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Liver Histopathological Change and Malondialdehyde Level of *Rattus Norvegicus* on Administration of *Curcuma Zedoaria* and Paracetamol Toxic Dose

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Abstract

High doses of paracetamol create necrosis in the liver and produce free radicals. When liver function decreased in a long time, it will lead to severe liver damage and it will be irreversible. Rhizome of *Curcuma zedoaria* has the potential effect as an antioxidant. It is assumed that its properties inhibit the formation of free radicals which formed from toxic doses of paracetamol. The aim of this study was to examine the histological structure of the liver and to determine malondialdehyde (MDA) levels in the administration of *C. zedoaria* toxic dose and paracetamol on the rats (*Rattus norvegicus*). The study was used twenty-four rats divided into four groups (positive control: carboxy methyl cellular (CMC) 0.5%; negative control: paracetamol 1.35g/kg body weight; treatment group 1 (T1): *C. zedoaria* 105mg/200g and paracetamol 1.35g/kg Body weight 2 hours later, and treatment group 2 (T2): paracetamol 1.35g/ kg body weight and *C. zedoaria* 105 mg/200g 2 hours later). The Kruskall-Wallis test results showed MDA level did not significantly different between groups ($p = 0.087$). Hepatocellular changes were observed descriptively with Hematoxylin-eosin staining. Positive control showed greater hepatocellular changes rather than other groups, hepatocyte cells were enlarged with cytoplasm showing eosinophilic granules infiltrates, enlarged irregular nuclei, nucleolus prominent, there are many necrosis cells.

Keywords

Antioxidant, *Curcuma zedoaria*, Hepatocyte, Malondialdehyde, Paracetamol.

INTRODUCTION

White Turmeric (*Curcuma zedoaria*) are found in tropical climates, which are often used as traditional medicine (1). Rhizome of *C. zedoaria* is widely used for the treatment of cancer, indigestion, chronic pelvic inflammation, blood clots, coronary heart disease and anemia (2).

C. zedoaria has bioactive components of flavonoids, curcuminoid polyphenols, terpenoids and essential oils. The Polyphenols are thought to be a potential antioxidants of this plant (3). The Phenolic or polyphenols were include to flavonoids, which are known to be modifiers biological response, functions as metal chelators and eradicating free radicals (4).

The ability of a component to capture free radicals and thus inhibit the oxidation process is called antioxidants (5). Free radicals including various other oxidants such as Reactive Oxygen Species (ROS) have detrimental effects and induce oxidative stress (6).

According to Ji et al (2), oxidative stress occurs when there is an imbalance between the formation and elimination of ROS. Free radicals are highly reactive and damage important molecules such as nucleic acids, proteins and lipids (2). Lipids will produce peroxidation when a free radical attack and break down the chain hydrogen from methylene, and form lipid radicals. When oxygen binds to lipid radicals form peroxyl lipid radicals which eventually become malondialdehyde (MDA) (6).

Inappropriate use of paracetamol or acetaminophen will induce necrosis and death in liver cells (7). Toxic doses of paracetamol leads to the production of the reactive metabolite N-Acetyle-p-benzoquinone imine (NAPQI) (8). NAPQI escalation and degradation of the amount of natural antioxidant hepatic gluthathione create hepatotoxicity (7).

The hepatoprotector effect of *C. zedoaria* to liver damage by paracetamol has been published yet, but the effect of administration of *C. zedoaria* and paracetamol toxic doses simultaneously has not been widely observed. The aims of this study was to examine the histological structure of the liver in the administration of *C. zedoaria* and paracetamol, conversely paracetamol and *C. zedoaria*, as well as the MDA level of *Rattus norvegicus.*

MATERIALS AND METHODS

Plants

The preparation of the extract from *C. zedoaria* and the experiment conducted in January, 2020. The *C. zedoaria* rhizome washed and cut into smaller pieces, then dried in a cold place. The pieces are mashed, filtered, until it obtains to a yellowish powder. The *C. zedoaria* powder then can be macerated using ethanol for 72 hours to extract all the compound in *C. zedoaria* that

dissolved with polar solvent (ethanol absolute). Futhermore, the extract was obtained by evaporation with a vacuum evaporator, extraction with evaporator temperature 60^{o} C and 100 rpm (9). Dose used in this study were 105 mg/ 200 g body weight (10).

