

The Effect of *Curcuma Zedoaria* (Berg.) Roscoe Extract on Liver Function Tests of *Rattus norvergicus* Exposure by Cigarette Smoke

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Abstract. *Curcuma zedoaria* (Berg.) Roscoe has been widely used traditionally as a valuable medicinal plant to treat many diseases due to its activity on repairing cell damage. This study aimed to evaluate the effect of ethanol extract of *Curcuma zedoaria* (CZE) on liver function test levels in male Wistar rats exposed by cigarette smoke. A total of 30 male rats were divided into six groups (n=5), i.e., control group(G1); negative control(G2); positive control(G3); cigarette smoke-exposure followed by administration of CZE 350 mg/kg b.wt group(G4), cigarette smoke-exposure followed by administration of CZE 525 mg/kg b.wt group(G5), and administration of CZE 525 mg/kg b.wt group (G6). Treatments were given for 45 days before blood collection and MDA measurements on rat liver tissue. The results showed that administration of 350 mg/kg b.wt., and 525 mg/kg b.wt reduced total protein, ALT, AST and ALP levels on rat exposed by cigarette smoke as well as decreased liver peroxidase level based on the average profile of MDA on rat liver tissue. In conclusion, administration of CZE showed the hepatoprotective effect on rats exposed by cigarette smoke.

Keywords: *Curcuma zedoaria*, cigarette

1 Introduction

Smoking is one of the most severe health-behavior problems considered as the causes of death in the world. The number of smokers' worldwide increase tremendously from about 5.8 billion in 2014 to more than 1.1 trillion in 2018. More than 6 million deaths in the world related to cigarette consumption, whereas about 890,000 of those are the result of exposure to secondhand smoke [1]. In Indonesia, there are approximately 50.6 million adults (aged 15 years and over) become active smokers [1], [2]. Riskesdas (2013) had identified that at least 25,000 passive smoker deaths are due to smoke exposure from active smokers near them.

Cigarette smoke has been known to contain many toxic components including various oxidants such as oxygen-free radicals or reactive oxygen species (ROS) and volatile aldehydes that allow damage into biomolecules [3], [4]. Activities of the free radical leading to oxidative stress have the potential risks to initiate or promote oxidative damage in the cellular level including damage to membrane lipids that make up cells, proteins, carbohydrates, and DNA [5]. It has been reported that oxidative damage caused by free radicals has implications for

various pathological conditions to tissues and vital organs such as the liver, kidneys, heart in both humans and animals [6].

A potential risk factor of liver cancer by increasing the risk of cirrhosis and affect the development of chronic liver disease could be triggered by prolonged ROS accumulation led by toxic chemical exposure [7]. Several studies showed that therapeutically use of components that counteract ROS activity was based on its progressive prevention on the pre-cancer stage [8], [9]. Nevertheless, either toxicants or drugs, including anticancer substances are known to have a substantial first-pass hepatic metabolism which potentially caused more damage to the liver [9], [10], [11]. Therefore, the development of natural compounds that have anticancer activity is expected to reduce the risk of liver damage compared to synthetic chemicals [12].

White Turmeric or *Curcuma zedoaria* (Zingiberaceae) had been used traditionally to treat many diseases, and the ethanol extract (CZE) has a hepatoprotective effect as well as high potency against cancer cell with less activity in the normal cell. Some studies suspected that the anticarcinogenic effect of CZE was due to its antioxidant activity [13], [14], [15]. Moreover, a cytotoxic test of CZE showed that the extract has IC₅₀ of 6.05, 17.84 and 55.50 µg/ml respectively to the lung cancer cell, prostate cancer and normal cells with an LD₅₀ value of 1000 mg/kg BW [16]. Although evaluation of the potential effect of CZE had widely studied, its hepatoprotective effect against secondhand smoke exposure in *Rattus norvegicus* model has not been reported. Therefore, the primary objective of this study was to observe the effect of CZE on reducing the effects of free radicals exposed from secondhand cigarette smoke through the parameters of liver function test in blood and liver organ.

