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Histology Study of Liver Changes Paracetamol-Induced Wistar Rats Treated with Sunkist (Citrus sinensis L. Osbeck) Extract

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Abstract

Unclear and high incidence and prevalence of hepatitis over the world. The peels extract of *Citrus sinensis* is riched in various phytochemical compound which may have antioxidant effects. This study is aimed to investigae the protection effect of ethanol extract from *Citrus sinensis* by histologic study of liver tissues in paracetamol-induced wistar rats. This study was using 15 rats which were divided into 5 groups included negative control, positive control, and ethanol extract groups (300 mg/kg b.w; 450 mg/kg b.w; 600 mg/kg b.w), after 14 days the rats was terminated and the liver was process to histology view. The result of this study showed that there was reducing in severity of degeneration following increasing of ethanol extract dosage. Based on the result study, ethanol extract of *Citrus sinensis* potentially has liver protection against paracetamol at highest dosgae by histology study.

Keywords: hepatitis; Citrus sinensis; copper; degeneration.

1. Introduction

Hepatitis is a term that is used to all type of inflammation involve hepatocytes. It can be caused by infection (viral, bacteri, parasite), drugs (traditional medicine), alcohol, excisive lipid, and autoimun diseases [1]. Various survey over the world show reltivily low incidencie of drug-induced hepatitis as cause of chronic or acute hepatitis. It was reported ncidence of drug-induced hepatitis was 1:10,000 to 1:100,000 patients.

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However, exactly incidence of drug-induced hepatitis was unclearly. It due to unsuficient of reporting system, dificulity in detection or diagnosis, and lack of survillence of drug-induced hepatitis [2]. The withdrawals of drug in United State was due to drug-induced hepatitis and around more than 50% were acute liver failure cases. Since 1998-2001 showed overall survival rate (includes liver transplantation case) was 72% [3]. Based on *Riset Kesehatan Dasar 2013* reported that increase incidence of hepatitis was diagnosed by clinical presentations 2-fold higher than in 2007 (1.2%). There were five provinces in Indonesia that had the highest prevalence of hepatitis included Nusa Tenggaraaa Timur, Papua, Sulawesi Sselatan, Sulawesi Tengah, and Maluku Utara [4]. Incidence of drug-induced hepatitis in North Sumatera was also unclear in the literature. However Riskesdas 2013 reported that Prevalence of Hepatitis in North Sumatera was 0.2% and one of thirteen province that had prevalence above average [4]. Recently, herbal drugs are popularly used as alternative drug over the world.. In United State, it was reported that 71% of Chile population and 40% in Columbia used herbal medicine [5]. Based on this fact, it needed to looking for natural source which can be used to protect the toxicity of drug against the liver. Indonesia are tropical country which rich in biodiversity after Brazil [6]. *Citrus sinensis* is commercial fruit crops which grown in all continent of the world, which are originated from South East Asia. This fruit had high nutritional value, source of vitamin, and other uses [7,8]. The peel of *Citrus sinensis* is rich in various phytochemical compound like phenolic acids, organic acids, and flavonoid which may have antioxidant effects [9]**.** Based on information above, this study was aimed to investigate liver protection effect of ethanol extract of *Citrus sinensis* against paracetamol toxicity through histology study in wistar rats.

2. Methods

2.1. Materials

Aquadest, ethanol, 10% buffer formalin, tween, sodium hydrogen phosphate, paracetamol, 0.5% Na CMC, concentrated alcohol, xylol, parafin, hematoxylin and eosin stain, peels of sunkist

2.2. Collection of Samples

The sample which was used in this study was peel of sunkist. It was gotten from one of subdistrict in North Sumatera named Pancur Batu. The sample was identified in Herbarium Medanense FMIPA USU.

2.3. Formulation of Extract from Sunkist Peel

Wistar rats which were used in this study were male wistars rats. They were housed in seperated cages according to their treatment group. In this study, there were 15 rats which were divided into 5 groups. These treatment groups were shown in table below.