Animals

The total of 24 adult male rats (*Rattus norvegicus*) were divided into 4 groups, namely 2 control groups (positive control and negative control), and 2 treatment groups (treatment 1 and treatment 2). The treatment was carried out for 7 days in the animal experimental laboratory of the Faculty of Medicine, Wijaya Kusuma University, Surabaya. Measurement of MDA levels was carried out at the Biochemistry Laboratory of the Faculty of Medicine, Wijaya Kusuma Surabaya University. Histopathological analysis was carried out at Laboratory of Pathology Anatomy, Airlangga University. This study received Ethical approval by Komisi Etik Penelitian Kesehatan Faculty of Medicine, Wijaya Kusuma Surabaya University (Reg. no. 06/SLE/FK/UWKS/2020.

Experimental Design

Hepatotoxicity effect in this study was performed by paracetamol at dose 1.35g/kg body weight (11). Oral route was used to paracetamol and *C. zedoaria* administration, because the treatment was done repeatedly for 7 days and consider this route was safer, it took slower onset of action which is important in drug effect experiment. Used of paracetamol single doses was performed in positive control. In negative control was indicated as normal indicator was administered by Carboxyl methyl cellulose (CMS) 0.5%. The treatment groups divided into 2 groups, which represent the effect of administration *C. zedoaria* extract at dose 105mg/ 200 g body weight and paracetamol at dose 1.35g/ kg body weight. The Treatment for group 1 (T1) was administered by paracetamol and 2 hours later was given *C. zedoaria* extract. The Treatment for group 2 (T2) was given *C. zedoaria* extract and administered by paracetamol 2 hours later. This study was conducted in 7 days.

Sample Analysis/ Sampling Analysis

On the day 8th after treatment, liver tissues were collected. Liver tissue were homogenized and measure of MDA levels by spectrophotometer. Spectrophotometric determination of liver malondialdehyde (MDA), according to the thiobarbituric acid (TBA).

Histopathological Analysis

The livers were collected in formaldehyde solution (10%). These samples were paraffined embedded, sectioned of 5 µm, and stained by hematoxylin-Eosin staining. Histological changes were observed by light microscope descriptively.

Statistical Analysis/Research Approach

The data were analyzed using the non-parametric Kruskal-Wallis test to analyze liver MDA levels, while descriptive analysis was used to observe changes in the structure of cells and tissues in the liver.

RESULTS

The differences in cell structure and tissue in the liver in each group are observed and analyzed descriptively. The results of this observations are presented in Figure 1. Histopathological changes observed by paraffin embedding methods use Hematoxylin-Eosin staining. Nucleus were stained by hematoxylin colored blue, and cytoplasm were red stained by eosin.

