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The Hepatoprotective Effects of Basil Leaf (*Ocimum sanctum L.*) Extract on Paracetamol Induced Liver Damage in Male Rat

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ABSTRACT

Basil leaf have antioxidants such as flavonoids, so it is thought to have a hepatoprotective effect. This study aims to investigate the effect of basil leaf extract on SGOT and SGPT levels in male rats induced by paracetamol. Basil leaf extract was carried out by the percolation method using ethyl acetate solvent, Some 20 male sprague dawley rats were randomly divided into 5 groups. Basil leaf extract (400 mg/kgBB and 600 mg/kgBB) and sylimarin (100 mg/kgBB) were carried out every day for 28 days, paracetamol was induced 24 hours after giving the last day of basil leaf extract. The parameters measured were SGOT and SGPT level to assess the effect of basil leaf extract on liver damage caused by paracetamol. The results showed that basil leaf extract (400 mg/kgBB dan 600 mg/kgBB) showed that the activities of SGOT and SGPT levels were statistically significant ($p<0,05$) to negative control. Basil leaf extract shows the effect of hepatoprotector on liver induced by paracetamol, however the effect given was not able to equate with positive control.

1. Introduction

The liver is one of the organs that is responsible for many functions, one of which is to process the metabolism of foreign materials in the body. Liver disease in Indonesia has a high prevalence. Liver damage occurs due to the presence of free radicals. Free radicals come from the environment such as UV radiation, pepticides, toxic chemicals, air pollution and cigarette smoke or due to excessive metabolism.[1]

Inflammation of the liver or known as hepatitis is caused by several factors such as viruses, bacteria, parasites, drugs, alcohol, worms, or malnutrition.[2] Hepatitis is a disease that has become a public health problem which affects the morbidity rate, life expectancy, mortality rate, public health status and has an impact on other socioeconomic conditions.[3]

Paracetamol can cause liver damage due to the use of drugs with inappropriate doses or at high doses. The use of paracetamol is widely known by the public as an analgesic and antipyretic which is widely sold in the market. At certain doses, paracetamol can cause hepatotoxicity and acute kidney.[4] Paracetamol is safe to consume at a dose of 4 grams / day, but its therapeutic effect is different when it is consumed more than 4 grams / day which results in severe hepatotoxies.[5]

Medicinal plants are scientifically studied and researched, the results prove and support the fact that

medicinal plants actually contain compounds and substances that are clinically proven to be beneficial to health[6]. Basil leaves are an example of a traditional plant that contains flavonoids as natural antioxidants that act as an antidote to free radicals[7]. Basil leaves have antioxidant compounds, namely phenolic compounds (flavonoids, phenolic acids, tocopherols), nitrogen compounds (chlorophyll derivatives, alkaloids, amines, and amino acids), and beta caratone.[8] Hydroxy free radicals (OH) can be prevented by flavonoids and can play a role as a donor so that it does not oxidize, protein, fat, and DNA, in cells that can result in death.[9] Results of research conducted by Adam et al (2018). states that basil leaves can affect the decrease in serum AST and ALT levels in mice injected with uric acid.

Parameters used to identify liver damage were serum SGOT, SGPT, Gamma GT, total protein including serum albumin, alkaline phostase. Measuring the levels of Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Piruvik Transaminase (SGPT) is one way to identify liver damage with a liver physiological test which aims to determine disorders that occur in the body and indications of liver disorders.[10] ALT levels in blood can increased in liver damage caused by paracetamol. [11]

Based on the above background, the author intends to carry out a study that aims to determine whether the basil leaf extract given to white mice induced by paracetamol affects the levels of SGOT (Serum Glutamic Oxaloacetic Transaminase) and SGPT (Serum Glutamic Piruvik Transaminase).

2. Method

Tool

The tools used in this research include standard animal cages, feeding containers, drinking containers, syringes, beaker glass, percolators, UV-Vis rotary evaporator, erlenmeyer, handscoon, test tubes, test tube racks, micro pipettes, yellow tip and blue tip.

