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The Antioxidant and Hepatoprotective Potential of Temulawak (*Curcuma xanthorrhiza Roxb*) Ethanol Extract in Paracetamol-induced Rats

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Paracetamol is widely used as analgesic and antipyretic drugs. Paracetamol can increase Radical Oxygen Species (ROS) if used exceeded the administered dose that might lead to hepatic and gastric damage. **The purpose** of this research is to investigate the antioxidant and hepatoprotective potential of Temulawak Ethanol Extract in Paracetamol-induced rats. This research was conducted using completely randomized design. The samples were 30 white rats, divided equally into 6 groups. The rats of group 1 were fed normally with regular feed and aquadest for 10 days (control). The rats of group 2 were fed using aquadest and temulawak extract with the dosage of 540mg/200g rats body weight for 10 days. The group 3 of rats were fed using aquadest and paracetamol with the dosage of 2g/kg rats body weight on the 7th day (positive control). The group 4,5, and 6 were fed using Temulawak ethanol extract, 270mg/200g, 540mg/200g, and 1080mg/200g rats body weight, respectively, for 10 days and were given 2g/kg rats body weight of paracetamol on 7th day. The rats blood samples were taken after 10 days treatment. The MDA, ALT and AST in rats blood serum were measured. The data were analyzed using ANOVA and LSD test. The treatment of 270mg/200g, 540mg/200g, and 1080mg/200g rats body weight Temulawak ethanol extract showed specific results of lowered ALT, ASP and MDA levels in paracetamol induced rats. It can be concluded from this research that the Temulawak ethanol extract is cosidered potential as antioxidant and hepatoprotector.

Keywords: Temulawak, ALT, ASP, MDA, Hepatoprotector

Backgrounds

Paracetamol, also known as acetaminophen, is one of the most used and famous analgesic and antipyretic drug with the therapheutic dosage of 4g/day (1). After getting into the body, paracetamol is metabolyzed by liver enzymes, especially cytochrome P450, producing toxic metabolites (2), such as N-acetyl-p-benzoquinone imine (NAPQI). This dangerous metabolite is detoxificated by liver antioxidant. In paracetamol overdose case, the cellular Gluthatione (GSH) supply was not sufficient (saturated), therefore the NAPQI could not be neutralized completely. In hepatocyte, NAPQI can form protein adduct with intracellular proteins. This might cause mitochondria disfuction, clear DNA fragmentation and high ROS production that lead to the high lipid peroxidation resulted in lipid peroxide product. The lipid peroxide can disperse into several compounds such as epoxide, hydrocarbon and aldehyde. One of the aldehyde compound is Malondialdehyde (MDA) (3). Beside, NAPQI can causes the hepatocyte death, the liver and gastric failure, and potential in causing patients death (1)(4). The hepatic cells death can be identified by the high level of intracellular enzymes such as transaminase (ALT and ASP) in blood serum.

The unbalance mechanism of ROS formation due to paracetamol overdose with and the level of endogenic antioxidant might cause the oxidative stress. There were several diseases reported caused by this oxidative stress, such as diabetes mellitus, neurodegenerative diseases (Parkinson-PD, Alzheimer-AD and Multipe schlerosis-MS), cardiovascular diseases (Atheroschlerosis and hypertension), respiratory disease (Asthma), cataract, rheumatoid arthritis and many kinds of cancer (colorectal, prostate, lungs and bladder cancer) (5). Therefore, the researches about exogenic antioxidants to decrease free radical and oxidative stress are needed to prevent many kinds of diseases.

Antioxidant can be obtained from nature or laboratory (synthetic). The nature antioxidants are more desirable due to the minimal side effects. Temulawak (*Curcuma xanthorrhiza Roxb*) is one of the nine superior plants, with various usages as medicines, according to Ditjen POM. Temulawak is widely used by people, for healthcare or medications, traditional medicine industries and cosmetics (6). Temulawak has properties as an antioxidant, antidiuretic, anticancer, antihypertensive, antihepatotoxic, antibacterial, and antifungal effect (7). this is related to the main compound content in C. xanthorrhiza, namely curcuminoids, essential oils and xanthorrhizols (6). Its wide use in traditional medicine, however there is still little information validating the potency of ginger for hepatoprotective anti-oxidant treatment. Based on the following backgrounds, the purpose of this research is to investigate the antioxidant and hepatoprotective potential of Temulawak Ethanol Extract in Paracetamol-induced rats.

Methods

This research used male Wistar white rats. The age of the rats were around 12-16 weeks (body weight 200-220 g) purchased from the animals house, Faculty of medicine, University of Airlangga. The rats were placed in a temperature controlled room (24 ± 1 ° C) with controlled light condition (12h light and 12h dark). The rats feed and water were given *ad libitum*. Before experiments, the test animals were acclimatized for a week.