2 Experimental

2.1 Preparation of *Curcuma zedoaria* Ethanol Extract (CZE)

The rhizomes of *C. zedoaria* were harvested from Sidrap district, South Sulawesi and determined at Laboratory of Biology, Mathematics, and Natural Sciences Faculty, Makassar State University. Preparation of CZE was based on the procedure explained elsewhere [17]. Shortly, the process started by a-24 hrs vacuum drying process at 45°C of clean-cut pieces of *C. zedoaria* rhizomes. The dried simplicia then achieved with mesh 18 and stored at the airtight container. A three-days conventional maceration process was used to extract the pulverized rhizomes with ethanol 70% as the solvent (1:10). The crude liquid of CZE was separated by filtration, and maceration was repeated twice. Evaporation of the solvent of CZE used rotary evaporator (Heidolph®) followed by storage in a desiccator containing dry silica gel to obtain the viscous extract.

2.2 Animals

Before the treatments, the male rats (*Rattus norvegicus*) age 8-10 weeks were acclimatized to environmental and experimental conditions for seven days. The rats were housed in controlled room temperature at 25±2°C under a light/dark cycle of 12 h in a cage with access to food and water *ad libitum*. The blood samples were withdrawn via a lateral vein to identify healthy animals, and a total of 30 male rats with an average weight of 150-250 g were divided into six groups (n = 5).

2.3 Experimental protocols

Experimental protocols on the animal use of these studies were approved by the Ethics Commission of Medical Faculty, Hasanuddin University (No. 292/H4.8.4.5.31/PP36-KOMETIK/ 2018). These studies were conducted in a posttest-only randomized experimental design that compared six groups of male Wistar rats (*Rattus norvegicus*). As the antioxidant activity against cancer cell proliferation of *C.zedoaria* determined in earlier studies, the dose is chosen accordingly with slight modification for dose conversion between animals [18]. The smoking chamber (a glass box size 50 x 60 x 40 cm) used in this study equipped with a vacuum pump for cigarette smoke and air supply. After the adaptation period of the animals, the treatments were as follow: Group I (G1): normal control rats received 1% w/v NaCMC (5 ml/kg b.wt); Group II(G2): Negative control rats exposed by the smoke of 10 cigarettes (contain 39 mg tar and 2.5 mg nicotine) in the smoking chamber for 30 min every day up to 45 days; Group III: Positive control rats, received cigarette smoke exposure as (G2) and 30 min further cotreated with Vit. C 10 mg/kg b.wt by oral gavages for 45 consecutive days; Group IV(G4): Rats treated as (G3) but Vit C replaced by administration of 350 mg/kg b.wt of CZE in 1% w/v NaCMC; Group V (G5): Rats treated as (G4) using CZE 525 mg/kg b.wt; Group VI (G6): Rats received CZE (525 mg/kg b.wt) alone. Rats were weighed every day to adjust the amount of CZE suspension administered. Blood samples were collected 24 hrs following the last treatments via a lateral vein then the rats were euthanized to harvest the liver for MDA assay.

2.4 Blood Biomarkers of Liver Function Tests

Blood samples were centrifuged (Hettich[®]) at 1500 g/min for 10 min to obtain serum. Clinical biochemistry parameters particularly the liver marker enzymes: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total protein were determined using ABX Pentra 400 diagnostic reagents and instrument (Horiba[®]).

2.5 Liver MDA Level Analysis

The concentration of lipid peroxide in liver tissues was measured by thiobarbituric acid (TBA) reaction, which measured the presence of malondialdehyde (MDA) as its product [19]. Individual rat livers were crushed and homogenized before weighed 400 mg using an analytical scale (Sartorius[®]). Two mL PBS pH 7.4 was added, and the mixture was centrifuged at 3000 rpm for 10 minutes. The supernatant was taken 0.5 ml then added with 1 ml of TCA (10% b / v) and 1 ml TBA (1% b / v) in a centrifuge tube. The mixture was heated at 90-100°C for 40 minutes, cooled to room temperature; then centrifuged again at 3000 rpm for 10 minutes. The absorbance was measured using a UV-Vis spectrophotometer (Shimadzu MR 2500[®]) at a wavelength of 532 nm [20].

2.6 Statistical Analysis

Data obtained were statistically analyzed using SPSS 20 software with Kolmogorov-Smirnov method. Normally distributed data, were analyzed by one-way analysis of variance

ANOVA measurement method then proceed with post hoc test using the LSD test of significance. $P < 0.05$ were considered as significantly different.

2.7 Results and Discussion

The characteristics of ethanol extract of *C. zedoaria* obtained after concentrated and stored in a desiccator was similar, as described in Farmakope Herbal Indonesia [17]. The yield of CZE was 27.461%, which showed a thick, viscous brownish extract with a bitter taste and characteristic odor of *C. zedoaria*.

