The Acute Toxicity Test of Methanolic Extract of Hyptis pectinata Poit on Liver Balb/c Mice

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The Acute Toxicity Test of Methanolic Extract of *Hyptis pectinata* Poit on Liver Balb/c Mice

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Abstract. Plants from Lamiaceae family has almost entirely reported having physiological activities. One of them is *Hyptis pectinata* Poit plant. Research on the toxicity of *Hyptis pectinata* needs to be done to protect people from the possibility of its harmful effects. This study aim to know the acute toxicity effects of *Hyptis pectinata* extract (HPE) on liver of Balb/c mice. This research was a laboratory experimental study using the post test only controlled group design. Balb/c mice were randomly divided into 4 groups. K (control group), P1, P2 and P3 (treatment groups; given HPE 200mg/kgBW, 1000 mg/kgBW, and 5000 mg/kgBW, respectively). The extract was orally given with gastric tube on the first day and the mice were terminated at the 8th day then the livers were observed. The Kruskal-Wallis test for macroscopic morphological and volume of the liver showed there were no significant difference with p=0.406 and p=0.054. The highest liver histopathological score was in P3 group. The Kruskal-Wallis test showed significantly difference (p=0.000). Continued with Mann-Withney test that showed a significant difference in K-P1 (p=0.009), K-P2 (p=0.009), K-P3 (p=0.009), P1-P2 (p=0.028), and P1-P3 (p=0.009). In conclusion, the HPE is safe to use which has no complication with liver of mice.

1. Introduction

There are at least 295 genera and 7775 species of plants from Lamiaceae family and most widely found in Mexico, Senegal, Brazil, Spain and in Asia such as India, Malaysia and Indonesia. Some species of this genus of plants, namely *Hyptis pectinata* Poit, *Hyptis suaveolens*, *Hyptis oblingofolia*, *Hyptis tumentosa*, *Hyptis verticillata*, *Hyptis capitata*, *Hyptis brevives* Poit, *Hyptis fruktikosa* and *Hyptis urticoide*, almost entirely reported to have physiological activities. The activities shown by this plant: anti-inflammatory, antipyretic, cancer [1, 2], ease breathing, skin diseases [3], disorders of the stomach, fever [4], and infections by bacteria [5].

In India, *Hyptis pectinata* Poit leaves are made in the form of tea which used as a beverage prevent abdominal pain [2]. Water extract of this plant has also been reported to increase liver regeneration [6].

Currently, in society the issue back to nature is growing and thus influence use of traditional medicine tends to increase. Meanwhile, many people assume that the use of herbs or traditional medicines are relatively safer than synthetic drugs. However it does not mean herbs or traditional

medicines do not have any adverse side effects when the use is not in the right path. The accuracy concerns to the right dose, the method and time of usage and also the selection of appropriate ingredients in accordance to indications of its use. Traditional medicine is usually used to maintain health, prevent disease, cure disease and restore health.

Research on the toxicity of *Hyptis pectinata* need to be done to protect people from the possibility of its harmful effects. Toxic effects of drugs are often seen in the liver because the liver has a central role in metabolizing a substance that goes into our bodies. The liver will change the structure of drugs that are lipophilic to be hydrophilic thus can easily removed from the body through urine or bile. Biliary excretion of a substance allows the accumulation of xenobiotics in the liver, causing hepatotoxic effects. Research on the toxicity and adverse reactions of *Hyptis pectinata*, both the single and multi herbal usage, has not been done. This makes the knowledge of the therapeutic dose and toxic dose of *Hyptis pectinata* herbal, especially to the liver, becomes ambiguous. Therefore, this study purpose to determine the safety and effects of this plant extract acute toxicity to the liver as the organ that functions in drug metabolism.

2. Material and method

Materials: *Hyptis pectinata* Poit plants, Liver materials tissue in mice for histopathological examination routine, standard feed mice, aquadest, buffered formalin. Additional material for immunohistochemical staining. Equipment: Tool making preparations for histological, mice cages, stomach probes, minor surgical tools, beakers, microscopes and digital cameras.

2.1. Colection anfd extraction of the plant

Hyptis pectinata poit plants was obtained from the village Kanayakan East Dago, Bandung. The specimens were stored at the herbarium of Department of Biology, Faculty of Science and Mathematica, Diponegoro University, Semarang. *Hyptis pectinata* leaves (1 kg) was dried under the sun for 8 days, and obtained 650g of crude simplicia (62%). The dried material result was then determined the moisture content by using the method of distillation (moisture content 7.6%) and smoothed by squeezing to obtain a powder. Furthermore, macerated using methanol (3x24 hours), then filtered and the solvent is evaporated using vacuum rotary evaporator (Buchi) until obtained thick extract (HPE) and ready for the next study.

2.2. Animal

Balb/c male mice (weight 25-35g) were obtained from Integrated Research and Testing Laboratory (LPPT) Unit IV, Gadjah Mada University. All the animals were maintained under standard condition, with temperature at 25±2 0C, 12 h dark-light cycle and relative humidity. The rat received food and water ad libitum. This study was approved by the Ethics Committee for Health Research and Medical University School of Medicine and Kariadi Hospital Semarang, Indonesia (Medical Letter No: 611/EC/FK/RSDK).