Table 1: The Treatment Groups of Rats

Group	Treatment
Negative	0.5% Na CMC once the day for 14 days 1 g/kg b.w of paracetamol in 14th (6 hours after 0.5% Na CMC)
Positive	200 mg/kg b. w of catechin rutin once the day for 14 days 1 g/kg b.w in 14th (6 hours after cathechin)
Ethanol Extract of Sunkist-I	300 mg/kg b. w of ethanol extract of sunkist peel once the day for 14 days 1 g/kg b.w in 14th (6 hours after cathechin)
Ethanol Extract of Sunkist-II	450 mg/kg b. w of ethanol extract of sunkist peel once the day for 14 days 1 g/kg b.w in 14th (6 hours after cathechin)
Ethanol Extract of Sunkist-III	600 mg/kg b. w of ethanol extract of sunkist peel once the day for 14 days 1 g/kg b.w in 14th (6 hours after cathechin)

2.4. Histology Investigation

After 14 days of treatment, the liver from each rat in the groups was washed by Normal Saline and fixed into 10% buffer formalin. After that, the liver was dissected 4-6 mm in thickness and dehydrated by alochol in various concentration (70%, 80%, 90%, 95%) for 24 hours and in concentrated alcohol (100%) for an hours three times. After the tissues were dehydrated, they were washed by xylol for an hour three times. Then, the tissues were infiltrated into parafin and dissected 4-5 micron in thickness. At the last these samples were put into object glass and stains by HE.

3. Result

After 14 days of treatment, In the macroscopy level, there were not any significant changes in liver tissue which could be seen. However, there were liver changes in microscopy level which were shown in table below.

Table 2: Histopathology Changes for Each Treatment Groups of Rats

Groups	Type of Liver Changes			
	Degeneration	**Congestion**	**Necrosis**	**Hemorrhage**
Negative	+++	+	+	-
Positive	+	-	-	-
Ethanol Extract of Sunkist-I	+++	-	-	+
Ethanol Extract of Sunkist-II	++	-	-	-
Ethanol Extract of Sunkist-III	+	+	-	-

Based on table above, the negative group showed severe degeneration and mild congestion and necrosis, it was similar like lowest dosage group sample that showed severe degeneration with little hemorrhage. While the positive group showed an opposite view that had mild degeneration, it was similar like highest dosage group sample that showed mild degeneration with mild congestion. On the other hand, remain group which was moderate dosage group only showed moderate degeneration in the microscopy view.

