase the activity of adenosine 5 monophosphate kinase which will facilitate glucose absorption, glucose oxidation will be converted into glycogen. This glycogen will be stored in the muscles, during a lack of energy, fat will be used continuously so that there is an increase in fatty acid oxidation which can lead to weight loss.[16,17]

Effect of Ethanol Extract of Peria fruit(*Momordica charantia L*) on Liver Relative Weight

In addition to weighing, all groups of rats were dissected on the 29th day for observation and weighing of rat organs and then the relative organ weight was measured. The organ measured is the liver. Based on the results of statistical processing of the normality test using the Shapiro Wilk test and homogeneity test, the overall rat organ weight data was normally distributed and homogeneous (p>0.05). To find out whether there was a difference in the relative organ weight of each group, a one-way ANOVA test was performed.

The results of the study can be seen in Table 3.

Based on Table 3, the average relative organ weight of the liver P1, P2, P3 showed an increase when compared to K but there was no significant difference seen from the value of p=0.365 in male rats and p=0.848 in female rats (p≤0.05). or the absence of the effect of giving the ethanol extract of bitter melon pulp with the above dose on

changes in the relative weight of the liver. So it can be said that there is no toxic effect of bitter melon on the liver.

Some plants can affect the weight of organs, especially the liver, because the liver is the main metabolic site that will detoxify and eliminate all toxins, both endogenous and exogenous. Toxic response in the liver can be indicated by the presence of cell degeneration, necrosis and fibrosis. The response of organs to toxic substances has been confirmed by research by Setia et al (2014) which showed that histopathological observation of giving 4.2 mg/20gbb of Ambon banana stem extract to mice which contained the same compounds as bitter melon, namely, flavonoids, tannins and saponins can affect the tissue structure in organs, especially the liver. Liver lesions show inflammation around the portal and central veins, fatty degeneration, and hepatocyte cell necrosis.[18]

Effect of Ethanol Extract of Peria Fruit(*Momordica Charantia L*) on Cholesterol Levels

In addition, the body weight was weighed, the whole group of rats were drawn blood on the 29th day for blood collection and then blood cholesterol was measured in the rats.

Based on the results of statistical processing of the normality test using the Shapiro Wilk Test and homogeneity test, the overall organ weight data of mice was normally distributed and homogeneous ($p>0.05$) can be seen in Appendix 6. To analyze whether there are differences in cholesterol levels in each group, a one-way ANOVA test was performed. The results of the study can be seen in Table 4.

Based on Table 4, the average total cholesterol levels of P1 in males and P1, P2, and P3 in females decreased compared to K. But there was no significant difference seen from the value of $p=0.202$ in male rats and $p=0.516$ in female rats. ($p>0.05$) or the absence of the effect of giving the ethanol extract of bitter melon flesh with the above dose on changes in total cholesterol levels in rats.

Some plants can affect liver metabolism, one of the substances metabolized by the liver is cholesterol. Liver damage can cause cholesterol metabolism disorders, so that cholesterol levels in the blood decrease. A damaged liver will cause an increase in insulin in the body so that there is an increase in the use of glucose in the body and reduce the use of fat. Ilahana's research (2016) confirmed that the administration of an ethanol extract of a combination of celery herb extract, cat whiskers leaf extract and noni fruit extract containing the same compounds as bitter melon, namely, flavonoids, tannins and

saponins at a dose of 1093.5 mg/kg body weight in male rats showed there is a decrease in levels cholesterol.[19]

CONCLUSION

Based on research that has been done, administration of ethanol extract of bitter melon (*Momordica charantia L*) at doses of 250 mg/kg, 500 mg/kg, and 1000 mg/kg did not cause death in rats and had no significant effect on weight loss. relative weight liver and cholesterol levels.

Further research needs to be carried out histopathological examination of the liver to see whether or not there are changes in the structure of the liver organ and SGOT and SGPT examination to see whether or not there is damage to the liver in terms of other functions.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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