Experimental design

The rats were chosen randomly and divided into 6 equal group (n = 5/group): Control group (C), Temulawak 540mg/200g rats body weight (T), Paracetamol (PCT), Paracetamol + Temulawak 270 mg/200g rats body weight (PCT+T1), Paracetamol + Temulawak 540mg/200g rats body weight (PCT+T2), Paracetamol + Temulawak 1080mg/200g rats body weight (PCT+T3). The **control** group were given aquadest orally everyday for 10 day consecutively. The **Temulawak (T)** group were fed with temulawak extract of 540mg/200g rats body weight. The **Paracetamol (PCT)** group were given aquadest orally everyday for 10 days consecutively and fed with paracetamol orally of 2g/kg/day on the 7th day. The **Paracetamol + Temulawak 1 (PCT+T1)** group were given temulawak extract of 270 mg/200g rats body weight, orally everyday for 10 days consecutively, and fed with paracetamol orally of 2g/kg/day on the 7th day. The **Paracetamol + Temulawak 2 (PCT+T2)** group were given temulawak extract of 540mg/200g rats body weight, orally everyday for 10 days consecutively, and fed with paracetamol orally of 2g/kg/day on the 7th day. The **Paracetamol + Temulawak 3 (PCT+T3)** group were given temulawak extract of 1080mg/200g rats body weight, orally everyday for 10 days consecutively, and fed with paracetamol orally of 2g/kg/day on the 7th day. During treatments, the rats were fed normally. After 10 days treatments, the rats were anesthetized and the blood samples were taken for ALT, AST and MDA analysis.

The rats serum collection

The unconcious rats (from anaesthetia), were placed on the operation table in supine position, and dissected using dissecting set. The blood were taken from the rat heart. The blood were placed in falcon tubes. The samples were precipitated for 2 hours, and centrifugated for 15 min (4°C; 5000 rpm) to obtain serum. The serum were kept in the temperature of -20°C for further observations.

Oxidative stress analysis

The MDA serum level measurement was conducted using TBA reagent based on Esterbauer dan Cheeseman (8). A 0.5mL of the rat blood serum was mixed with 9mL of cold PBS. Then, 4 ml of the supernatant was taken and mixed with 1mL of 15% TCA, 1mL of 0.37% TBA in 0.25N HCl. The mixture was heated in a waterbath 80°C for 15 min, then cooled down in room temperature for 60 min. Then, the mixture was centrifugated in 3000 rpm for 15 min. The MDA-sample supernatant absorbance was measured using spectrophotometer in λ 532 nm. The MDA level (nmol/mL) was determined using linear regression equation from the standard curve of 1,1,3,3-tetramethoxypropane solution.

Biochemical analysis

The ALT, AST level in blood serum was measured using Diasys (*Diagnostic System*) reagent and spectrophotometry methods (9)

- AST
  A 100 µL of blood serum was mixed with 1000µL of reagent 1 and incubated for 5 min. Then, 250 µL of reagent 2 was added and the absorbance was measured in λ 340 nm. The measured absorbance was AST.
- ALT
  A 100 µL of blood serum was mixed with 1000µL of reagent 1 and incubated for 5 min. Then, 250 µL of reagent 2 was added and the absorbance was measured in λ 340 nm. The measured absorbance was ALT.

Results and Discussions

Oxidative stress analysis

Malondialdehyde (MDA) is widely used as oxidative stress indicator, especially from unsaturated fatty acid. Malondialdehyde is the final product of lipid peroxidation, especially unsaturated fatty acid, and resulted from the free radical oxidation. The chemical method to measure MDA level uses MDA reaction with thiobarbituric acid (TBA), forming a TBA-MDA complex bond, which is resulting in red coloured solution. The intensity of the coloured solution is determined using spectrophotometer.

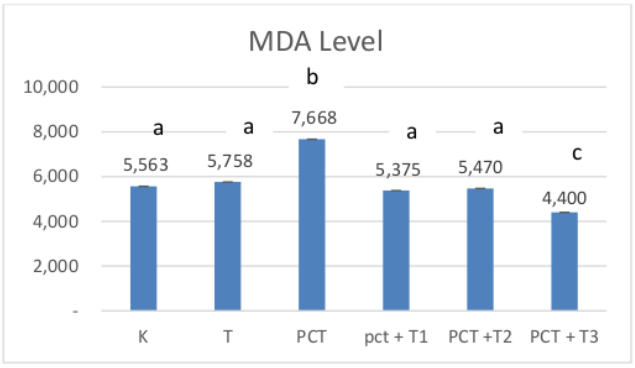

Fig. 1. The average MDA level in rats blood serum. The abc superscript: if notated in identical alphabet, means insignificant difference; if notated in different alphabet, means significant difference, between the treatment groups, according to *Pos Hoc Mann-whiteney* test.

Based on Fig. 1, the paracetamol treatment increased the MDA level compared to the control group ($P < 0.01$). Meanwhile, the ethanol extract of Temulawak in concentration of 270, 540 and 1080 mg/200g rats body weight turn the MDA level back to almost normal level approaching the MDA level of control group.

