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Effect Of Butterfly Pea Flower (*Clitoria Ternatea*) On Histopathology Of Male Rats Wistar (Rattus Norvegicus Strain Wistar) Hepatocyte Induced By Carbon Tetrachloride (CCl_4)

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ABSTRACT

Background: ILO stated that 2.3 million workers died due to occupational diseases , which one of them caused by chemicals. The chemical that can damage the liver is CCL4. That can increase *reactive oxygen species* have an important role in the pathogenesis of liver disease. CTE may have the potential hepatoprotector because contains antioxidants against oxidative stress. **Purpose:** To determine the effect of CTE on the histopathology of hepatocyte cells of Wistar rats induced by CCL4. **Method:** true experimental, post test only control group design with 4 groups. The positive group induced by CCL4 1 ml / KgBW subcutaneously dissolved in *olive oil* (1: 1) and the treatment group was given CTE 200, 400, and 800 mg/KgBW/day was induced by CCL4. **Results**: CTE had an effect on decreasing the number of steatosis cells. CTE affected 76.2% on the number of steatosis cells ($R^2 = 0.762$). The effective dose of CTE in this research is 800 mg/KgBW/day. The content of flavonoids and anthocyanins in CTE can againts free radicals, contribute hydrogen to radicals and isolate radical reactions so as to prevent steatosis in the liver. **Conclusion:** CTE has an effect on decreasing the number of hepatocyte cells, steatosis in male wistar rats induced by CCL4.

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1. INTRODUCTION

Indonesian society can no longer be protected from exposure to chemicals. Related to the wider and more free use of chemicals for various purposes. One of them is in jobs such as firefighters, farmers, industrial workers, and laundry workers. The International Labor Organization (ILO) stated that 2.3 million workers die each year due to occupational diseases [1]. More than 160 million workers suffer from occupational diseases and 313 million workers experience non-fatal work related diseases occur annually. Approximately 651,279 of them were caused by chemicals [2]. One of the chemicals that can damage the liver is carbon tetrachloride (CCL4).

CCL4 is often used by the household and industrial sectors as a cleaning agent, asphalt production, electrical equipment production, rubber production and used in fire extinguishing, besides that Indiana Department of Health stated CCL4 is also found in vehicle fumes5. The National Center for Biology Information says CCL4 can cause occupational diseases because the material evaporate easily so it has the potential to enter the body by inhalation, ingestion, eye contact or direct contact with the skin and it's very slow degradation in the

air so CCL4 is easy inhaled by humans [3]. In the body this compound can cause steatosis in the liver by attacking endoplasmic membrane lipids which can result disruption of protein synthesis which result in suppression of conjugation of triglycerides with lipoproteins which causes impaired lipid transport. High exposure of CCL4 can increase reactive oxygen species (ROS) which play an important role in the pathogenesis of liver disease21. ROS are highly reactive and classified as a free radical group and an increase of ROS can disrupt liver homeostasis which results in oxidative stress which can lead to liver damage [4] [5].

To neutralize these free radicals, antioxidants are needed. Antioxidants work to inhibit the formation of lipid peroxidation through accepting or donating electron to eliminate the unpaired condition of the radical. Antioxidants provide an electron to a free radicals so that free radicals becomes stable [6].

In Indonesia, there are many natural ingredients that contain antioxidants. One of these herbs is butterfly pea (Clitoria ternatea). Kazuma et al, 2003 have identified the content of butterfly pea petals containing flavonoids and anthocyanins. One of the main benefits of flavonoid compounds as natural plant antioxidants which act as reducers of hydroxyl radicals, superoxide and peroxyl radicals [7]. These antioxidant components act as a hepatoprotector against liver damage.

2. METHOD

This research is an experimental study with post test only control group design. This research was conducted at the Biomedical Laboratory of the Faculty of Medicine, University of Muhammadiyah Malang. 16 samples of Rattus norvegicus wistar strains aged 2-3 months with a body weight of 150- 250 grams, in good condition and characterized by active movement were divided into 4 groups namely positive control group and 3 treatment group with different dosage.

