Combination of three species of Zingiberaceae prevents doxorubicin-induced hepatotoxicity

Heny Ekowati*, Sarmoko*, and Retno Widiastuti**

ABSTRACT

BACKGROUND
Doxorubicin as an anticancer drug has hepatotoxic side effects. Curcuma xanthorrhiza, Curcuma longa and Zingiber officinale are commonly used as herbals in Indonesia and around the world. Several compounds in these plants have antioxidant activities and are known to exhibit protection against doxorubicin-induced toxicities. This study aimed to observe the hepatoprotective effect of a combination of C. xanthorrhiza, C. longa, and Z. officinale extract on doxorubicin-induced hepatotoxicity in rats.

METHODS
A total of 28 Wistar male rats were divided into four groups: 1) control group (0.9% NaCl); 2) doxorubicin 5 mg/kg intraperitoneally (ip) four times in 14 days (days 1, 5, 9, 13); 3) doxorubicin + combination of C. xanthorrhiza, C. longa, and Z. officinale (temulawak, kunyit, and jahe merah, designated as Tekuja) 250 mg/kg/day orally for 14 days; and 4) doxorubicin + Tekuja extract 500 mg/kg/day orally for 14 days. Measurements of parameters based on liver histopathology and the parameters of serum alanine amino transferase (ALT) and aspartate amino transferase (AST).

RESULTS
Doxorubicin caused significant elevation in serum ALT and AST enzymes after 14 days of treatment. Rats treated with doxorubicin + Tekuja extract 250 mg/kg/day orally for 14 days had decreased levels of ALT and AST.

CONCLUSION
This study indicates that the combination of C. xanthorrhiza, C. longa, and Z. officinale has a protective effect in rats against liver damage induced by doxorubicin.

Key words: Doxorubicin, Curcuma xanthorrhiza, Curcuma longa, Zingiber officinale, hepatotoxicity, rats
Kombinasi tiga spesies Zingiberacea mencegah hepatotoksitas yang diinduksi dokorubisin

LATAR BELAKANG
Doksorubisin adalah antikanker yang memiliki efek samping hepatotoksik. Curcuma xanthorrhiza (Temulawak), Curcuma longa (kunyit), dan Zingiber officinale (jahe), adalah herbal yang biasa digunakan di Indonesia dan di dunia. Senyawa-senyawa yang terkandung dalam ketiga herbal tersebut dilaporkan dapat menurunkan toksisitas yang disebabkan penggunaan dokorubisin. Penelitian ini bertujuan untuk menilai efek hepatoprotectif dari kombinasi ekstrak C. xanthorrhiza, C. longa, dan Z. officinale (Temulawak, kunyit dan jahe merah) pada tikus yang diinduksi dokorubisin.

METODE
Hewan tikus galur Wistar jantan sejumlah 28 ekor dibagi dalam empat kelompok: 1) kelompok kontrol (0.9% NaCl); 2) dokorubisin 5 mg/kg intraperitoneal, empat kali selama 14 hari (hari 1, 5, 9, 13); 3) dokorubisin + kombinasi ekstrak temulawak, kunyit dan jahe merah (Tekuja) 250 mg/kg/hari per oral selama 14 hari; dan 4) dokorubisin + kombinasi ekstrak Tekuja 500 mg/kg/hari per oral selama 14 hari. Pengukuran parameter berdasarkan gambaran histopatologi organ hati dan parameter alanine amino transferase (ALT) dan aspartate amino transferase (AST).

HASIL
Doksorubisin menyebabkan peningkatan yang signifikan pada enzim ALT dan AST setelah 14 hari perlakukan. Hewan yang diberi ekstrak tidak menunjukkan perbedaan pada gambaran histopatologi namun memberikan gambaran penurunan level ALT dan AST. Dokorubisin menyebabkan kenaikan jumlah yang signifikan enzim ALT dan AST serum.

KESIMPULAN
Studi ini menunjukkan bahwa kombinasi ekstrak Tekuja memiliki efek protektif pada organ hati yang diinduksi dokorubisin.

