Utilization of mango ginger (*Curcuma mangga* Val.) rhizome extracts to decrease serum bilirubin in male rats (*Rattus norvegicus* L.)

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Abstract. This study was conducted to observe the effect of ethanol extract of mango ginger rhizome (*Curcuma mangga* Val.) on the level of total bilirubin and direct bilirubin liver damage induced by carbon tetrachloride (CCl₄). We categorized 24 Sprague-Dawley male rats (*Rattus norvegicus* L.) into six treatment groups (NC, TC, T1, T2, T3 and T4). The NC group is a normal control group that was not injected with CCl₄ and 0.5 % CMC-fed. The TC group is a treatment control group that was intraperitoneally injected with CCl₄ treatment in the amount of 1 ml/kgBW and 0.5 % CMC-fed. T1, T2, T3, and T4 are treatment groups that were injected with CCl₄ 1 ml/kgBB and were orally administered mango ginger rhizome ethanol extract each at a dose of 10 mg/kgBW, 20 mg/kgBW, 40 mg/kgBW, and 80 mg/kgBW, respectively. The results of Kruskal & Wallis non-parametric test (α = 0.05) shows that the 10 mg/kgBW, 20 mg/kgBW, 40 mg/kgBW and 80 mg/kgBW doses impacted total bilirubin and direct bilirubin levels. Dunnett’s (α = 0.05) multiple comparison test result showed that the dosages had no significant differences with KK1 group. In conclusion, the dosages could have curative effects because they successfully reduce the level of total and direct bilirubin until it approached normal level.

Keywords: Mango ginger, rhizome extracts, bilirubin, male rat

1. Introduction

The Zingiberaceae family is the most widely used tribe as a traditional medicine [1] *Curcuma* is one of the Zingiberaceae genera that is widely spread in Indonesia [2]. Some well-known members of the *Curcuma* genus are *Curcuma xanthorrhiza* [3] *Curcuma domestica* [4] and *Curcuma zedoaria* [5], yet *Curcuma mangga* [6] (mango ginger) is scarce. Therefore, research on the utilization of mango ginger as traditional medicine needs to be examined more deeply. Mango ginger is believed to have potential health benefits because it produces secondary metabolite compounds such as phenolics (curcuminoids and flavonoids), terpenoid, alkaloid, and saponin [7, 8]. The rhizome of mango ginger has antitumor, antidiarrheal, and wound healing properties. Some researchers have found that the mango ginger rhizome has a curative effect that decreases the levels of alkaline phosphatase (ALP) [9], serum glutamic pyruvate transaminase (SGPT), and serum glutamic oxaloacetic transaminase (SGOT) [10]. Liver damage can be assessed by measuring total and direct bilirubin levels in serum. The present study
assessed the curative effect of Curcuma mangga Val. ethanolic extract against CCl₄ induced hepatotoxicity.

2. Materials and method

2.1. Plant materials
Samples of Curcuma mangga rhizomes were obtained from the Indonesian Medicinal and Spices Research Institute (BALITRO) Cimanggu, Bogor, West Java. The plant rhizomes were identified and authenticated at the Botani Unit of the same institution. Then, all of the samples were deposited to the Department of Biology, Universitas Indonesia.

2.2. Mango ginger rhizome extract preparations
All of the Curcuma mangga Val. rhizome samples (figure 1) were washed with tap water. Samples were cut and dried in the oven at 40 °C until the dried weight was constant. Then, the dried rhizome was refined using a blender and then soaked in ethanol solvent in a 1:10 ratio (weight/volume). After 72 h of maceration, the filtrate was separated and filtered using filter paper [Whatman No.1: 125 mmØ]. Residue of Curcuma mangga Val. rhizome simplicia was re-macerated and filtered until the filtrate was colorless. Filtrate was concentrated using a rotary vacuum evaporation at 40 °C.

2.3. Test animals
Normal healthy male Sprague-Dawley albino rats (Rattus norvegicus) L. (150–250 g) were used in the present investigation. Animals were housed under standard environmental conditions at room temperature (25 ± 2 °C) and light and dark (12:12 h). Rats were fed with a standard pellet diet (Agency for Health Research and Development - Ministry of Health) and water ad libitum.