Administration of CCL4 and Clitoria ternatea Extract

Rats used as many as 16, were divided into 4 groups and each group consisted of 4 rats. The positive control group was given standard BR-1 feed and induced by CCL4 1 ml /KgBW subcutaneously dissolved in olive oil (1:1) 4 times in 2 weeks and the treatment group was given CTE 200, 400, and 800 mg/KgBW/day was induced by CCL4 for 14 days. The extract of Clitoria ternatea was carried out in the Herbal Laboratory of Materia Medica Batu using ethanolic 96% as a solvent. At the end of the treatment, all rats were killed to observe histophatology steatosis cells in liver, hematoxylin eosin has been stained. Observation were made by counting steatosis cells using a microscope with 400x magnification in 5 visual fields.

3. RESULTS AND DISCUSSION

This research was conducted on 16 rats, which did not obtain drop out criteria during the experiment. The results of the histopathological observation of the rat liver showed that the hepatocytes experienced steatosis in positive control group (C+) and treatment (T1, T2, T3) as shown below.

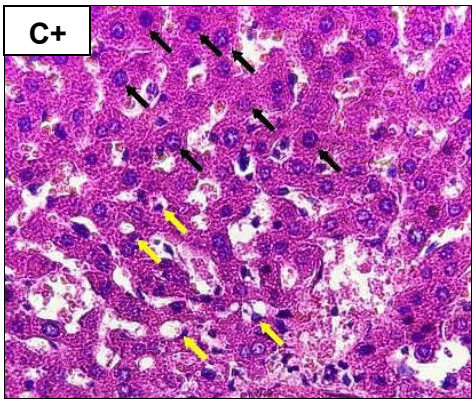

Figure 1. Positive Control Group
(yellow arrow) steatosis cells; (black arrow) normal hepatocyte cells

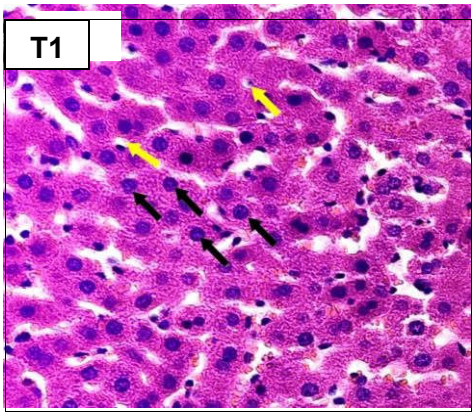

Figure 2. Treatment Group 1
(yellow arrow) steatosis cells; (black arrow) normal hepatocyte cells

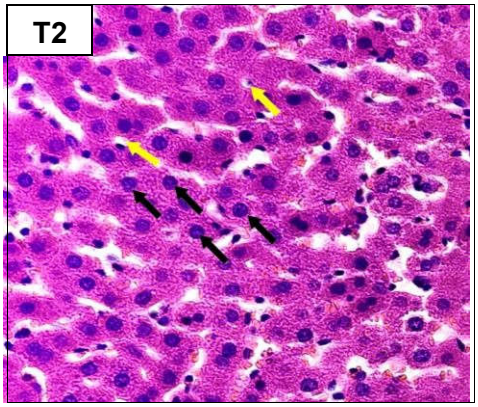

Figure 3. Treatment Group 2
(yellow arrow) steatosis cells; (black arrow) normal hepatocyte cells

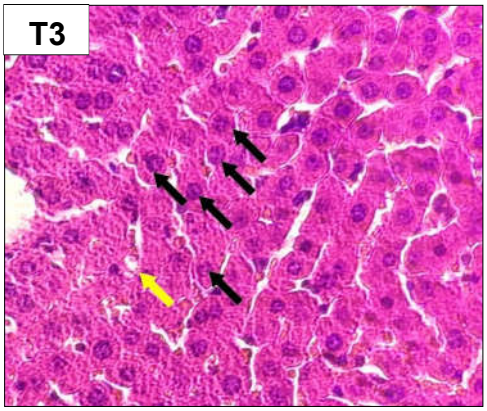

Figure 4. Treatment Group 2
(yellow arrow) steatosis cells; (black arrow) normal hepatocyte cells

Table 1. The Number of Steatosis Cells in Each Group.