Kata kunci : Dokorubisin, Curcuma xanthorrhiza, Curcuma longa, Zingiber officinale, hepatotoksitas, tikus

INTRODUCTION
Natural products and their active constituents as a source of new drugs for the treatment of disease have attracted attention in recent years. Medicinal applications of spices or herbs have been gradually increasing in developed countries. Curcuma xanthorrhiza, Curcuma longa and Zingiber officinale are commonly used as spices as well as herbals in Indonesia and around the world. These spices are an indispensable component of curry, and belong to the Zingiberaceae family. Several compounds in these plants have antioxidant activities and are known to exhibit protection against doxorubicin-induced toxicities. C. xanthorrhiza is known as temulawak or Javanese turmeric and its extract reportedly possesses anti-inflammatory and antitumor activities, particularly one of its active principles, xanthorrhizol. Z. officinale or ginger, is one of the most commonly used spices in Indonesia and around the world. The active compounds of Z. officinale are gingerol, paradol, shogaol, and
The major chemical constituent of *C. longa* rhizomes is a yellow pigment, 1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3, 5- dione, known as curcumin (diferulolylmethane). Curcumin has shown antioxidant, anti-neoplastic and anti-inflammatory properties. Doxorubicin (DOX) is an anthracycline glycoside antibiotic that possesses a potent and broad spectrum antitumor activity against a variety of human solid tumors and hematological malignancies. The DOX antitumor effects include mechanisms related to alterations of DNA and the production of free radicals. However its use in chemotherapy has been limited, largely due to its diverse toxicities, including cardiac, hepatic, hematological and testicular toxicity. Several studies have shown that the combination of the inflammatory process, free radical oxidative stress, and lipid peroxidation is frequently associated with liver damage, induced by toxic agents such as DOX. Persistent and irreversible liver damage has been a well-known side effect of DOX therapy. It has been observed that there is an increase in the apoptotic processes in liver tissues after a single dose of DOX. It was confirmed that the therapeutic dose of DOX enhanced lipid peroxidation in microsomes and mitochondria in the liver, especially in the presence of Fe3+ ions. DOX-mediated hepatotoxicity includes focal damage in hepatocytes, vascular damage and steatosis.

Although the antioxidant activities of *C. xanthorrhiza*, *Z. officinale* and *C. longa* were well-known, their protective effect against DOX-induced hepatotoxicity has not yet been reported. Several studies have shown that using a combination of plants increases their effectiveness because of synergy with minimal side effects. In our study, we used a combination of three herbs, *C. xanthorrhiza* (temulawak), *C. longa* (kunyit) and *Z. officinale* (jahe merah) (which we will designate as Tekuja). A study on the combination of *C. longa* and *C. xanthorrhiza* showed that it had a protective effect against doxorubicin-induced liver damage in rats. An earlier study, using *Z. officinale* only, suggested that *Z. officinale* protected against nephrotoxicity either by enhancing the renal antioxidant status that had been reduced by DOX, or by exerting a direct antioxidant activity.

Therefore the current study was aimed at evaluating the hepatoprotective activity of an aqueous ethanolic extract of a combination of *C. xanthorrhiza*, *Z. officinale* and *C. longa* (Tekuja) on doxorubicin-induced hepatotoxicity in rats.

**METHODS**

**Study design**

An experimental laboratory study was conducted from March to October 2012. A total of 28 male rats was randomized into 4 groups (control and experimental groups) of seven rats each. The minimum group size as derived from the formula (t-1) (r-1) ≥15, where t = number of treatments and r = number of rats, was 4 rats per group. Animals from group I (control group) received NaCl 0.9%; animals from group II received doxorubicin (DOX) (5 mg/kg) intraperitoneally (ip) 4 times in 14 days (on days 1, 5, 9,13); group III was given Tekuja extract 250 mg/kg/day orally for 14 days and DOX (5 mg/kg) intraperitoneally (on days 1, 5, 9,13); animals from group IV were given Tekuja extract 500 mg/kg/day orally for 14 days and DOX (5 mg/kg) intraperitoneally (on days 1, 5, 9, 13). DOX-treated groups received the drug every fourth day, while the other groups instead of DOX received 0.9% sodium chloride (10 mL/kg BW) intraperitoneally. The time intervals between two DOX administrations were similar to the most frequently-used treatment schedules in humans. On day 15 (1 day after the last dose of DOX) all animals were sacrificed.

**Experimental animals**

Laboratory-bred Wistar albino rats (8-week-old and weighing 140 ± 30 g) were
Ethical Clearance

Animals were handled according to the rules and regulations of the Animal Care Committee of the Faculty of Medicine, Gadjah Mada University.

Data analysis

All results were expressed as mean and standard deviation. Differences between groups were assessed by one-way analysis of variance (ANOVA), followed by Tukey HSD. Statistical significance was defined as p<0.05.

RESULTS

Observation was terminated at 14 days after the last DOX treatment. Macroscopic observation of the livers showed no perceptible differences between groups (Figure 1). The histopathological appearance of the livers in rats treated with DOX and DOX + Tekuja extract also showed no differences between groups (Figure 2).