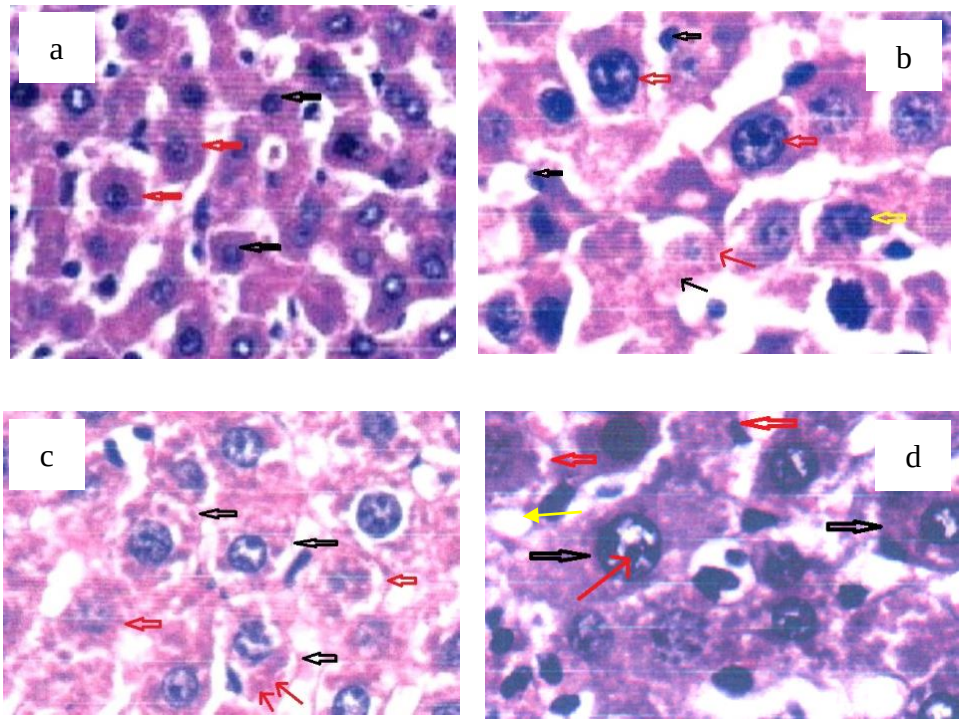

Figure 1. Hepar Histopathology in (a) negative control, (b) positive control, (c) treatment 1/ T1, (d) and treatment 2/ T2: Normal cell (thick red arrow) and nucleus (thick black arrow). Positive control: observed enlarged hepatocytes with nucleus (thick red arrow), eosinophils granules were found within cytoplasm (thin black arrow). Cell death and necrosis were found (thin red arrow) with inflammatory cells (thick black arrow). Irregular shaped of nucleus (thick yellow arrow). T1: showed enlarged nucleus of hepatocytes (thick black arrow), necrosis hepatocytes with pale and fragmented nucleus (thick red arrow) and granule eosinophils within cytoplasm (thin red arrow). T2: showed enlarged nucleus of hepatocytes (thick black arrow), more death or necrosis cell than T1 (Thick red arrow) and prominent of nucleolus (thin red arrow), found hepatic vacuolization (thin yellow arrow).

Histological changes at the Negative control group, the liver is in normal conditions, the normal hepatocyte cells were seen. The Positive control group was induced by toxic doses of paracetamol show enlarged hepatocyte cells with cytoplasm showing eosinophilic granules, enlarged nucleus and irregular shape of cells. The nucleolus was prominent, there are many cells in the mitosis phase. The liver histological in Positive control are also appear in the necrosis of cell. In addition, around the central vein appear inflammatory cells such as lymphocytes, histiocytes and polymorphonuclear (PMN) cells. The hepatocytes also experienced necrosis characterized by pale colored and fragmented nucleus, or even the cells loss of their nucleus. Meanwhile, T2 group show the cell histology that was not slightly different from the T1 group, but there were more necrotic of hepatocyte.

Liver oxidative stress represented by Malondialdehyde level in liver. The mean of MDA levels for each group are presented in Figure 2.