Table 1. Body weight of rats after 45 days of treatments.

Treatment Groups	Mean BW \pm SD (g)
Normal (G1)	230 \pm 17.3
Cigarette smoke (G2)	218 \pm 30.3
Cigarette smoke+vitamin C (G3)	234 \pm 25.1
Cigarette smoke+CZE 350 mg/kg b.wt (G4)	246 \pm 24.1
Cigarette smoke+CZE 525 mg/kg b.wt (G5)	220 \pm 9.6
CZE 525 mg/kg b.wt (G6)	227 \pm 9.6

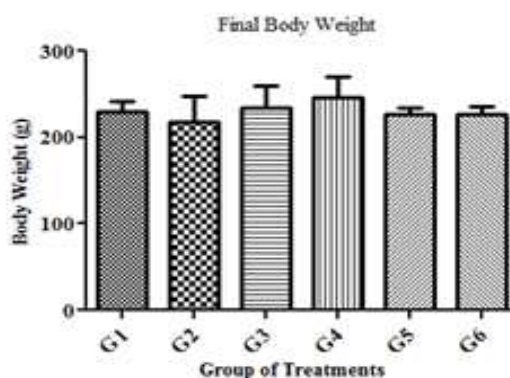


Fig. 1. Effects of treatments on the Body Weight of *Rattus norvegicus*. Values are mean \pm SD (n=5).

After the adaptation period of the animals, the baseline level of blood biomarkers was measured one day before treatment (day-0). The rats which showed results in the range of rat's reference values as experimental animals [21] were used for this study. Every day, rats were weighed to adjust the number of CZE administered. Data in Table 1 demonstrates the mean body weight of the rats after 45 days of treatments. It is seen that the mean body weight of rats exposed cigarette smoke without other treatments (G2) slightly decreased although still showed no significant differences among other groups (Figure 1). These data were entirely different from other studies which revealed the fast response of weight loss in line with the

reduction of food consumption and positively associated with increasing doses of nicotine even on short-term cigarette smoke exposure [22], [23]. However, others also concern about the fact that heavy smokers tend to be less active to move physically than non-smokers and potentially gaining more weight as a result [24]. The limitations of this study are the exposure of cigarette smoke that given to rats simultaneously in the smoking chamber causing the amount level of cigarette smoke that inhaled for each rat was unknown, and the habit differences did not observe detailly. However, in general, visual observations, when rats exposed to cigarette smoke, they became restless, which suddenly show slower movements afterward. These observations were by habitual evaluation studies of rodents, which showed anxiety and fatigue after exposure to cigarette smoke [25].

Table 2. The level of blood biomarkers for Rat's Liver Function Tests after 45 days of treatments

Treatment Groups	Mean \pm SD			
	Protein Total (g/dl)	AST (U/L)	ALT (U/L)	ALP (U/L)
Normal (G1)	5.7 \pm 0.2	118.0 \pm 7.5	51 \pm 2.6	70.0 \pm 20.0
Cigarette smoke (G2)	5.7 \pm 0.1	146.0 \pm 2.0	66.3 \pm 14.0	104.0 \pm 38.5
Cigarette smoke+vitamin C (G3)	5.4 \pm 0.4	115.0 \pm 3.5	58.3 \pm 8.0	98.8 \pm 60.6
Cigarette smoke+CZE 350 mg/kg b.wt (G4)	5.4 \pm 0.3	116.0 \pm 4.3	54.0 \pm 5.3	85.0 \pm 10.0
Cigarette smoke+CZE 525 mg/kg b.wt (G5)	5.4 \pm 0.4	114.3 \pm 10.8	55.0 \pm 2.6	83.8 \pm 38.6
CZE 525 mg/kg b.wt (G6)	5.6 \pm 0.1	115.0 \pm 4.6	56.0 \pm 9.5	86.7 \pm 46.2

After 45 days of treatments, the blood samples were withdrawn to evaluate the rat's liver function based on its biochemical parameters (Table. 2). Evaluation of the level of total protein in smokers tends to be lower than non-smokers. Although the increasing level of oxidative stress due to cigarette-smoke exposure proved to stimulate more proteins production for rebalancing [26], higher oxidation of the albumin also rapidly cleared from the circulation and degraded [27]. In this study, total protein in the blood of cigarette smoke-rat groups and non-cigarette smoke groups showed nearly similar levels (Figure 2A). Nevertheless, these levels were slightly lower than the reference levels (6.3-8.6 mg/dl) [21], meaning that all the treatments given could affect the level of protein total in rats.