Groups	The Number of Steatosis Cells in Each Group				Average ± SD
	1	2	3	4	
K+	61	77	68	86	73 ± 10,8627
T1	46	32	49	45	43 ± 7,52772
T2	22	19	21	23	21,25 ± 1,70782
T3	18	17	10	14	14,75 ± 3,59397

Note :
C+ : Positive control group
T1 : Treatment group with 200mg/KgBW/day Clitoria ternatea extract
T2 : Treatment group with 400mg/KgBW/day Clitoria ternatea extract
T3 : Treatment group with 800mg/KgBW/day Clitoria ternatea extract

Homogeneity test was performed with $p < 0.05$ and ANOVA test with $p = 0.000$. Based on the One-Way ANOVA result, there was significant difference between each groups. The results of the Post Hoc Tamhane test showed that there was a significant difference in the decrease in the number of hepatocyte cells experiencing steatosis in the control group with the P1, P2, and P3 groups ($p<0.05$). Based on the results of the linear regression test, it was found that the effect of Clitoria ternatea extract on the number of steatosis cells in the rat liver was 76.2% based on the results of the linear regression test which had a value (R-square: 0.762) and 13.8% influenced by other factors besides the Clitoria ternatea extract. The form of the equation connecting the dose of Clitoria ternatea extract given to the number of steatosis cells is as follows :

Y =62,300 +(-0,069)X
Note:
Y: The number of steatosis cells in rat liver
X: Clitoria ternatea extract dosage

The equation is significant by CCL4 administrasion, the number of steatosis cells is 62,3 cells and increasing dose of Clitoria ternatea extract 1 mg/KgBW/day decreasing 0.069 steatosis cells. Then the strength of a correlation is R: 0.873 which means had a strong correlation between telang flower extract and the decrease in steatosis cells.

Based on the calculation of the number of steatosis cells in the group of rats induced by CCL4 1 ml/KgBW subcutaneously 4 times in 2 weeks at a dose of dissolved in olive oil 1: 1 can cause steatosis cells in the liver. This study is in line with Maulina (2013) [8] used in this study that there was an increase in the number of steatosis cells in the control positive group induced by CCL4 ($P < 0.05$). This is also supported by the research of Gao et al. (2017) [9] which stated that the administration of CCL4 as much as 1 ml / KgBW occurred steatosis in the control positive group.

CCL4 undergoes biotransformation to become reactive which is catalyzed by the cytochrome enzyme P450 become CCL3 radicals which result in lipid peroxides, thereby causing susceptibility to the mitochondrial membrane and endoplasmic reticulum (RE). CCL3 radicals will undergo oxidation to become CCL3O2 radical which are more reactive so that lipid peroxidation occurs more rapidly resulting in disruption of calcium homeostasis [10] [11]. The mechanism of cell steatosis due to CCL4 involves the formation of free radicals that cause lipid peroxide and a decrease in antioxidant enzymes such as SOD, catalase, GPx, GSH, and glutathione-S-transferase [12]. The formation of steatotis cells begins with a disturbance in the cell membrane causing the liver cells to lose calcium homeostasis from the mitochondria and ER, resulting in increased calcium in the cytosol. This increase in cytosolic calcium concentration results in the activation of a number of catabolic enzymes, one of which is the ATPase-enzyme [13]. This enzyme activation causes a decrease in ATP synthesis, resulting in disruption in protein synthesis. Disruption in protein synthesis will inhibit the synthesis of protein units from lipoproteins and suppress the conjugation of triglycerides with lipoproteins. This results in lipoproteins not being formed so that lipid transport is disrupted. The disruption of lipid transport will cause the accumulation of lipids in the hepatocytes, resulting in steatosis [14].

