

Effect of Basil Leaves (*Ocimum sanctum* L.) Infusion as Hepatoprotective Agent Induced by Paracetamol

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Abstract

Indonesia has biodiversity potential to be developed as medicinal plants, such as basil leaves (*Ocimum sanctum* L.). Basil was reported to have a very high antioxidant activity in vitro. The aim of this study was to determine the effect of basil leaves (*Ocimum sanctum* L.) infusion to liver based Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) value in each dose. 18 rats were divided into 6 groups., control group, negative control group treated with CMC Na 0.5%, positive control group treated with Curcuma 3.6 mg/200gBB, groups of 4-6 consecutive given a 80 mg dose infusion basil/200 gBB, 160 mg/200 gBB, 320 mg/200 gBB for 8 consecutive days, on the day of the 4th and 8th all treatment groups induced by toxic doses of paracetamol (500 mg/200 gBB) except the normal control group I, The research data in the form of enzyme activity of AST and ALT were analyzed using parametric and nonparametric ANOVA, and Friedman test with the level of trust then followed by SNK test and Bnj test. The statistical test result with a 95% of level of trust that shown basil infuse with a dose of 80mg/200gBB, 160mg/200gBB, 320mg/200gBB have hepatoprotective effects in rats induced by paracetamol 500mg/200gBB. Based on the result of changes in average levels of AST on the fourth day and the eighth day of the three treatment infusion, infusion at a dose of 160 mg/200gBB most effectively reduce average levels of AST and a group that has the average AST closest to the control group is positive, but infusion at a dose of 320mg/200gBB the group that has the closest average ALT positive control group.

Keywords : hepatoprotective, *Ocimum sanctum* L, Paracetamol

INTRODUCTION

According to Minister of Health, traditional medicine is the treatment and/or care in a way, drugs and treatments that refers to the experience, skills hereditary, and/or education/training, and applied in accordance with the norms prevailing in society. Traditional medicine is the ingredient in the form of plant material, animal material, mineral materials, galenic, or a mixture of these materials which has been used for treatment based on experience (Regulation of the Minister of Health of the Republic of Indonesia No. 003 of 2010).

Traditional medicine was divided into jamu, standardized herbal medicine, and phytopharmaca. Jamu is crude drug and manufactured by very simple manner, such as boiling or brewed with hot water. Jamu has been used traditionally, based on experience, and does not need quality control assurance.

Standardized herbal medicine used extract as basic ingredient. Standardized herbal medicine have to meet quality, efficacy, and safety requirement, such as preclinical pharmacology test and chemical content (Moelyono, 2007).

Indonesia have biodiversity potential to be developed as a medicinal plant, such as basil (*Ocimum sanctum* L.). *O.sanctum* often used for the treatment and also consumed as a salad. *O.sanctum* is widely used as a cough, runny nose, fever, skin medications, drug hepatitis and kidney stones remedy. *O.sanctum*. Flavonoid contained in *O.sanctum* have been studied widely and have any activity.

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Flavonoids act as antioxidants that inhibit blood clotting stimulates the production of nitric oxide leading to relaxation of blood vessels, and inhibits cancer cells growth. Flavonoids also have some activity such as hepatoprotective, antithrombotic, anti-inflammatory and antiviral. Flavonoids could interact with metal and scavenged free radicals, such as superoxide ion and lipid peroxy radical (Winarsi, 2007). Based on previous research by Ikhlas (2013) *O.sanctum* extracts categorized as a very strong antioxidant activity with IC₅₀ of 21.8989 ppm with AAI (antioxidant activity index) of 1.8006.

O.sanctum leaves have been used to treat fever, cough, runny nose, liver (liver dysfunction), and increase breast milk production. Moreover, *O.sanctum* also have various effects on organisms such as bacteria, fungi, and viruses. Among treat liver dysfunction. This present study aimed to explore scientific fact regarding the effectiveness of *O.sanctum* leaves infusion to treat liver function disorders, especially hepatitis.

MATERIALS AND METHODS

Instrument

The instrument used in this study were: syringe 3mL, 5mL and hose NGT No. 14; analytical balance (Kern, type EW 220-3NM), thermometer (BOECO), UV-Vis spectrophotometer (Shimadzu type W-1700); centrifuge; micropipette.

Materials

Materials used in this study were basil leaves, distilled water, female mice blood, Paracetamol, Na.CMC, Curcuma (Soho), ether, 2N HCl, FeCl₃.