Evaluation on the transaminase enzymes after 45 days of treatments showed that there was a significant increase (about 25%) in mean AST levels in the negative control group received cigarette-smoke exposure compared to the other groups (Figure 2B). A statistically significant reduction ($p < 0.05$) in AST level between G2 and G3 which received Vit.C were similarly observed on other study used chemicals to induce oxidative stress in rat's liver [28,29]. Therefore, relatively similar data which showed declining AST levels on the groups treated with CZE 350 mg/b.wt (G4) or 525 mg/b.wt (G5) could be associated with the antioxidant properties of CZE to protect hepatotoxicity effect of cigarette smoke.

Evaluation of liver function using more specific biochemistry parameters ALT and ALP also showed an elevation of about 30% and 50% respectively on cigarette-smoke exposure group (G2) compare to the negative control group (G1). However, the statistical analysis evaluates that there were no significant differences in all treated groups, which indicates that induction of liver hepatotoxicity through cigarette-smoke exposure for 45 days was

inadequate. Alteration of AST level considers as a sensitive marker to detect damage in liver cells, yet this transaminase enzyme also presents in large numbers in heart, kidneys, skeletal muscles, and pancreas. Whereas, ALT is widely found in the liver and its elevation level in blood explicitly associated with the liver cell damage caused by drugs or other toxicants [30]. The results in this study found contrary to another study which discovered a high increase in ALT level of the rats after induced by cigarette smoke for 6 weeks [31].

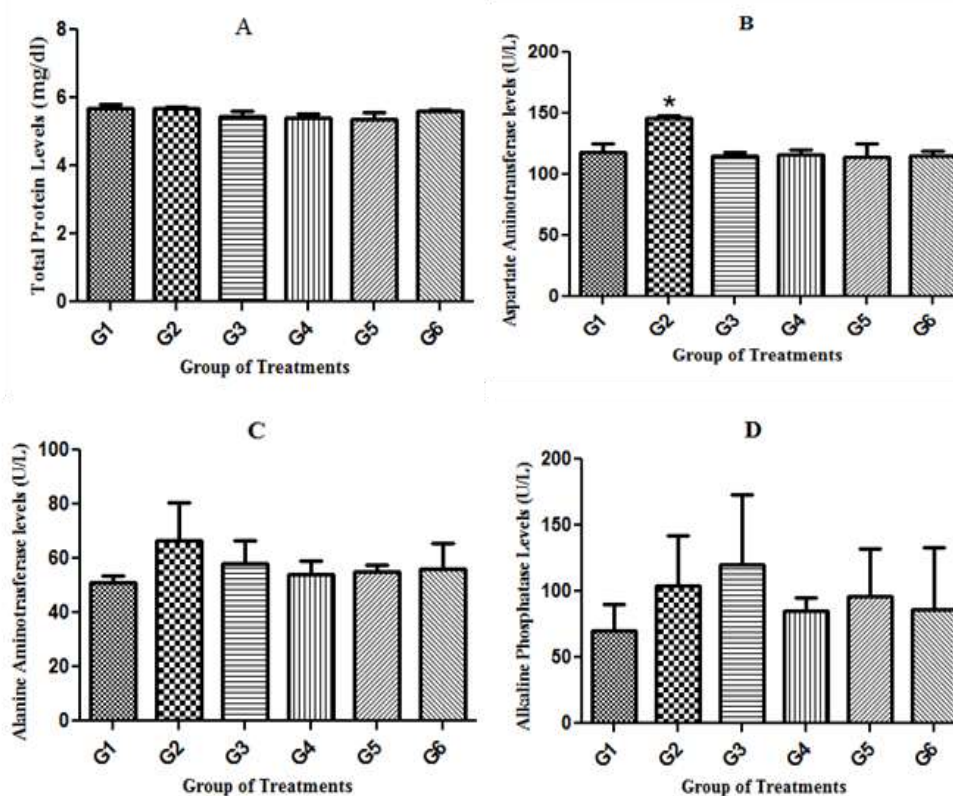


Fig. 1. Effects of ethanol extract of *Curcuma zedoaria* on the blood biomarkers of *Rattus norvegicus* exposed by cigarette smoke: total protein levels (A), AST levels (B), ALT levels (C), and ALP levels (D). Values are mean±SD (n=5). G1, control rats; G2, cigarette-smoke exposed rats; G3, cigarette smoke+Vit. C; G4, cigarette smoke+CZE 350 mg/kg b.wt; G5, cigarette smoke+CZE 525 mg/kg b.wt; G6, CZE 525 mg/kg b.wt. *P<0.05 statistically significant as compared with other groups.