Material

The materials used in this study include male rats, special rat feed, mineral water, husks, basil leaves, ethyl acetate, paracetamol, basil leaf extract, 0.5% Na CMC, sylarin, alcohol swab, ether, and inj. Ketamine HCl.

Sample preparation

In this basil plant research, the part taken is the leaves. Drying the leaves is done by being aerated without exposure to sunlight or in an oven at a temperature of 20 °C. After the leaves are dry then mashed in a blender then sifted into a simplicia powder.

Basil leaf extraction

The extraction method used in this research is percolation which is carried out by slowly flowing the solvent into a percolator. In the initial stage of percolation, 500 grams of dried basil leaf powder were taken and then immersed in 5 liters of ethyl acetate as a solvent. Before the extraction process is carried out, the sample is immersed for at least 1 hour in a closed vessel then goes into the extraction process on the percolator for 2 days until the liquid dripping from the percolator is clear white and produces a liquid extract. The liquid extract will be vacuum distilled with the help of a rotary evaporator in a temperature of 30-40 °C and a thick extract is produced.[12]

Basil leaf phytochemical screening

1. Test flavonoids

   The filtrate is made by dissolving 1 g of the extract in 100 ml of hot distilled water, then adding 5 ml of the test solution with Mg powder, 2 ml of ethanol and HCl. The addition of Mg and HCl in this test will reduce the flavonoid compounds present, causing a red, orange or yellow reaction.[13]
2. Alkaloid test

   The 10 mg sample extract was added with 5 ml of 25% NH3 crushed, then 20 ml CHCl3 was added. 10 ml of filtar was extracted with 10 ml of 1: 10 HCl solution then dropped on filter paper and dripped with dragendroff reagent if it was red or orange and the addition of mayer reagent to the filtrate would

produce a white precipitate indicating the presence of alkaloid compounds.[13]

3. Saponin test

   10 ml of the solution obtained from the identification of flavonoids was put into a test tube and shaken quickly. On examination of the saponin content, a positive test was shown by the formation of foam and was stable for 10 minutes[13]

4. Test the tannins

   10 ml of the solution obtained from the identification of flavonoids was inserted into the test tube. Then filter and add 1 ml of 10% FeCl3 until the color changes. The examination of the positive test for tannin content is indicated by the appearance of a blackish blue or blackish green color[13]

Assignment Test

1. Flavonoids

Maintain 1000 ppm main solution, weigh 100 mg of quercetin and dissolve it with ethanol to 100 mL. Quercetin dilution was made with a concentration of 6, 8, 10, 12, 14 ppm. Pipette 1 mL of quercetin standard solution at each concentration series. Add 0.2 mL of 10% $AlCl_3$ mL and add 0.2 mL of potassium acetate add 10 mL of aquadest. After that it was incubated for 30 minutes at room temperature and measured the absorbance on UV-Vis spectrophotometry with the maximum wavelength. The basil leaf extract sample was weighed 10 mg dissolved in 10 mL 96% ethanol. Take 1 mL of the test sample, add 0.2 mL of 10% $AlCl_3$, and add 0.2 mL of potassium acetate add 10 mL of aquadest. After that it was incubated for 30 minutes at room temperature and measured the absorbance at the maximum wavelength. Total flavonoids were calculated using the linear regression equation from the previously measured quercetin calibration curve.[14]

2. Tannins

Made a standard solution of 1000 ppm gallic acid, weigh 100 mg gallic acid and dissolve it with ethanol up to 100 mL. Gallic acid dilution was made with a concentration of 10, 20, 30, 40, 50 ppm. Gallic acid solution was pipette 1 mL and added 1 mL of Folin Ciocalteau reagent, then shaken and let stand for 3 minutes. To the solution was added 4 mL of 7.5% Na_2CO_3, shaken until homogeneous, and the absorbance was measured at a wavelength of 600-800 nm. Weigh 20 mg of extract and dissolve it with 10 mL of p.a ethanol, then add 1 ml of pipette with 0.4 mL of Folin-Ciocalteau reagent, shake it and leave it for 4-8 minutes, then add 4.0 mL of 7% Na_2CO_3 solution, shake until homogeneous. Add distilled water to 10 mL and let stand for 2 hours at room temperature. Measure the absorption at a maximum absorption wavelength of 630 nm. Do 3 repetitions.[14]