Extraction

8g *O.sanctum* leaves were washed, added with 100mL distilled water and heated. After the temperature reaches 90°C, the solution was stirred occasionally for 15 minutes. Then filtered while hot and added distilled water to obtain volume of 100 mL. Basil leaves infusion (BLI) was kept in a referigerator at 4°C until used.

Phytochemicals Screening

Alkaloids

A few drops of the sample was added with 1 mL of HCl 2N, then heated for 2 minutes, and filtered. Three drops of the filtrate then transferred into a test tube and add 2 drops bouchardat reagent. The possitive result could be found by precipitates (Anonymous, 1995).

Flavonoids

A few drops of the sample was added with 2 mL of 95% ethanol, 0.5 g of zinc powder P, and 2 mL of 2N HCl, and incubated for 1 minute. The solution then added with 10 drops of concentrated HCl. Flavonoid was observed by orange-red color (Anonymous, 1995).

Saponin

1 mL of the were diluted with 10mL of water and shake vigorously for 10 minutes. Foam was observed which is as high as 1 cm to 10 cm and stable for 10 minutes. Positive result could be determined by addition of HCL 2N. Saponin formed stable foam after addition of HCl (Anonymous, 1995).

Tanin

A few drops of the sample was added with 3 drops of FeCl₃. Green to blue color formed were indicating the presence of tannins (Harborne, 1987) (Table 1).

Experimental Animals

Animals used were female rats, 2-3-month-old, 100-250 grams. 18 rats were divided into six groups. Rats were adapted for 7 days, feeding with food and drink ad libitum. Those groups were group I as a normal group (only 0.5% CMC-Na), group II as a negative control group (CMC-Na 0.5% + Paracetamol), group III as a positive control (Curcuma® + Paracetamol), group IV to VI as treatment group (infuse basil + Paracetamol).

Hepatoprotective Activity Assay

Group I and II were treated with Na CMC 0.5% as a normal group and negative control group. Group III as a positive control, was treated with Curcuma® at the dose of 3.6 mg/200g BW rats. Groups IV to VI were with basil leaves infusion 80mg/200g BW, 160mg/200g BW and 360mg/200g BW mice respectively. The treatment for each groups were given orally for 8 days. Paracetamol were given on day 4 to 8, except for group I. AST and ALT levels were measured on day 4 and 8.

Table 1. Phytochemical Screening of Basil Leaves Infusion

Compound	Qualitative Test	Bibliography	Conclusion
Alkaloids	No sedimentation	In case it contains alkaloids (Anonymous, 1995)	-
Flavonoid	Red colour	formed red color orange intensive shows flavonoids (Anonymous, 1995)	+
Saponins	foam does not disappear for 10 minutes	foam does not disappear, show the saponins (Anonymous, 1995).	+
Tanin	green to blue	green to blue color is formed, showed the presence of tannins (Harborne, 1987).	+

(Source: Primary Data Research 2015)

Description:

+ : Contains

- : Does not contain

Venous blood sampling was done through the eyes (retro-orbital plexus) using a capillary tube. Blood was collected on test tube (centrifuge tubes) and allowed to stand for 15 minutes then centrifuged at 3000 rpm for 15 minutes. Therefore, blood cells were precipitated and separated from the plasma (above deposition).

The Doses of basil infusion used in this study were 80mg/200gBB, 160mg/200gBB, and 320mg/200gBB respectively. Those variations aim to determine the potential dose of basil leaves infusion to reduce alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzyme activity after induction of paracetamol. ALT is an enzyme widely found in the liver because it is produced in the liver (hepatocytes), whereas AST is an enzyme with high metabolism rate and found in the heart, liver, muscle, spleen, pancreas and lungs.

RESULTS AND DISCUSSIONS

The results showed ALT and AST activity decreased after rats were induced with paracetamol on day 4 compared with normal levels. AST level was less specific in liver function tests compared the levels of ALT because the AST enzyme produced not only in the liver in the kidneys, but also muscles,

skeleton, brain, and heart. Meanwhile, the enzyme ALT is only produced in the liver. Results of AST levels were taken on day 4 (T4), and day 8 (T8) as shown in Table 2.

The average of AST level in rats treated with basil infusion was changed at T4 to T8. Based on the result, infusion at a dose of 160mg/200gBB can lower the average of AST level and a group that has an average of AST near to the control group positive. The group which similar to the positive control group was a group that has the best protection. From the results of further SNK and HSD test showed the treatment group infuse basil dose of 80mg/200gBB, 160mg/200gBB dan 320mg/200gBB have activity of hepatoprotective by blocking increased levels of the enzyme AST after paracetamol induced at day 4 and decreased levels of the enzyme AST after administration of infusion simultaneously with paracetamol from day 4 to day 8 in rats compared with positive group.