Table 3. Malondialdehyde (MDA) levels on rat's liver after 45 days of treatment

Treatment Group	Mean ± SD (µg/mL)
Normal control (G1)	0,340 ± 0,274
Negative control (G2)	0,548 ± 0,197
Positive control (G3)	0,293 ± 0,178
Cigarette smoke + extract 350 mg/kg b.wt(G4)	0,296 ± 0,072
Cigarette smoke + extract 525 mg/ kg b.wt (G5)	0,313 ± 0,114

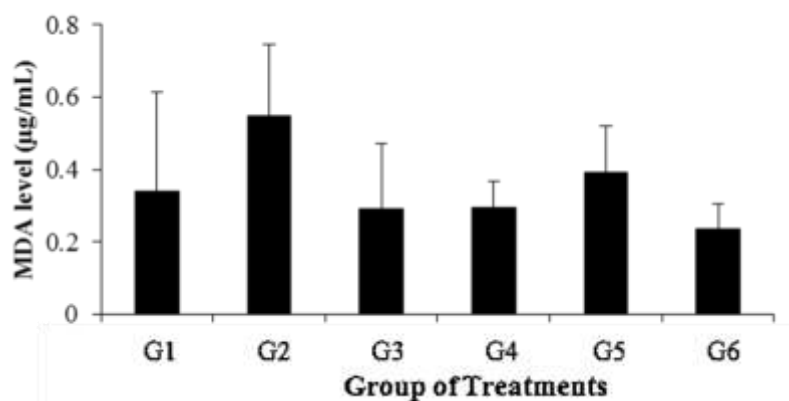


Fig. 3. The level of malondialdehyde (MDA) on rat liver after 45 days of treatments. Values are mean±SD (n=5). G1, control rats; G2, cigarette-smoke exposed rats; G3, cigarette smoke+Vit. C; G4, cigarette smoke+CZE 350 mg/kg b.wt; G5, cigarette smoke+CZE 525 mg/kg b.wt; G6, CZE 525 mg/kg b.wt.

Measurement of Malondialdehyde (MDA) levels as an indicator of lipid peroxidation carried out by thiobarbituric acid (TBA) method describes in Table 3. The mean MDA level of the negative control group was higher 37.97% than the normal group, and 46.53% higher than the positive control group receiving Vit.C. The higher level of MDA shows that exposures to cigarette smoke for 45 days was able to increase levels of free radicals in the body. This result is in line with the statement that cigarette smoke contains components that increase levels of free radicals in the body, thus potentially caused damage to liver cells [32],[33].

Meanwhile, the treatment groups receiving cigarette smoke and coadministered by Vit.C or CZE subsequently showed lower levels of liver's MDA compare to negative control and normal groups. The average level of MDA in G4 (CZE 350 mg/kg b.wt) is almost the same as the average MDA level of G3-the positive control group given vitamin C as a standard antioxidant meaning that orally administered of CZE has a reducing effect to MDA levels in rat liver. Moreover, the group which only received CZE 525 mg/kg b.wt had an average MDA level lower than all other treatment groups as well as showed no different statistically on AST, ALT, ALP levels to the normal control group (G1). This finding was in accordance by other studies which identified that the ethanol extract of some turmeric plants had free radical scavenger's activity to protect damage on hepatocytes but relatively has no toxic effect to liver cells [16][13]. The hepatoprotective effect of *Curcuma zedoaria* was supported by the antioxidant activity of curcumin and other essential oils as the active compounds which contain monoterpenes and sesquiterpenes (furanogermone) [34].

2.8 Conclusion

Administration of *Curcuma zedoaria* (Berg.) Roscoe Extract 350 mg/kg b.wt., and 525 mg/kg b.wt. Reduced total protein, ALT, AST, and ALP levels as well as decreased liver peroxidase level based on the average profile of MDA on rats exposed by cigarette smoke. Therefore, it can be concluded that the administration of *Curcuma zedoaria* (Berg.) Roscoe

Extract 350 mg/kg b.wt., and 525 mg/kg b.wt. showed the hepatoprotective effect on rats exposed by cigarette smoke.

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