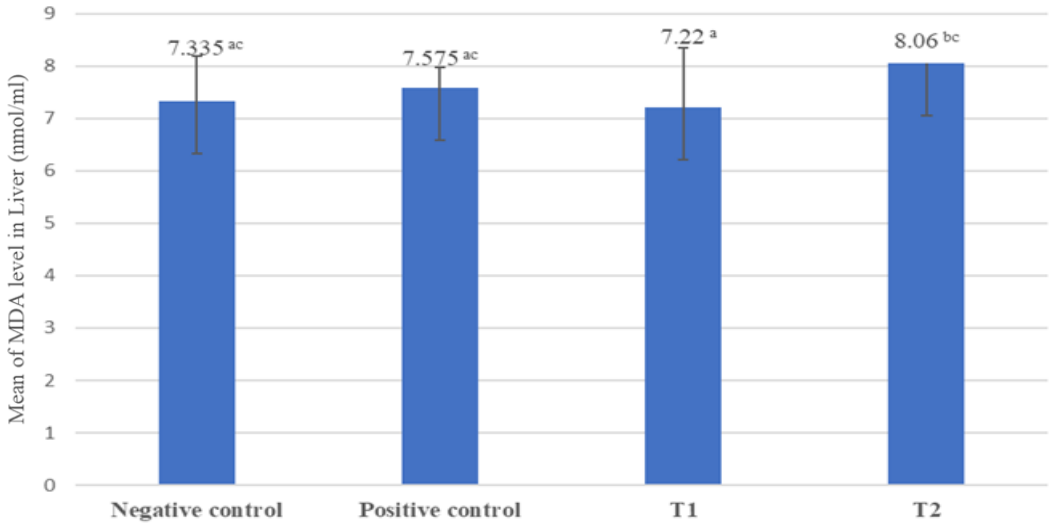

Figure 2. The Mean of MDA level on Negative control, Positive control, T1 and T2.

The highest mean level of MDA from the table above described in the T2 group, which is received *C. zedoaria* extract before giving paracetamol (8.18 nmol/mL). whereas administered of paracetamol before *C. zedoaria* extract (T1) show the lowest average of MDA level, which was 7.22 nmol/mL. Giving *C. zedoaria* after paracetamol (T1) compared to give *C. zedoaria* before paracetamol (T2) show that MDA levels were significantly different. The Statistical analysis using SPSS 16.0 software with the non-parametric Kruskal-Wallis test.

The results of statistical data analysis obtain p-value of 0.087 ($\alpha > 0.005$). The difference in liver MDA levels between groups is not significantly different.

DISCUSSION

The toxic doses of paracetamol used are known create a hepatic damage and hepatoxic. In accordance with the research of Rivera *et al* (12) it is known that the acute and repeated administration of paracetamol for 4 days found severe histological changes the sinusoidal dilatation, ballooning hepatocytes, lymphocyte infiltration (inflammation), hemorrhage and extensive pericentral hepatic necrosis. Acute and repeated administration of paracetamol lower the expression of Peroxisome Proliferator-activated Receptor alpha (PPARα). PPARα is a receptor that plays a role in controlling metabolic homeostasis, inflammation and fibrogenesis. The expression of hepatic PPARα receptors will decrease when there is an advanced inflammation and the fibrosis condition of Non-alcoholic Steatohepatitis (NASH) (13). This also illustrates that a decrease in PPARα indicates a lack of anti-inflammatory agents and an increase in ROS (12).

The results show an inconsistency, especially in the Positive control group where the level of MDA in the liver show a lower value than the T2 group. Paracetamol administration in positive control showed lower MDA when compared to T2 group. It is presumed because the levels of MDA show greater changes in the kidneys rather than the liver, as demonstrated in the study of Hamza and Al-Harbi (14) giving paracetamol dose of 2g/kg for 30 consecutive days can increase MDA levels in the liver and kidneys. MDA level in the liver were 12.35 nmol/g and MDA level in the kidney were of 38.68 nmol/g. MDA level of the liver in control group was 1.74 and 8.67 nmol/g in the kidney. Paracetamol will show higher levels of MDA in the kidneys.

The used of paracetamol dose to induce liver damage for seven days and demonstrate toxicity were insufficient. In the study of Bharatham et al (15), the toxic dose of paracetamol used are 3000mg/kg or 600mg/200g per day given for 7 days, whereas in this study are 1.35g/ kg body weight per day.

The Effect of *C. zedoaria* which is given 2 hours before paracetamol administration is not effective because of the short time of *C. zedoaria* activity to neutralize the oxidant from paracetamol. To analyze the effect of *Curcuma* as hepatoprotector and reduce liver enzymes, when antioxidants properties of *Curcuma* are given for seven days, the paracetamol is given on day eight of a single dose (16). The research model or design used to describe the hepatoprotector activity of *Curcuma* should be given antioxidants and paracetamol together several days in one group, followed by a single dose of paracetamol in the next day. Research by Dogaru et al (17) was given paracetamol along with Sylimarin antioxidant properties for five days and after that, the next day paracetamol was given. In group T2 of this

study, *C. zedoaria* as an antioxidant was given two hours before paracetamol without administered of paracetamol again in the next day. It is the possibility that antioxidant effect of *C. zedoaria* is not visible.