Kamajaya (2006) states that the result of careful measurement is obtained by choosing a measuring instrument and measurement proper way. Measuring instrument imperfections can cause measurement errors. Some aspects of measuring instruments that must be considered include the aspect of accuracy, calibration aspects, aspects of accuracy and sensitivity aspects.

Table 2. Average examination AST in rats

Group & Treatment	Average AST (IU/L)±SD		
	at T ₄	at T ₈	Δ T ₄ - T ₈
Normal (only CMC 0.5%)	152.8±26.510	111.7±10.072	41.1±16.438
Negative Control (CMC 0.5% + Paracetamol)	184.0±13.180	149.0±7.750	35±5.43
Positive Control(Curcuma + Paracetamol)	113.1±3.360	118.4±1.750	5.3±1.61
Treatment I (BLI 80 mg / 200gBB + Paracetamol)	140.8±24.645	124.4±14.446	16.3±10.509
Treatment II (Infusa dose 160 mg + Paracetamol)	122.8±11.873	116.4±4.331	8.6±7.796
Treatment III (Infusa dose 320 mg + Paracetamol)	139.0 ±8.063	127.1±1.154	11.9±6.909

Table 3. Average examination results ALT In Rats

Group & Treatment	Average ALT (IU/L)±SD		
	At T ₄	At T ₈	Δ T ₄ - T ₈
Normal (only CMC 0.5%)	57.8±2.302	57.9±9.702	0.1±0.161
Negative Control (CMC 0.5% + Paracetamol)	67.8±2.274	68.2±2.463	0.4±0.979
Positive Control(Curcuma + Paracetamol)	47.0±4.215	46.8±3.253	0.2±-4.416
Treatment I (Infusa dose 80 mg + Paracetamol)	53.2±8.707	52.8±8.631	0.4±4.199
Treatment II (Infusa dose 160 mg + Paracetamol)	53.0±7.451	47.8±4.508	5.2±5.705
Treatment III (Infusa dose 320 mg + Paracetamol)	45.4±2.100	44,93±1.746	0.47±2.1

Levels of ALT is a more specific indicator of liver function tests compared to the levels of AST. ALT enzyme was only produced in the liver, while the enzymes AST is also produced in the kidneys, muscles, skeleton, brain and heart not only in the liver. ALT level was measured on day 4 (T₄), and day 8 (T₈) and shown in Table 3.

In Table 3, the average of ALT examination in mice treated infuse was decreased from T₄ to T₈ in the positive group and group treatments I-III, while the normal group and negative groups were increased from T₄ to T₈. Results of change in average levels of ALT on day 4 and 8 of the three treatments infusion, infusion at a dose of 160mg/200g BW decreased levels of ALT. Meanwhile, infusion at a dose of 320m/200g BW has a flat ALT level. Decreased levels of ALT occurred in the group control positive and three treatment groups had not significant. Based on SNK test and HSD, the treatment group of basil infusion at the dose of 80mg/200g BW, 160mg/200g BW and 320mg/200g BW have hepatoprotective activity by inhibit levels of the enzyme ALT after paracetamol induced at day 4 and decreased ALT enzyme levels after administration of infusion simultaneously with paracetamol day 4 until day 8 in mice compared with positive group. Basil infusion group, positive control group and normal group had differences result with the negative control group.

The treatment group with a subset value for alpha = 0.05 (mean) closest to the positive group is a group that has the most excellent inhibition of the normal approach is the treatment group III infusion 320mg/200gBB.

Alteration of ALT and AST enzymes level due to flavonoids content in basil leaves. The flavonoids have antioxidant activity that inhibits oxidation thus protecting the lipid membrane from damage due to the oxidation reaction. The antioxidant activity of flavonoids

in basil leaves infusion reduced the level of enzymes ALT and AST of paracetamol-induced hepatotoxicity in rats.

CONCLUSION

Based on the result is the average value of aspartate AST were not normally distributed, but there was a decrease in the levels of the treatment group infuse basil and average values ALT normally distributed and there are decreased levels in the treatment group infuse basil leaves. It is concluded that there is significant infusion administration basil leaves to rat liver after induced by paracetamol based on the value of aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

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