2.4. Experimental design
In total, 24 rats (18 CCl₄ hepatic toxicity-induced rats and 6 normal rats) were categorized into 4 groups of 6 rats each: Normal Control Group I (NC): Rats received carboxyl methyl cellulose (0.5 %); Normal Treatment Group II (TC): Rats received 1ml/kg body weight of CCl₄ as a CCl₄ hepatic toxicity-induced control; Treatment Group I (T1): Liver injured rats received an ethanol extract of Curcuma mangga Val. rhizome at 10 mg/kg body weight; Treatment Group II (T2): Liver injured rats received an ethanol extract of Curcuma mangga Val. rhizome at the dose of 20 mg/kg body weight; Treatment Group III (T3): Liver injured rats received an ethanol extract of Curcuma mangga Val. rhizome at 40 mg/kg body weight; Treatment Group VI (T4): Liver injured rats received an ethanol extract of Curcuma mangga Val. rhizome at the dose of 80 mg/kg body weight.

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\text{Treatment volume of } \text{CCl}_4 = \frac{\text{weight of the test animal (g)}}{100 \text{ g}} \times 1 \text{ mL CCl}_4
\]  

(1)

Figure 1. Curcuma mangga Val. rhizomes
2.5. Biochemical design

Blood from the orbital sinus was collected into an Eppendorf tube and was allowed to clot. Serum was separated by centrifugation at 5000 rpm for 10 min. Total bilirubin and direct bilirubin levels in serum were photometrically measured using the method of Jendrassik–Groff.

2.6. Statistical analysis

Data were expressed as the mean ± SD. Differences among the means were analyzed using the Kruskal–Wallis non-parametric test and Dunnett test. p < 0.05 were considered to be statistically significant using SPSS Software version 19.

3. Results and discussion

Total and direct bilirubin level results in all treatment groups are shown in table 1. The result of Kruskal–Wallis statistical tests on these data indicate that the data were homogeneous, and there was no difference between the treatment groups (p > 0.05) (table 1).

Table 1 shows that the level of total and direct bilirubin before treatment in all groups was 0.02–0.04 mg/dL [normal range, (total bilirubin: 0.00–1.00 mg/dL) (direct bilirubin: 0.00–0.3 mg/dL)] [11-15]. Thus, total and direct bilirubin levels before treatment were within the normal ranges. Total and direct bilirubin levels were measured before treatment to ensure that bilirubin levels in the normal group and treatment group were treated under normal and homogenous conditions. Therefore, it can be concluded that the preliminary data were representative and the normal control group (NC) can be used as a comparison group for the other groups.

The results of Kruskal–Wallis statistical tests (p < 0.05) showed the differences among the groups. The Dunnett’s statistical tests indicate significant differences between NC with TC and T3 in total bilirubin, and NC with T2 on direct bilirubin, but there was no significant difference between NC and T1, T2, or T4 in total bilirubin and NC with TC, T1, T3 or T4 on direct bilirubin. The results were not suitable because there were one or more data points that deviated too far from the average. Statistical results could be improved with repeated experiments. In contrast, the levels of total and direct bilirubin were increased after administering CCl₄ (table 2, figure 2 and figure 3).

### Table 1. The average total and direct bilirubin levels before treatment.

| Group            | Total bilirubin (mg/dL) | Direct bilirubin (mg/dL) |
|------------------|-------------------------|--------------------------|
| Normal control   | 0.02 ± 0.02             | 0.02 ± 0.02              |
| Normal treatment | 0.03 ± 0.02             | 0.02 ± 0.01              |
| Treatment 1      | 0.03 ± 0.01             | 0.04 ± 0.02              |
| Treatment 2      | 0.04 ± 0.01             | 0.04 ± 0.04              |
| Treatment 3      | 0.03 ± 0.02             | 0.04 ± 0.04              |
| Treatment 4      | 0.04 ± 0.03             | 0.02 ± 0.02              |

