The effect of combination ethanol extracts of bitter melon leaves, white turmeric rhizome and bangle rhizome on the sgot-sgpt levels and the liver histopathology profile of rats

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Abstract. The leaves of bitter melon (Momordica charantia L), as well as rhizomes of White Turmeric (Curcuma zedoaria Rosc.) and Bangle (Zingiber cassumunar Roxb.), are some of the most commonly used plants in Indonesian herbal medicines. It was found that a combination of the three extracts was able to exhibit antimicrobial activity against Mycobacterium tuberculosis in vitro. We aim to observe the toxicity subchronically effect of the ethanol extracts of the plants, particularly in terms of the liver histopathological examination of rat models (Rattus norvegicus). The mixture extract was made into a suspension with various concentrations given for 28 days for 20 rats which were divided evenly into 4 groups. In this present study, The measurement results of SGOT (Serum Glutamic Oxaloacetic Transferase) and SGPT (Serum Glutamic Pyruvic Transferase) levels from rat blood were taken post-treatment period analyzed using One Way ANOVA showed that SGPT and SGOT did not increase significantly compared to the control group. Histopathological results showed group 1 had a mild level of hepatocyte cell damage compared to groups 2 and 3 which had moderate to severe damage.

1. Introduction

Natural products have been the centre of traditional remedies for most diseases caused by microbes which have been used either topically or systemically. The main reason behind this is due to the production of naturally occurring antimicrobial, antiprotzoal, and insecticidal agents [1]. Tuberculosis (TB) is one of the diseases that is caused by the infection of Mycobacterium tuberculosis, affecting mostly the organs of respiratory system. The infection of respiratory TB spread more easily through the air [2]. According to data from Indonesia Health Profiles, there were 360,700 deaths was reported following the infection of respiratory TB in the whole region of Indonesia as recorded in January 2018 [3]. In the recent years, people interests in using naturally derived medicines have also increased. Drugs derived from natural products might become one of the efficient approaches to develop antituberculosis drug with an excellent activity [4].

Some plants which have been used colloquially in Indonesia to treat respiratory disorder are the leaves of bitter melon (Momordica charantia), rhizomes of Curcuma zedoaria and Zingiber cassumunar. These plants have been reported to exhibit antibacterial activity against Mycobacterium tuberculosis as well as good mucolytic activity in vitro [5, 6, 7]. Extract from the mixture of the three plants also inhibited mycobacterial growth at a concentration of 0.5 %w/v. Phytochemical screening of these plants showed that there are at least several types of secondary metabolites have been detected. These includes flavonoids, Saponins, terpenes and alkaloids [8]. Although the activity of
the extract have been reported, the toxicity of these naturally derived sources needs to be investigated. In this paper, we aim to study the toxicity of the extract of these mixture against the liver of rats as one of the organs responsible for metabolizing toxic substances.

2. Methods

2.1 Extraction

As much as 200 g of powder consisting a mixture of dried bitter melon leaves, C. zedoaria and Z. cassumnar rhizomes at a ratio of 1:1:1 was macerated in 2 L of 70% ethanol for 3 x 24 hours. After 3 days, the filtrate was collected and evaporated using rotary evaporator to obtain viscous extract. The remaining material was then remacerated using 1.5 L of solvent to maximize the extraction.

2.2 Animal handling

In this report, 20 white male Wistar rats (Rattus norvegicus) were used as the animal model for the toxicity study. The rats were kept and fed for 2 weeks in order to allow adaptation to the surrounding environment. The rats were then divided into four groups. Group 1 was given suspension of extract at a concentration of 1 %w/v. Group 2 was given suspension of extract at a concentration of 0.5 %w/v. Group 3 was given suspension of extract at a concentration of 0.1 %w/v. As a control, group 4 was only given NaCMC. The extract suspension was administered once a day for 28 days with a maximum volume 1 ml/100 g of each animal bodyweight.

2.3 Preparation of the Extract Suspension

Each concentration of the extract suspension was prepared based on a calculation where 0.25 g (1%), 0.125 (0.5%), and 0.025 g (0.1%) of viscous extract were subsequently suspended in a 1% colloidal solution of NaCMC. The total volume of each preparation was 25 ml. The suspension was prepared by slowly grinding and mixing the extract with the medium until is homogenous.

2.4 Blood collection

Blood collection was done prior and post intervention for 28 days to compare the SGOT and SGPT levels. As much as 2 ml of blood was taken via the intravenous and plexus retro orbitalis routes each times the blood was collected.

2.5 Histopathological examination

2.5.1 Fixation by Formalin 10%

The liver is taken by dissecting the abdomen of the white rat. The liver is removed and cleaned with a 10% NaCl solution. The liver is then placed in a 10% formalin solution for fixation [9].

