Hepatoprotective Effect of *Trigona spp.* Bee Propolis against Carbon Tetrachloride-Induced Liver Injury in Rats

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Abstract

Background: Oxidative stress reaction can cause liver injury. This process can be prevented by antioxidant activities which can break the destructive chain caused by free radical substances in the liver. Propolis produced by *Trigona spp.* bee is known to have a high level of antioxidant. The aim of this study was to examine the effect of *Trigona spp*. bee propolis on liver histological toxicity caused by carbon tetrachloride-induced oxidative stress.
Methods: This experimental study was conducted in September 2013 at the Animal Laboratory of Departement of Pharmacology and Therapy, Faculty of Medicine Universitas Padjadjaran. Twenty-four healthy male Wistar rats as objects were adapted for one week and randomly divided into 3 groups. Group I was the control negative, group II was given carbon tetrachloride on day 14, group III was given *Trigona spp.* bee propolis on day 1-14. On day 14, group III was injected CCl4 intraperitoneally. The quantitative data were statistically analyzed using the one way ANOVA and Tukey test with p value < 0.05.
Results: Group I showed the liver contained normal cells, without significant injury of the membrane, round and complete nucleus. The average number of liver cell was 464 ± 9.59281 cells/field; group II underwent necrosis and the average of the cells was 146 ± 7.56885 cells/field; group III showed some normal liver cells, and some necrotic area with the normal liver cells average was 263 ± 14.10860 cells/field. The p-value=0.00.
Conclusions: *Trigona spp.* bee propolis has a hepatoprotective effect against CCl4-induced liver injury histologically. **[AMJ.2016;3(3):482–6]**

Keywords: Carbontetrachloride, hepatoprotective, propolis, *Trigona spp.*

Introduction

Hepatitis is still a serious health problem in Indonesia. According to *Riset Kesehatan Dasar* (Riskesdas) Nasional 2007, Indonesia was placed as the second largest country among other SEARO countries with hepatitis patients.[1] In October 2007–2009, the Ministry of Health of the Republic of Indonesia reported about 17.999 positive hepatitis C cases. Infection with hepatitis C is associated with increased levels of Reactive Oxygen Species (ROS)/Reactive Nitrogen Species (RNS) and decreased antioxidant levels.[2] ROS/RNS is a cause of oxidative stress.

Oxidative stress is a condition where there is a high level of free radicals; one of the potential substance is carbon tetrachloride which has strong connection with pathologic process like cell destruction.[3] Currently, it is commonly used for inducing hepatitis model in laboratory experimental animals. When carbon tetrachloride enters the body, this substance will be metabolized in the liver and will produce free radical such as trichloromethyl radical (CCl3) and trichloromethyl peroxy radical (CCl3O2) that induce lipid peroxidation and death of the cell.[4] One substance that can prevent lipid peroxidation is antioxidant. Furthermore, propolis is a natural product, which is a mixture of resin and beeswax collected from plants particularly from flowers and leaf buds by honey bees (*Apis spp.* and *Trigona spp.).* It contains many chemical substances and varies depending on the plants resin geographically. The *Trigona spp.* bee are found on many islands in the Indonesian region and it has already been studied that it has more antioxidant substance than the

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Table 1 Average Number of Normal Hepatocyte from Each Group

Group	Number of Normal Hepatocyte (mean±SD)	p *
Group I	463.50±9.59281	0.00
Group II	146.07±7.56885	0.00
Group III	263.24±14.10860	0.00

Note: p* = one way ANOVA

propolis produced by the Apis mellifera.[5,6] Until now, there has been only few studies about its hepatoprotective effect.[7] The objective of this study was to examine whether the *Trigona spp.* bee propolis has a hepatoprotective effect against oxidative stress mechanism, hence after further preclinical and clinical studies has been performed, it expected that this propolis could be used effectively by hepatitis high risk patients and also by patients with chronic hepatitis.

Methods

This experimental research was conducted at the Animal Laboratory of the Pharmocology Department, Faculty of Medicine Universitas Padjadjaran, in September 2013. Twenty-four healthy male Wistar rats as objects were used.[4] All laboratory experimental animals in this study was approved by the Health Research Ethics Committee Faculty of Medicine Universitas Padjadjaran. The animals were randomly divided into 3 groups and given standard food and water. This study was performed in fifteen days, excluded from the one week of adaptation. Group I was the control negative, which was given standard food and water. Group II was the control positive which was given 1ml/kgBW CCl4 50% intraperitoneally at day 14 to induce necrosis of hepatocytes.[8] Group III was given 1ml/kgBW Trigona spp. propolis on day 1–14, and on day 14, three hours after given propolis, the rats were injected with 1 ml/kgBW CCl4 50% intraperitoneally.[9] Twenty-four hours after the last treatment, all rats from all groups were sacrificed. First the animals were anaesthetized using ketamine intramuscularly, and then laparotomy was performed to expose the liver organ. Afterward, the liver was perfused using NaCl 0.9% through the left ventricle, and blood was cleared from the body through inferior vena cava. The liver was also washed with 10% formalin, then excised and transferred to a 10% formalin fixative