2.3. Acute toxicity study

This study uses a post-test only controlled group design using adult Balb/c mice males (age 2-3 months), in health physical condition and not disabled. The study was conducted in May-July 2016 in microanatomy laboratory of Veterinary Medicine Faculty, University of Gadjah Mada, Yogyakarta, Indonesia. Research carried out for two weeks with each of the seven days for acclimatization and seven days for a treatment time of *Hyptis pectinata* Poit extract (HPE).

Sample 20 mice and were divided into four treatment groups:

1. The control group (K) which is only given aquadest
2. P1 group is group that exposed HPE with dose of 200 mg/kg
3. P2 group is group that exposed HPE with dose of 1000 mg/kg
4. P3 group is group that exposed HPE with dose of 5000 mg/kg

Then observed for 7 (seven) days and terminated on the eighth day for observation of its liver. After that, the picture of macroscopic morphology, volume, and liver histology of Balb/c mice are observed.

2.4. Statistical Analysis

The data were analyzed and the results are presented in tables and boxplot. To determine the normality of the data normality test using the Shapiro-Wilk test. When the data were normally distributed and homogeneous, the parametric analysis will do. Where the conditions are not met then the parametric analysis performed in non-parametric analysis. Limit when significance level was P≤0.05 with 95% confidence interval. The data analysis done using software SPSS Ver. 15.0 for Windows.

3. Results

3.1. The Liver Macroscopic Image

Table 1. Results of liver volume measurement

Groups	*Liver volume (ml)*		
	Median	**Minimum**	**Maximum**
Control	4	4	4
P1	3.5	3.0	4.5
P2	3.5	3.0	4.0
P3	2.5	2.5	3.5

*difference test non-parametric Kruskal-Wallis p = 0.054

p> 0.05: there are no differences in liver volume significantly.

3.2. The Liver Microscopic Image

The highest mean score of histopathological changes in the treatment groups of three (P3). Scores were assessed include changes in the form parenkimatosa degeneration, hydropic degeneration and necrosis. The mean value of changes in the liver histological structure in each treatment group are shown in Table 2 as follows:

Table 2. The mean score of histopathological changes image of liver cells of Balb/c mice

Groups	***Mean score of histopathological changes image of liver cells of Balb/c mice***	
	Median	**Standard deviation**
Control	314.20	66.53
P1	430.80	70.06
P2	651.20	33.38
P3	757.60	32.11

* Kruskal-Wallis test: p=0.000

p <0.05: there are no significant differences in the structure of the histopathology of liver cells in two groups. Furthermore, Mann-Whitney test was used to assess the differences between groups. The results of the Mann-Whitney test can be seen in Table 3 below:

Table 3. The p-value at the Mann-Whitney test between groups Group K, P1, P2 and P3

Groups	*K*	*P1*	*P2*	*P3*
K		0.175	0.009	0.009
P1	0.175		0.009	0.009
P2	0.009	0.009		0.028
P3	0.009	0.009	0.028	

4. Discussion

Results of macroscopic observation and analysis of the liver showed that the *Hyptis pectinata* extract (HPE) acutely not have a significant effect on the macroscopic image of the liver, in this case assessed morphology and the volume of the liver. Kinds of substances contained in a material, the administered dose, and duration of exposure are factors that affect the liver damage [7]. The duration of the exposure can be categorized as acute (≤ 2 weeks), sub-chronic (2 weeks-3months) and chronic (>3months). Small degeneration often does not cause macroscopic changes in the liver and these changes usually occur in chronic administration [8]. Besides the liver also have high regeneration ability, tissue loss due to toxic substances or due to surgery can stimulate the regeneration of liver cells so that the mass of the liver tissue back to normal [9].

Results of statistical analysis of the microscopic image showed that the control group (K) and the treatment group one (P1) was not significantly different ($p = 0.175$), whereas the control group (K) with treatment group two (P2) and the treatment of three (P3) there significant differences with each value of $p = 0.009$. The control group (K) compared with the treatment group (P1) was not significantly different it is because the administered dose is the smallest dose and still a safe dose thus the resulting effect is also small. The control group compared to the treatment group two (P2) and the treatment of three (P3) had a significant difference, it indicates that the concomitant increase in the dose administered, the effects are also getting bigger then offset by the increasing number of cells undergoing necrosis.

The score changes in the microscopic image of liver cells increases in accordance with the increasing of dose given of *Hyptis pectinata*. This is consistent with the dose-response theory, where the higher the dose given, the higher the response generated and a considerable range will be found therapeutic doses, toxic and lethal doses [10].

Changes of histopathologic image seems increase with the increasing of doses. These changes are also consistent with a dose-response theory. Large doses can cause liver cell damage also increase further.

5. Conclusion

In the acute treatment of HPE, the macroscopic appearance of liver between the control group compared with the treatment groups did not significantly difference and the microscopic appearance of the liver between the control group which compared with treatment groups was significantly difference. In conclusion, the HPE is safe to use which has no complication with liver of mice.

6. Acknowledgements

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