Repetitive paracetamol administration for 7 days could be resistance to hepatic damage. Hepatotoxic due to upregulation of the enzymes Glucose-6-Phospatease Dehydrogenase (G6PD) and Glutathione Reductase activity as an adaptive and protective response due to oxidative stress and lack of glutathione (18). Repetitive dose of paracetamol in Positive control did not shows statistically differences of MDA level to T2.

The effect of paracetamol and *Curcuma* towards MDA level slightly different with the histopathological changes. Administration of paracetamol show many changes in the hepatocellular. The effect of paracetamol at dose 1.35g/ kg body weight on liver histopathology is observed in the Positive control group. In this group, liver tissue damage was seen with hepatocellular necrosis, which is indicated by enlargement of the hepatocytes with granulated cytoplasm or vacuolation. It is related with several studies which illustrate that paracetamol overdose will produce centrilobular hepatic necrosis which can be fatal (19). Enlarged of nucleus and hepatocytes due to cell were prepared to undergo proliferation and mitosis. Liver is an organ has capability to regenerate after damaged by paracetamol. Exposure of paracetamol induce cell death and necrosis that can be recover by proliferating hepatocytes.

According to Thonda and Shivalinge (20), paracetamol administration causes necrosis in centrilobular hepatic necrosis which is characterized by nuclear picnosis and eosinophilic cytoplasm followed by excessive hepatic lesions. The covalent bond of N-acetyl-P-benzoquinone imine (NAPQI) which is an oxidative product of paracetamol, with sulfidrile group proteins, results in lipid peroxidation, degradation of gluthathione levels and produces necrotic cells in the liver.

Histopathological changes in the T1 and T2 groups show several enlarged hepatocyte cells and nuclei with irregular shapes. Administration of *C. zedoaria* before paracetamol (T2) show more necrotic cells and inflammatory cells rather than in T1. However, when those are compared with Positive control given toxic doses of paracetamol, it shows improvement in morphology and cell necrosis, although not all of the hepatocytesbecomes normal. Inflammatory cell appearance in Positive control and T2 indicate that necrotic hepatocytes produces by paracetamol are phagocyted by lymphocytes and PMN as inflammatory cells. Previous study reported that administration of paracetamol dose 1,000 mg/kg show the increase number of inflammatory cells i.e. neutrophil (21).

Between all groups, it shows that T1 is the group that point out the hepatocellular recover from paracetamol toxic dose. It is consistent with the MDA level, in T1 is the lowest level of MDA as well.

The role of *Curcumin* as an antioxidant agent is the scavenging activity against ROS. The antioxidant capacity of *Curcumin* is also related to the inhibition of lipid peroxidation and induction of translocation of Nuclear Factor E2 related Factor 2 (Nrf2) to the nucleus. *Curcumin* administration can reduce serum transminase, MDA and inflammatory cytokines in experimental animals given APAP (22), which is indicated by the absence of inflammatory cells such as PMN or lymphocytes. In this study represented by T1 where MDA showed lowest level than in other groups.

The limitation of this study is the inaccurate research design, then the exact biomarker testing that should be tested is Metalloproteinases (MMPs) as a marker of liver damage. The antioxidant and anti-inflammatory activities were more precisely observed with the ability to overcome ROS and improve cell viability in reducing cell death.

CONCLUSIONS

The administration of *C. zedoaria* and paracetamol on MDA levels has no effect. There were no differences in MDA levels between treatments. The most Histopathological changes are shown in paracetamol alone within 7 days of treatment.

AUTHOR CONTRIBUTIONS

Putu Oky Ari Tania: conceptualization, writing-reviewing, validation. Puja Ayu Misuari: data analysis, methodology. Satya Yudhayana: sampling and data collection. K. Ayesha Eldelwise Prayoga: writing-original draft preparation.

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CONFLICT OF INTEREST

The Authors declare no conflicts of interest regarding of this research.

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