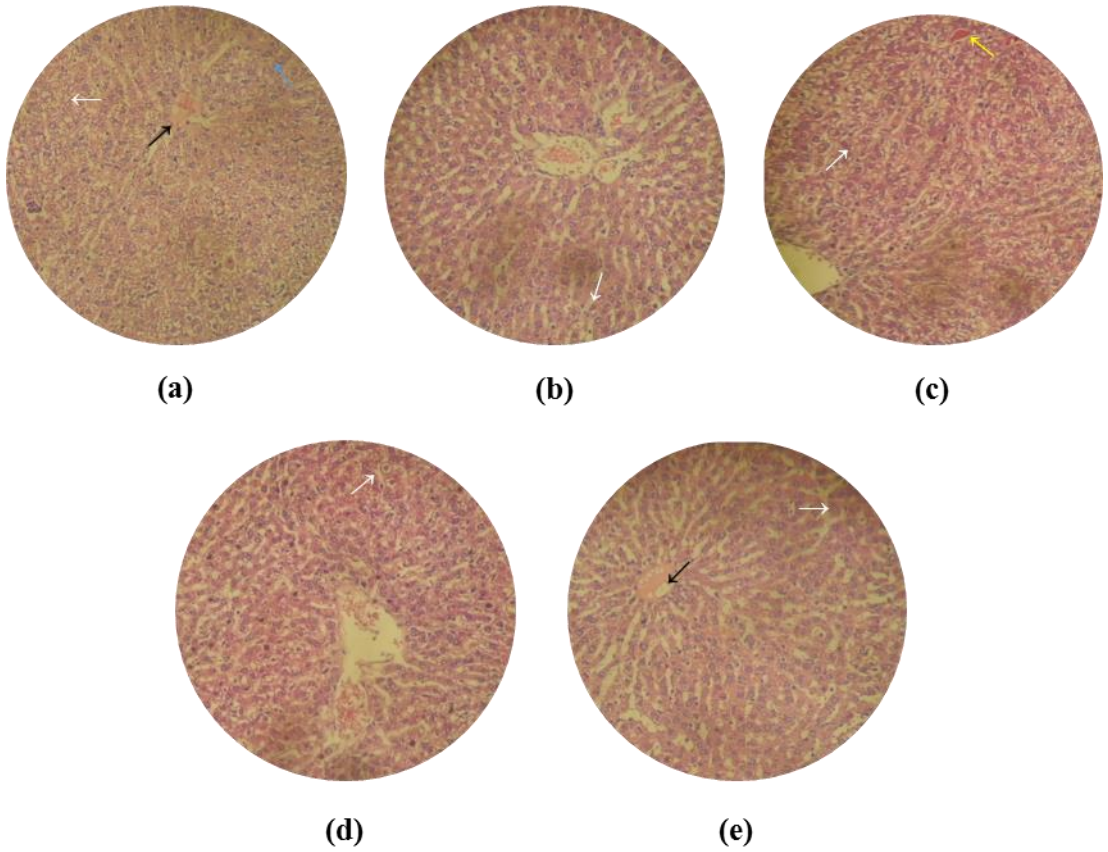

Figure 1: Microscopy view of Liver Histology. (a) Negative group; (b) Postive group; (c) Ethanol Extract of Sunkist-I; (d) Ethanol Extract of Sunkist-II; (e) Ethanol Extract of Sunkist-III. Stain: Hematoxylin and Eosin (H&E). Medium Magnification

The microscopy view of liver tissues in each groups sample was shown in figure above. The arrow tailed color show some histopathology change. The white, black, blue, and yellow arrow tailed showes degeneration, congestion, necrosis, and hemorrhage changes, respectively.

4. Discussion

Liver is the largest internal organ (except skin), weighing approximately 1,500 g and accounting for nearly 2-2.5% of adult body weight. It is located in the upper right and partially in the upper left quadrants of the abdominal cavity, protected by the ribcage. Basic structure of liver includes: parenchyma which is formed by hepatocyte which divided in to 3 zone (centrolobular, midzonal, and periportal area), connective tissue stroma, sinusoidal capillaries, and perisinusoidal space (spaces of disse) which lie between the sinusoidal endothelium