The results of the One Way ANOVA $P < 0.05$ showed that there were differences between groups and the results of the test post hoc tamhane proved that the group of rats Clitoria ternatea extract in all treatment groups

had a significant effect on decreasing the average number of damaged hepatocyte cells experiencing steatosis compared to the positive control.

This is in accordance with previous studies that the dose of 400mg/KgBB of telang flower extract was significant able to increase the level of glutathione, catalase and superoxide dismutase (SOD) which can reduce liver enzyme levels in rats, significantly reduce damage to rat liver histopathology and reduce levels of malondialdehyde (MDA) which in the event of an increase in MDA in the liver indicates an increase in lipid peroxide and mechanism failure. antioxidant defenses [15] [16]. So that the researchers carried out variations in the dose of the telang flower extract in which P1 was given a dose of ½ n and P3 was given a dose of 2n.

The administration of Clitoria ternatea extract at a dose of 200 mg/KgBW/ day can significantly reduce steatosis cells compared to control positive with an average of 43 cells. Meanwhile, the dose of 800 mg/KgBW/day was significantly able to reduce the steatosis cells the most compared to the dosage of 200 mg/KgBW/day and 400 mg/KgBW/day with an average of 14.75 cells. The results of T1 (Clitoria ternatea extract 200 mg/KgBW) with T2 (Clitoria ternatea extract 400 mg/KgBW) and T2 (Clitoria ternatea extract 400 mg/KgBW) with T3 (Clitoria ternatea extract 800 mg/KgBW) were not significant differences ($p > 0.05$) although based on the mean number of steatosis T2, T3 was lower than the T1 group. This shows that giving a dose of 200 mg/KgBW with 400mg/KgBW and a dose of 400 mg/KgBW with 800 mg/KgBW there is no significant difference in reducing the number of steatosis cells.

Researchers utilized the antioxidant content of Clitoria ternatea extracts to repair histopathological damage to the liver by preventing an increase of steatosis cells because the antioxidant content of Clitoria ternatea can neutralize free radicals [17]. Another study stated confirmed the hepatoprotective effect of Clitoria ternatea extracts against the model hepatotoxicant acetaminophen and paracetamol because of their antioxidant content [18]. The antioxidant activity of flavonoids and anthocyanins contained in Clitoria ternatea can provide hepatoprotective effects through different mechanisms of action. Flavonoids scavenge free radicals thereby stabilizing and reducing free radicals thus protecting from increased lipid peroxide 25,19, while anthocyanins donate hydrogen to radicals and help end the radical chain reactions [21].

Based on the results of the linear regression test, it was found that the effect of Clitoria ternatea extract on the number of steatosis cells in the rat liver was 76.2% based on the results of the linear regression test which had a value (R-square: 0.762) and 13.8% influenced by other factors besides the Clitoria ternatea extract. Other factors that were not researched or the influence of other variables as a endogenous factors such as endogenous antioxidants (GSH, catalase, and superoxide dismutase) in rats [22] and research limitation that could not be controlled were exogenous factors such as the mouse environment before becoming the experimental animal of this study, adaptability and nutritional intake from food and drink rats that can affect the number of steatosis cells in this study [23].

The equation obtained from the linear regression test in this study ($y = 62{,}300 + (-0.069)\ X$. Increasing Clitoria ternatea extract 1 mg/KgBB/day decreasing 0.069 cells. The correlation results show the strength of the relationship with $R = 0.873$ which means had a very strong correlation between the Clitoria ternatea extract and the decrease in steatosis cells.

Based on the facts found in this study and through statistical studies, it can be concluded that the effective dose of Clitoria ternatea extract in this study was 800 mg/KgBW/day and the hypothesis regarding the provision of Clitoria ternatea extract induced CCL4 was proven to prevent an increase in rat liver steatosis cells.

4. CONCLUSION

The administration of Clitoria ternatea extract for 14 days effectively reduced the damage to hepatocytes in male wistar rats induced by CCL4 and the effective dose of telang flower extract in this study was 800mg/kgBW /day.

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