2.5.2 Tissue Prosessing and Embedding

The liver specimens were then put in a tissue processor before they were taken to the embedding machine to create paraffin blocks of the tissue. The blocks were then cooled and trimmed using microtome to cut undesired parts of the tissue. The trimmed tissues were then allowed to sit on a surface of water inside a water bath which have been set at a 37°C. The tissue was then taken using a slide which has been with PA followed by heating on a hot plate at 60°C until the paraffin around the tissue melted down. Following this process, the tissue slides were dried at a room temperature [10].

2.5.3 Staining by Hematoxylin-Eosin

Staining was conducted using hematoxylin-eosin reagent. The slide was immersed in xylol I for about 30 minutes followed by immersion in xylol II for 30 minutes as well. Subsequently, the slide was
then let to dry completely. Then, it was immersed in ethanol for 5 minutes followed by 96% ethanol for around 5 minutes and eventually with 70% ethanol for 5 minutes. The slides were then rinsed with running water and were immersed in hematoxylin for 5-8 minutes then washed with running water for 10 minutes. The slides were then soaked with eosin for 10 seconds then transferred to ethanol I, II, and III solutions three times each followed by xyloil I, II and III solutions for 5 minutes each. Finally, few drops of Entellan® were added on the top of the slides before each slide was covered with deck glass [10].

2.6 Interpretation of Observation Results
The prepared slides were then observed under using microscope at 400 times magnification. The results were then analysed descriptively using Mitchell scoring system.

Table 1. Scoring system of the hepatocytes obstruction level based on the morphology of the histopathological observation.

| Level of Damage | Type                                      |
|-----------------|-------------------------------------------|
| Normal          | Normal Hepatocyte                         |
| Very Mild       | Hydrophic degeneration+                   |
| Mild            | Hydrophic degeneration +, Lipid degeneration+, Necrosis+ |
| Moderate        | Hydrophic degeneration ++, Lipid degeneration++, Necrosis++ |
| Severe          | Hydrophic degeneration+++, Lipid degeneration++++, Necrosis++++ |

Describe:  
+ (25% damage in eight fields of view)  
++ (50% damage in eight fields of view)  
+++ (75% damage in eight fields of view)

2 Results and Discussion
3.1 SGOT and SGPT Levels

| Biomarkers | Replications | Group | 1 | 2 | 3 | 4 |
|------------|--------------|-------|---|---|---|---|
|            |              |       |   |   |   |   |
|            |              | 1     | 278| 257| 318| 227|
|            |              | 2     | 209| 213| 421| 206|
|            |              | 3     | 501| 168| 222| 207|
|            |              | 4     | 291| 271| 167| 210|
|            |              | 5     | 141| 240| 442| 314|
| Mean       | 284          | 229   | 314| 232|

| Biomarkers | Replications | Group | 1 | 2 | 3 | 4 |
|------------|--------------|-------|---|---|---|---|
|            |              |       |   |   |   |   |
|            |              | 1     | 59 | 123| 83 | 67 |
|            |              | 2     | 111| 99 | 313| 65 |
|            |              | 3     | 120| 91 | 101| 112|
|            |              | 4     | 63 | 93 | 92 | 89 |
|            |              | 5     | 76 | 91 | 89 | 145|
| Mean       | 85           | 99    | 135| 95 |

Describe:  
reference value of SGOT (249 K.U)  
reference value of SGPT (141 K.U)[12]

Measurements of SGOT (Serum Glutamate Oxaloacetic Transaminase) and SGPT (Glutamate Serum Pyruvic Transaminase) levels were carried out after the administration of mixed extracts over 28
days. SGOT and SGPT are enzymes that become biomarkers of liver damage that are characterized by increased levels in the blood due to the release of each biomarker into the plasma [11].

In table 2. It shows the measurement results of SGOT and SGPT levels. SGOT levels increased in all rats in the post-treatment population. While the increase in SGPT levels only occurs in a few tails in each group. In group 3 all rats experienced elevated levels of SGPT. Increased levels of SGOT and SGPT indicate that there has been damage to the liver due to exposure to toxic substances. Toxic substances can be sourced from exposure to medicinal products, natural materials, and uncontrolled environmental pollution, causing necrosis of hepatic cells [13].

**Table 3.** Statistical analysis of SGOT levels between each treatment group and the control group using One Way ANOVA (Analysis of Variants)

|                | Sum of Squares | Df | Mean Square | F    | Sig.  |
|----------------|----------------|----|-------------|------|-------|
| Between Groups | 25188.950      | 3  | 8396.317    | .918 | .454  |
| Within Groups  | 146343.600     | 16 | 9146.475    |      |       |
| Total          | 171532.550     | 19 |             |      |       |

The results of the statistical test in table 3. using the One Way ANOVA method indicate that the significance value between the treatment group and the control group is 0.454 (> 0.050). which means that there was no significant effect between the administration of extracts and the control group which was only given Sodium CMC suspension.