Treatment of experimental animals

There are 25 experimental animals in this research which will be grouped into 5 groups and each group contains 5 animals. The rats were caged with the husk mat in groups, the standard food given to the rats was 20 g / head / day with drinking water ad libitum. Before the experimental process was carried out, it was adapted for 7 days, this was done so that the diet and way of life between mice were the same, and could adapt to the environment in their cage. During the study, the five groups of mice were given standard feed.[15]

The grouping of experimental animals was divided into 5 different treatment groups, as follows:

1. Rats in group one were the normal group (K0) which were given standard feed during the study and were given 0.5% Na CMC on day 1 to day 28.
2. Group II rats were the negative control group (KN) which were given 0.5% Na CMC on day 1 to day 28 and were given paracetamol dose 2000 mg / kgBB on day 29 without giving basil leaf extract.
3. Tikus kelompok III merupakan kelompok kontrol positif (KP) yang diberi jamu Silymarin pada dosis 100 mg/kgBB di hari ke-1 sampai hari ke-28 dan diberi parasetamol dosis 2000 mg/kgBB pada hari ke-29.
4. Rats in group IV were the first test group (KU1) which were given basil leaf extract 400 mg / kgBW on day 1 to day 28 and were given paracetamol dose 2000 mg / kgBB on day 29

5. Group V mice were the second test group (KU2) which were given basil leaf extract 600 mg / kgBW on day 1 to day 28 and given paracetamol dose 2000 mg / kgBB on day 29.

Blood sampling

Blood samples were taken via the retro orbital plexus vein on day 31 (after 48 hours of the last treatment). The orbital sinuses of the eyes of the test animals on the 31st day were collected into the eppendorf tube and centrifuged for 10-15 minutes at 3000 rpm, using a vacutainer tube containing serum separators. In the process of measuring SGPT and SGOT, it is carried out in the Pertamina Bintang Amin Hospital laboratory.

Data collection

In this study, data collection was carried out by calculating the average results of SGOT and SGPT levels in serum, expressed as UI / L data in each research group.

Data analysis

To determine the effect of basil leaf extract (Ocimum sanctum L.) on SGOT and SGPT levels in rats, the results of the data obtained from the measurement of SGOT and SGPT levels were compared using the ANOVA (Analysis of Variance) test with a significant value at P <0, 05. The test begins with the normality distribution test with the saphiro wik test, variance homogeneity and continues to the ANOVA and Post Hoc Multiple Comparation LSD tests.

3. Results and Discussion

Result of determination

Basil leaf samples (Ocimum sanctum L.) were obtained from the Sumberejo area, which have been determined in the Laboratory of FMIPA, University of Lampung. Based on the letter of determination, it states that the determination of basil leaves is in accordance with the literature, so the plant sample used as the test material in this study is Basil leaves (Ocimum sanctum L.).

Basil leaf extraction results

A total of 500 grams of basil leaf powder was extracted by percolation with ethyl acetate. The filtrate obtained is dried with a rotary evaporator at a temperature of 30-40 ° C in order to obtain a thick extract. Viscous extraction obtained after evaporation was carried out at the FMIPA Laboratory of the University of Lampung to remove the remaining solvent used. So that the extract was obtained as much as 32 grams with a percentage yield of 6.8%.

Phytochemical screening of basil leaf extract

Identification of the chemical content of basil leaves (Ocimum sanctum L.) was carried out to determine the content of active metabolites extracted by the solvent used. The results of the identification of chemical contents can be seen in table 1.

Determination of basil leaf extract content

Determination of secondary metabolite levels in basil leaves (Ocimum sanctum L.) was carried out to determine the amount of flavonoid and tannin compounds extracted by the solvent used. The results of the assay can be seen in table 2.

Results of measurement of blood serum SGOT and SGPT levels

The results of measuring the levels of SGOT and SGPT in blood serum in rats, namely in the normal control group (N0), the negative control group (KN), the positive control group (KP), the test group 1 400 mg / kgBW (KU1) and the test group 2 600 mg / kgBW (KU2) can be seen in table 3.