and hepatocyte [10,11]. Liver exhibit repeating hexagonal unit, know as lever lobules. Each center of liver lobule had a central vein, which radiate through plates of liver cells, called hepatocyte, and sinusoids toward the periphery. Each lobule consist of three to six portal areas. Portal areas or portal canals were formed by branches of hepatic artery, hepatic portal vein, bile duct, and lymph vessels [12] Necrosis is the last outcome from cell injury. Cell injury is resulted from cells that are no longer able to adapt to inherent damaging agent or suffer from intrinsic abnormalities. There are two type of cell injury includes reversible cell injury and irreversible cell injury (Cell death). Morphology changes of reversible cel injury includes cellular swelling and fatty changes while cell death divided into apoptosis and necrosis. Apoptosis is a physiologic process in body, however necrosis is an pathology process [13]. The commonest pathology process which will cause cell injury is ischemic or hypoxia. Furthermore, ischemic or hypoxia will progress to reduce ATP formation which will shift the metabolic pattern into anaerobic metabolism. Anaerobic metabolism will cause lowering cytoplasm pH that lead to nucleus clamping. At the next step, all of cellular derangements lead to cellular membrane damage. If the membrane damage cannot be recovery , it will be ended by necrosis [13,14].Necrosis is type of cell death which is associated with loss of membrane integrity and leakage of cellular contents, and ended by dissolution of cell, largely resulting from degradative action of enzyme on lethally injured cell. The leaked of cell content initiate local host reaction called inflammation and start the subsequent repair process. The enzyme responsible for the digestion come from lysosomes of dying cell or leucocyte which have come when the inflammation was initiated. There are some characteristic of necrosis includes cytoplasmic changes (increased eosinophilia), Nuclear changes (karyolysis, pyknosis, and karyrrhexis), and fates of necrotic cells (calcified) [13].There are some injury can cause necrosis of liver cells include microbiologic, toxic, circulatory or traumatic. There are some varies of necrosis in hepatic lobule, which divided into 3 type: diffuse (submassive to massive), zonal, and focal. Diffuse (submassive to massive) necrosis where there is extensive and diffuse necrosis of liver, while zonal necrosis occurs in 3 different zone of hepatic lobule, each type of zonal necrosis is caused by different etiologic factor. The commonest type of zonal necrosis is centrolobular necrosis which is characterized by ischemic injury due to this zone is the farthest zone from blood supply and it usually occurs in poisoning with chloroform, carbon tetrachloride, and certain drugs. The last type is focal necrosis which involves small groups of hepatocytes irregularly distributed in hepatic lobule and it's usually caused by microbial infection [14]. Acetaminophen which were widely known as Paracetamol. Normally, acetaminophen is mostly conjugated by glucoronide or sulphate in liver. About 5% or less it is metabolizes to NAPQI (N-Acetyl-p-benzoquinoneimine) by hepatic P-450 system. Very large doses of acetaminophen, NAPQI accumulation can cause centrolobular hepatic necrosis. NAPQI can reduce number of glutathione (GSH) that lead to increase formation of reactive oxygen species (ROS) [13]. According to Bander (2015) whether free radicals are highly reactive molecular species with an unpaired electron, which persistent for only a very short time before they collides with another molecule and either abstract or donate an electron in order to achieve stability. ROS (Reactive Oxygen Species) or Oxygen radicals are the most damaging radical in body, especially superoxide, $^{\bullet}O_2^-$, hydroxyl, $^{\bullet}OH$, and perhydroxyl, $^{\bullet}O_2H$. Free radicals can cause membrane damage which take important role in cell injury. Some mechanisms will cause membrane damage by free radicals includes lipid peroxidation, oxidation of protein, DNA damage, and cytoskeletal damage [14,15]. There are some factors that protects body against oxygen radical damge are known as antioxidants [15]. Ethanol extract of *Citrus sinensis* is riched by some phytochemicals includes saponins, tannins, terpenoid, alkaloid, steroids, cardiac glycoisdes, and flavonoid [7].

Polyphenols are a group of chemical compound which are found in plants that had aromatic ring (s) bearing on ormore hydroxyl group. Polyphenols are broadly divided into four classes including flavonoids (e.g., flavonols, flavones, isoflavones, and anthocyanidins), stilbenes, lignans, and phenolic acids. These compound will protect against effect of free radical by scavenging action through delocalization of the gained electron over the phenolic antioxidant and the stabilization by the resonance effect of the aromatic nucleus, which prevent the continuation of the free radical chain reaction [16]. Based on information above, mechanism of protection for liver against paracetamol due to presence of some phytochemicals which have antioxidant effect. It will prevent centrolobular necrosis in liver tissue due to impact of ROS which are formed by NAPQI.

5. Conclusion

There were protection effect of ethanol extract from peel of Sunkist for liver against paracetamol due to there was no significant microscopic changes of liver tissue.

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