**Table 4.** Statistical analysis of SGPT levels between each treatment group and the control group using One Way ANOVA (Analysis of Variants)

|                | Sum of Squares | Df | Mean Square | F    | Sig.  |
|----------------|----------------|----|-------------|------|-------|
| Between Groups | 7107.400       | 3  | 2369.133    | .791 | .516  |
| Within Groups  | 47892.400      | 16 | 2993.275    |      |       |
| Total          | 54999.800      | 19 |             |      |       |

In table 4 is the result of statistical tests the effect of giving extracts on SGPT levels showed a value of 0.516 (> 0.050) which means the same as SGOT levels that did not experience a significant effect between the group given extract and the control group.

This is related to table 2. There were several rats whose SGOT and SGPT values exceeded the experimental animal reference value. In some experimental animals of mammalian species, increased levels of SGOT and SGPT are also indicators of tissue damage in the heart organs [14].

In this research, only one control group was used (negative control) according to the Provisions of OECD guidelines for testing of chemicals in 2008 number 407 that in administering the test dose, at least 3 test groups and a group is only given a vehicle of the test material [15]. This is also included in the guidelines for in vivo non-clinical toxicity testing by Head of BPOM Republic of Indonesia number 7 in 2014 [16].
3.2 Histopathological test results

In Table 5 shows the results of histopathological observations of the rat liver after a mixture of extracts were given for 28 days with varying degrees of damage. The Normal level indicates no visible damage. All rats from group 1 did not experience damage to hepatocyte cells. Whereas in groups 2 and 3 they were damaged with levels varying from mild (25% damage), moderate (50% damage) to severe (75% damage). Scoring for several types of damage parameters such as hydropic degeneration, hyperemia, and necrosis to determine the level of damage seen in the observation [17].

**Table 5.** The results of histopathological examination of hepatocyte cells that have been given mixed extract for 28 days.

| Group | Replications | Type of Damage                          |
|-------|--------------|----------------------------------------|
| 1     | 1            | NORMAL                                 |
| 1     | 2            | NORMAL                                 |
| 1     | 3            | NORMAL                                 |
| 1     | 4            | NORMAL                                 |
| 1     | 5            | NORMAL                                 |
| 2     | 1            | MODERATE = Hydropic Degeneration ++    |
| 2     | 2            | MODERATE = Hydropic Degeneration ++    |
| 2     | 3            | MODERATE = Hydropic Degeneration ++    |
| 2     | 4            | MILD = Hydropic Degeneration +         |
| 2     | 5            | MILD = Hydropic Degeneration +         |
| 3     | 1            | SEVERE = Hydropic Degeneration +++     |
| 3     | 2            | SEVERE = Hydropic Degeneration +++     |
| 3     | 3            | MODERATE = Hydropic Degeneration ++    |
| 3     | 4            | MODERATE = Hydropic Degeneration ++    |
| 3     | 5            | SEVERE = Hyperemia, Hydropic Degeneration +++ |
| 4     | 1            | NORMAL                                 |
| 4     | 2            | NORMAL                                 |
| 4     | 3            | NORMAL                                 |
| 4     | 4            | MILD = Hydropic Degeneration +         |
| 4     | 5            | MILD = Hydropic Degeneration +         |

Hydropic degeneration is a condition characterized by vacuolized cytoplasm or cell vacuoles that have enlarged and clearly. This is because cells store more fluids than normal because of the disruption of ion channel function on the cell surface. Fluids will accumulate in the cytoplasm and be difficult to remove so that the cells will experience swelling. Generally, this condition starts with lipid degeneration to cell necrosis [18].

Hyperemia is the presence of erythrocytes in large numbers in organs or tissues. Hyperemia sometimes occurs due to the role of regulation of metabolism by the bloodstream. Blood flow increases because of the need for blood from an organ that causes arteries to become vasodilation [19]. Many erythrocytes in the liver tissue are most likely because the liver's need for blood increases as the liver's workload increases to metabolize the exposure of the toxic substance.

Some derivative compounds from the alkaloid group can be toxic to liver cells. Chronic exposure can cause a temporary increase in some serum enzyme levels such as SGOT, SDH, and GGT. However, the clinical signs shown are only minimal focal hepatocyte necrosis. The next effect is gradual damage to hepatocytes starting from inflammation, fibrosis and then cirrhosis. Failure of liver function will trigger damage to other organs [20].
Figure 1. the Appearance of mouse hepatocyte cells with 400x magnification. 1) Normal liver cells from group 1. 2) level of mild damage in group 2, the appearance of hydrophic degeneration. 3) the level of severe damage in group 3, there is hydrophilic degeneration and hyperemia. 4) liver cells that appear normal in the control group. DH (Hydrophilic Degeneration), H (Hyperemia), E (Erythrocytes).

4 Conclusion
The effect of mixed leaves of bitter melon, rhizome of white turmeric and bangle did not have a significant effect on the increase in SGOT and SGPT levels in rats. On histopathological observations, the level of tissue damage from the liver also follows the level of concentration given, from the lowest concentration (0.1%) to the condition of normal liver tissue and the highest concentration (1%) with the level of severe damage to the tissue of rats liver.

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