Experiments that have been carried out show that the results of SGPT and SGOT levels in the negative group show high scores compared to other groups after treatment and in the positive group shows low scores compared to other groups after treatment.

The results of SGOT and SGPT measurement data performed One Way ANOVA with a significant level of

0.05 indicated that the normal control group, positive control, negative control, test group 1 (400 mg / kgBB) and test group 2 (600 mg / kgBB) obtained significant values. <0.005, this indicates that there are significant differences between treatment groups in both parameters.

The results of the treatment group seen from the results of the LSD post hoct test showed that the normal group, the positive group, the test group 1 and the test group 2 had differences in the negative group. In test groups 1 and 2 with doses of 400 mg / kgBW and 600 mg / kgBW showed low values compared to negative controls who were only given 0.5% Na-CMC and induced paracetamol during the study. This shows that the administration of basil leaf extract can prevent damage to the liver caused by induction of paracetamol doses of 2000 mg / kgBW. In the second test group with a dose of 600 mg / kgBW was better at preventing paracetamol damage compared to the 400 mg / kgBW dose, the higher the dose used increased the ability of basil leaf extract to prevent liver damage due to induction of paracetamol. However, in this study the positive control group still had a better effect on reducing SGOT and SGPT levels, so it is necessary to increase the dosage of basil leaf extract or increase the time of administration so that the resulting effect is better.

Paracetamol-induced hepatotoxicity is a widely used model for the hepatoprotective screening of plant / medicinal extract activity. In this study, a significant increase in SGOT and SGPT in serum was observed after paracetamol administration. This marker enzyme originates in the cytoplasm and is released into the circulation after cell damage[16] The increase in the AST enzyme is usually accompanied by an increase in the SGPT level, which plays an important role in the conversion of amino acids to keto acids. Leakage of large amounts of enzymes into the bloodstream is associated with centrilobular necrosis and balloon degeneration of the liver. However, the increase in the levels of this enzyme was significantly decreased by treatment with basil leaf extract (Ocimum sanctum L.) which indicates that basil leaf extract is able to prevent liver damage.

Table 1. Results of basil leaf extract phytochemical screening

Identification	**Observation result**	**Information**
Flavonoids	A red solution is formed	(+) Positive
Alkaloids	White deposits are formed	(+) Positive
Saponins	Forms foam and is stable for 5 minutes	(+) Positive
Tannins	A turquoise color solution is formed	(+) Positive

Table 2. Results of determination of secondary metabolite levels on basil leaves

Grading	**Recurrence**	**Absorbance**	**Concentration (ppm)**	**Content (%)**	**Average Levels (%)**
Flavonoids	1	0.383	6.127	6.127	6.229
	2	0.387	6.209	6.209	
	3	0.394	6.353	6.353	
Tannins	1	0.352	48.96	24.48	23.87
	2	0.338	46.84	23.42	
	3	0.342	47.45	23.72	

Table 3. Average blood serum SGOT and SGPT levels

Group of Rats	Average SGPT and SGOT U/L	
	SGOT (s.d)	SGPT (s.d)
K0	73.7750 ± 16.03151	27.9250 ± 6.86895
KN	128.9250 ± 15.24366	66.0750 ± 8.62028
KP	76.7750 ± 2.88141	27.0250 ± 4.59882
KU1	87.6750 ± 8.46458	38.2000 ± 12.70722
KU2	81.3000 ± 7.51931	31.1500 ± 5.34197

4. Conclusion

From the results of the research on the hepatoprotective activity test of the administration of basil leaf extract (Ocimum sanctum L.) on the enzyme activity of SGPT and SGOT in male white rats induced by paracetamol at a dose of 2000mg / kgBW, it was concluded that the administration of basil leaf extract (Ocimum sanctum L.) at a dose of 400 mg / kgBW and 600 mg / kgBW showed that the SGPT and SGOT activities were statistically significant ($p \leq 0.05$) against the negative control. Basil leaf extract at a dose of 600 mg / kgBW is a dose that is more effective in preventing liver damage when compared to a dose of 400 mg / kgBW.

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