The increased MDA level in rats blood serum from Paracetamol group (PCT), indicated there was oxidative stress occured in this group, which played an important role in liver pathogenesis. The unbalanced mechanism of ROS formation and natural antioxidant in the body (Glutathione, superoxide dismutase) could trigger the oxidative stress (10). In stress oxidative condition, ROS reacts to unsaturated lipid in cell membrane and plasma lipoprotein resulted in the formation of lipid peroxide (Malondialdehyde) which is chemically modifies proteins and nucleic acid bases. Furthermore ROS modifies amino acid in proteins directly thus the proteins could not be identified as *self*, but as *nonself* by immune system(11).

The ethanol extract of Temulawak treatment decreased the MDA level of rats blood serum that were inducted with paracetamol. The most effective dosage of Temulawak extract was 1080 mg/200g rats body weight, giving a significant difference compared to the other treatment groups. This result demonstrated that the active metabolites of Temulawak were able to suppress free radicals formation caused by paracetamol induction. Curcumin is the main compound C. Xanthorrhiza can inhibit GST and cytochrome P450 activity. [35,36]. Mechanism Curcumin prevention is thought to be related to its inhibitory effect on lipid peroxidation and oxidative stress. Curcumin restores Bcl-2 / Bax ratio, thereby reducing PCT-induced hepatocyte apoptosis. The Bcl-2 protein is commonly known as an

antiapoptotic factor that inhibits cell apoptosis by preventing it depolarization of the mitochondrial membrane.(12)

ALT and AST enzymes activity assay

The presence of specific enzymes in blood, in significant amount, could indicate the tissues damage. The raised of specific enzymes in liver, AST (aspartate aminotransferase) and ALT (Alanine transaminase), are indicator for liver damage or injury.

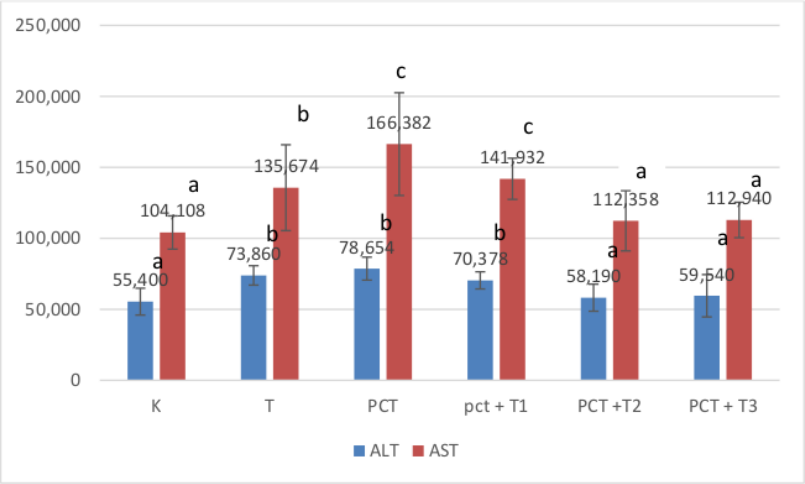

Fig. 2. Average activities of ALT and AST enzymes in rats blood serum. abc superscript: if notated in identical alphabet, means the insignificant difference; if notated in different alphabet, means the significant difference, between the treatment groups, according to *Pos Hoc Mann-whiteney* test.

Based on Fig. 2, paracetamol treatment enhanched the activities of ALT and AST enzymes compared to the control group. Meanwhile the ethanol extract of Temulawak treatments in the concentration of 540 and 1080 mg/200g rats body weight turn the ALT and AST levels back to almost normal level as control group.

Overdose on PCT will cause a buildup of NAPQI, which will bind with glutathione (GSH) to form a conjugation that will cause oxidation and conversion of GSH to glutathione disulfide, resulting in decreased blood and liver GSH levels (13). These events initiate liver necrotic cell death and abnormality. Necrotic cell death releases biochemical markers such as ALT and AST into the circulation(13). Thus the ALT and AST levels in blood serum have increased as shown in Fig.2.

The ethanol extract of Temulawak reduced the ALT and AST enzymes activities in paracetamol-induced rats blood serum. The 540 mg/200g rats body weight and 1080 mg/200g rats body weight dosage of temulawak ethanol extract, demonstrated almost the same effects (insignificant difference) in reducing ALT and AST activities. Yet, the 540 mg/200g rats body weight and 1080 mg/200g rats body weight dosage of temulawak ethanol extract, showed better effect compared to 270 mg/200g rats body weight group.

The rhizome of C. xanthorrhiza contains essential oils, xanthorrhizol, curcuminoids, flavonoids, ar-curcumene, terpenoids, phenols, and saponins (6). C. xanthorrhiza essential oil contains a lot compounds such as xanthorrhizol, diepi-α-cedrene, α-curcumene, champor, and B. Germacrene (14).

Xanthorrhizol is the active compound of C. Xanthorrhiza responsible for hepatoprotective activity. Xanthorrhizol has better hepatoprotective activity than curcumin. The mechanism of xanthorrhizol as a hepatoprotective effect may be associated with its ability to regulate transcription factor DNA binding, nuclear factor kappa B (NF-αB) and AP-1(15).

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