### Table 2. The average total and direct bilirubin levels after CCl₄ treatment.

| Group            | Total bilirubin (mg/dL) | Direct bilirubin (mg/dL) |
|------------------|-------------------------|--------------------------|
| Normal control   | 0.05 ± 0.01             | 0.02 ± 0.01              |
| Normal treatment | 0.22 ± 0.05             | 0.20 ± 0.02              |
| Treatment 1      | 0.03 ± 0.02             | 0.04 ± 0.02              |
| Treatment 2      | 0.13 ± 0.12             | 0.06 ± 0.03              |
| Treatment 3      | 0.06 ± 0.06             | 0.02 ± 0.02              |
| Treatment 4      | 0.19 ± 0.03             | 0.05 ± 0.06              |
Figure 2. Diagram of average total bilirubin (mg/dL). Different letters indicate significant differences (p < 0.05).

Figure 3. Diagram of average direct bilirubin (mg/dL). Different letters indicate significant differences (p < 0.05).
Table 3. The average total and direct bilirubin levels after given oral doses of mango ginger extract.

| Group               | Total bilirubin (mg/dL) | Direct bilirubin (mg/dL) |
|---------------------|-------------------------|--------------------------|
| Normal control      | 0.04 ± 0.01             | 0.04 ± 0.02              |
| Normal treatment    | 0.15 ± 0.02             | 0.13 ± 0.05              |
| Treatment 1         | 0.18 ± 0.07             | 0.17 ± 0.06              |
| Treatment 2         | 0.23 ± 0.12             | 0.16 ± 0.02              |
| Treatment 3         | 0.14 ± 0.04             | 0.10 ± 0.06              |
| Treatment 4         | 0.20 ± 0.18             | 0.20 ± 0.09              |

Elevation of total and direct bilirubin levels shows that CCl₄ successfully induces liver damage. The mechanism of liver damage due to carbon tetrachloride (CCl₄) starts from the conversion of carbon tetrachloride (CCl₄) into trichloromethyl radical (·CCl₃) by cytochrome P-450 enzymes, especially CYP2E1, in the liver endoplasmic reticulum. Trichloromethyl will then bind to oxygen to form a highly reactive trichloromethyl peroxide free radical (CCl₃−O₂). Trichloromethyl peroxide (CCl₃−O₂) will undergo chain reaction with polyunsaturated fatty acids (PUFA), which initiates lipid peroxidation in liver cell membranes, destruction of Ca²⁺ homeostasis, and finally, results in cell death [16].

Bilirubin is a yellow pigment produced when heme is catabolized. Serum bilirubin is considered to be one of the truest tests of liver function as it reflects the ability of the liver to take up and process bilirubin into bile. Elevated serum bilirubin levels may indicate several illnesses. High levels of total bilirubin in CCl₄-treated rats may be because of CCl₄ toxicity. CCl₄ administration has been postulated to induce liver injury via lipid peroxidation. In the present study, elevated levels of lipid peroxidation end products in the livers of CCl₄-treated rats were observed.

The result of Kruskal–Wallis (p < 0.05) test shows that there were significant differences between groups. The results of the Dunnett test on total bilirubin showed no significant differences between the normal control group (NC) and the treatment groups T1, T2 or T3 (table 3). The Dunnett’s test on direct bilirubin also showed no significant differences between the normal control group (NC) and T1, T2, T3, or T4. The Dunnett’s test showed that the mango ginger extract successfully decreased the total and direct bilirubin levels after CCl₄ induction and successfully approached the normal control group. In conclusion, the results of this study demonstrate that the ethanol extract of Curcuma mangga Val. rhizome had a potentially curative action against CCl₄-induced hepatic damage in rats. The antioxidant potential of whole plant extract could be the result of the antioxidant activity in individual phytochemicals, i.e., flavonoids and curcumin present in Curcuma mangga Val. rhizome [17].

4. Conclusion
The present study concluded that the ethanol extract of Curcuma mangga Val. rhizomes has a curative effect against CCl₄ induced hepatic damage in rats. The ethanol extract of Curcuma mangga Val. rhizome at the oral dose of 10 mg/kgBW significantly decreased the elevated total and direct bilirubin in serum approach the normal level.

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