However, we found a significant increase in ALT and AST levels (p<0.05) in the group treated with DOX only, compared with the control group (Table 1). Administration of Tekuja (250 and 500 mg/kg BW) plus DOX significantly (p < 0.05) attenuated the decrease in ALT and AST levels observed after administration of DOX alone. The ALT levels were restored to normal in the Tekuja plus DOX groups (Tukey HSD Test, p<0.05) (Table 2).

DISCUSSION

The results of the present study indicate that the aqueous ethanolic extract of the combination of C. xanthorrhiza, C. Longa and Z. officinale (Tekuja) significantly protected rats against DOX–induced hepatotoxicity. A study on the combination of C. longa and C.xanthorrhiza showed that it had a protective effect against DOX-induced liver damage in rats. However, another study using C. longa alone
Figure 1. Absence of morphological differences in livers of rats treated with *C. xanthorrhiza*, *Z. officinale* and *C. longa* combination and doxorubicin

(A) NaCl control group; (B) Doxorubicin group; (C) Doxorubicin+250 mg/kgBW *C. xanthorrhiza*, *Z. officinale*, and *C. longa* combination; (D) Doxorubicin+500 mg/kgBW *C. xanthorrhiza*, *Z. officinale*, and *C. longa* combination

Figure 2. Histological evaluation in liver tissues of control and experimental group. (A) NaCl control group; (B) Doxorubicin group; (C) Doxorubicin + 250 mg/kgBW *C. xanthorrhiza*, *Z. officinale*, and *C. longa* extract combination; (D) Doxorubicin + 500 mg/kgBW *C. xanthorrhiza*, *Z. officinale*, and *C. longa* extract combination
Table 1 Mean of on ALT and AST bases on Treatment groups in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>169.57 ± 7.79</td>
<td>34.43 ± 2.76</td>
</tr>
<tr>
<td>DOX</td>
<td>312.14 ± 8.71</td>
<td>73.14 ± 5.38</td>
</tr>
<tr>
<td>DOX+Tekuj 250mg</td>
<td>256.14 ± 9.21</td>
<td>60.43 ± 3.95</td>
</tr>
<tr>
<td>DOX+Tekuj 500mg</td>
<td>221.86 ± 8.71</td>
<td>53.86 ± 1.86</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.D. of seven animals

Table 2. Multiple comparison test (Tuckey HSD) of ALT (U/L) and AST (U/L) levels between treatment groups

<table>
<thead>
<tr>
<th>ALT levels (U/L)</th>
<th>Mean Difference</th>
<th>Std. Error</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-DOX</td>
<td>-142.57</td>
<td>4.48</td>
<td>0.000</td>
</tr>
<tr>
<td>Control-DOX+Tekuj 250mg</td>
<td>-86.57</td>
<td>4.48</td>
<td>0.000</td>
</tr>
<tr>
<td>Control-DOX+Tekuj 500mg</td>
<td>-52.28</td>
<td>4.48</td>
<td>0.000</td>
</tr>
<tr>
<td>DOX-DOX+Tekuj 250mg</td>
<td>56.00</td>
<td>4.48</td>
<td>0.000</td>
</tr>
<tr>
<td>DOX-DOX+Tekuj 500mg</td>
<td>90.28</td>
<td>4.48</td>
<td>0.000</td>
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<tr>
<td>DOX+Tekuj 250mg-DOX+Tekuj 500mg</td>
<td>34.28</td>
<td>4.48</td>
<td>0.000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AST levels (U/L)</th>
<th>Mean Difference</th>
<th>Std. Error</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-DOX</td>
<td>-38.71</td>
<td>1.73</td>
<td>0.000</td>
</tr>
<tr>
<td>Control-DOX+Tekuj 250mg</td>
<td>-26.00</td>
<td>1.73</td>
<td>0.000</td>
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<tr>
<td>Control-DOX+Tekuj 500mg</td>
<td>-19.42</td>
<td>1.73</td>
<td>0.000</td>
</tr>
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<td>DOX-DOX+Tekuj 250mg</td>
<td>12.71</td>
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<tr>
<td>DOX-DOX+Tekuj 500mg</td>
<td>19.28</td>
<td>1.73</td>
<td>0.000</td>
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<tr>
<td>DOX+Tekuj 250mg-DOX+Tekuj 500mg</td>
<td>6.37</td>
<td>1.73</td>
<td>0.006</td>
</tr>
</tbody>
</table>

showed that *C. longa* has multiple therapeutic activities by blocking cardiac, hepatic, and renal toxicities induced by doxorubicin. An earlier study using *Z. officinale* only, suggested that *Z. officinale* protected against nephrotoxicity either by enhancing the renal antioxidant status that had been reduced by DOX, or by exerting a direct antioxidant activity. The present study explored the effect of a combination of *C. xanthorrhiza*, *C. longa* and *Z. officinale* extract (Tekuja) on DOX-induced hepatotoxicity in rats. The Tekuja extract was given during the DOX induction period. Treatment with the combination of *C. xanthorrhiza*, *Z. officinale* and *C. longa* extract was designed to prevent metabolic activation of DOX and suppress liver damage. The liver is the central organ of metabolism and acts as a storage organ. Liver cells metabolize toxic agents, including DOX. The liver is extremely vulnerable to damage by chemical agents, presumably as a result of its central role in the metabolism of foreign substances. In our study, histopathological examination of the livers showed no appreciable differences between groups. These results may be interpreted as indicating that administration of DOX does not result in liver damage, although the serum transaminase was elevated.

DOX in the form of DOX semiquinone has been suggested to play a major role in its hepatotoxic action. Semiquinones are unstable under aerobic conditions, thereby generating superoxide anion radicals by reacting with molecular oxygen. Hepatocytes are the likely targets of attack by reactive oxygen species (ROS) in the failing liver. It is conceivable that free radicals cause damage at their formation. Consequently the mitochondria as the major source of ROS production, could also be the major target susceptible to attack by ROS.
Serum transaminases have long been considered a sensitive indicator of hepatic injury. Injury to the hepatocytes alters their transport function and membrane permeability, leading to leakage of enzymes from the cells, causing a decrease in the levels of ALT and AST in hepatic cells but an increase of these enzymes in serum. In this study, administration of DOX to rats significantly increased serum ALT and AST levels. AST is a more liver-specific enzyme. On the other hand, an increase in ALT activity is usually proportional to the extent of cardiac damage.

Treatment with the combination of *C. xanthorrhiza*, *Z. officinale* and *C. longa* (250 and 500 mg/kg) resulted in a significant decrease in enzyme activities in DOX-treated animals, thus offering considerable protection against hepatotoxicity. The data presented in this study demonstrate that DOX increased serum indices of liver function including ALT and AST. These elevations of ALT and AST are attributable to hepatocellular damage and decreased liver functions. These elevated levels of serum indices for hepatocellular damage have been previously reported in a DOX-induced hepatotoxicity model.

The effectiveness of *C. xanthorrhiza* in lowering the serum enzyme levels of AST, ALT, and glutamate transferases demonstrates the hepatoprotective effect of this plant against cisplatin-induced hepatotoxicity. The plant also acts to prevent fatty degeneration of the liver, which can cause irreversible functional breakdown, inevitably leading to death.

Yemitan et al. tested the effect of an ethanol extract *Z. officinale* rhizomes against carbon tetrachloride (CCl₄) and acetaminophen-induced liver toxicities in rats. Carbon tetrachloride and acetaminophen induced many histopathological changes and increased the activities of ALT, AST, ALP, LDH and SDH in serum. The protective effect of *Z. officinale* extract against carbon tetrachloride- and acetaminophen-induced damage was confirmed by histopathological examination of the liver.

*C. xanthorrhiza*, *C. longa* and *Z. officinale* all contain the active compounds curcumin, xanthorrhizhol and oleoresin. Curcumin has shown antioxidant, anti-neoplastic and anti-inflammatory activity. Many of the activities associated with curcumin relate to its ability to suppress acute and chronic inflammation. An in vivo study showed that rats fed curcumin for 7 days prior to being treated with cyclophosphamide to induce lung injury, exhibited an increase in antioxidant defense mechanisms. Thus, curcumin exhibits substantial antioxidant properties in a wide variety of experimental settings.

The antioxidant properties of [6]-gingerol, which is a very effective agent for anticipation of ultraviolet B (UVB)-induced reactive oxygen species production and COX-2, and a promising therapeutic agent against UVB-induced skin disorders, have been studied both in vitro and in vivo. The compound also has a protective role against the toxicity and lethality induced by agents such as carbon tetrachloride and cisplatin.

The present study provides the information that Tekuja extract protected against the hepatotoxicity of DOX. Tekuja was shown to protect liver tissue, as indicated by the histopathological profile, and ALT and AST levels. These results suggest the possible use of Tekuja as a novel agent against DOX-induced organ toxicities and a potential candidate to be further evaluated. However, the parameters in our present study could not explore the activity of DOX in DOX-induced decline in hepatic antioxidant status or its direct antioxidant activity. Therefore further studies should be conducted to explore hepatic antioxidant status and direct antioxidant activity.

**CONCLUSION**

The results of the present study indicate that the combination of *C. xanthorrhiza*, *C. longa*, and *Z. officinale* has a protective effect against liver damage induced by DOX.
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