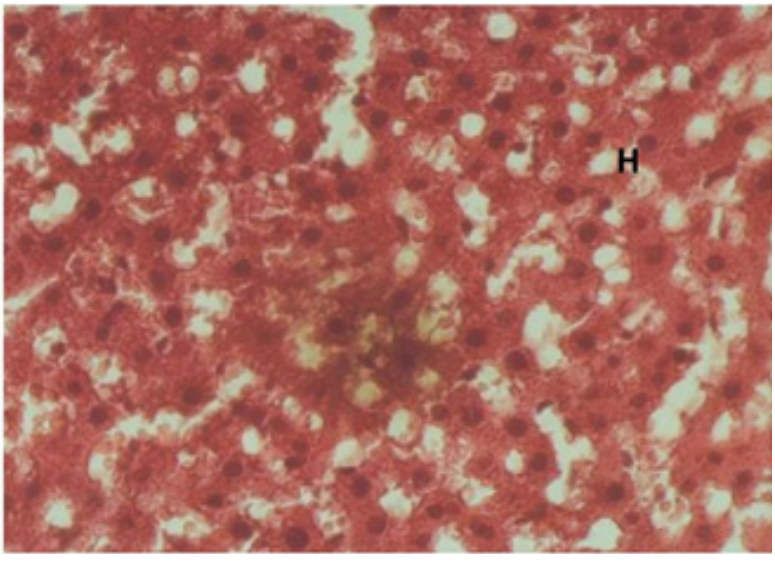

Figure 1 Liver Histologic View from Group I
Note: H=normal hepatocyte

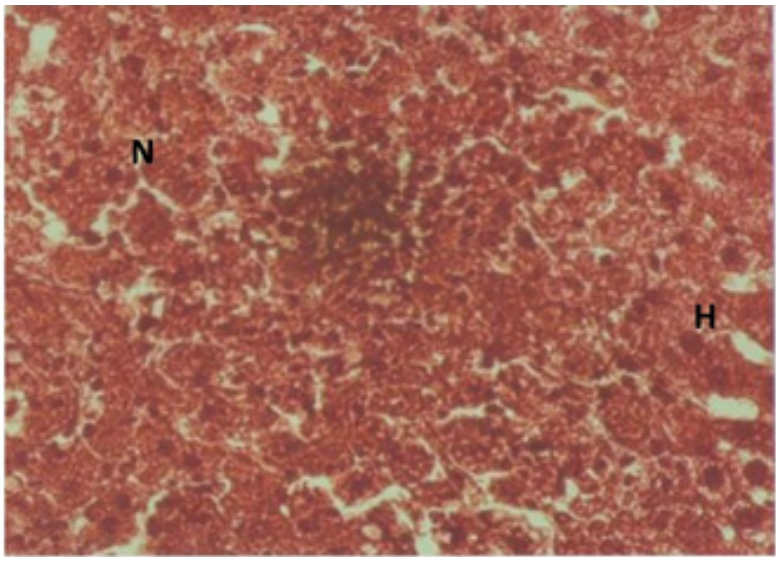

Figure 2 Liver Histologic View from Group II
Note: H=normal hepatocyte, N=necrotic area

solution. The liver tissues were processed for paraffin embedding and sections (5 µm thick) were taken in a microtome. After staining with hematoxylin and eosin, slides were examined under the microscope (400x) for histopathological changes and counted for the average of the normal cells in each group The existence of a necrotic area on hepatocytes, was characterized by rupture of the cell membrane, vacuolization of the cytoplasm, karyolisis, pyknosis, and karyorrhexis. Moreover, the quantitative data were statistically analyzed using the One Way Analysis of Varian (ANOVA) followed by the Tukey test using the Statistical Product and Service Solutions (SPSS) for Windows version 13.0. A p-value of less than 0.05 was considered as statistically significant.

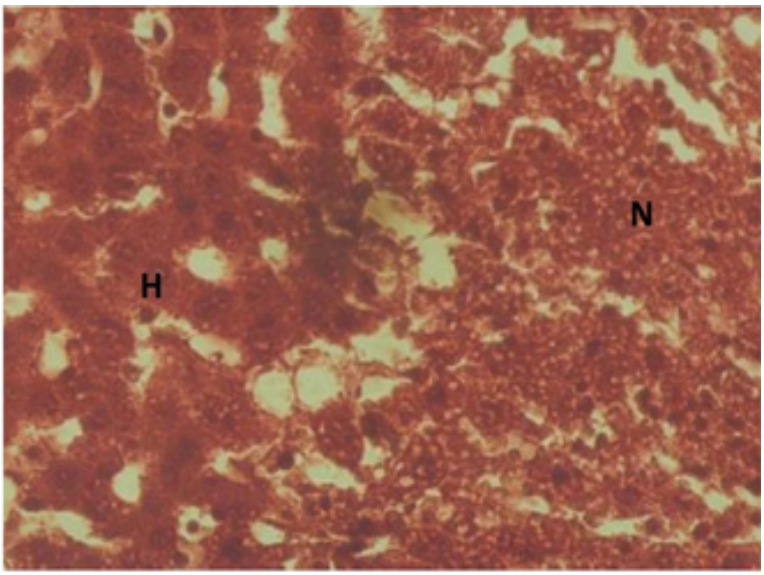

Figure 3 Liver Histologic View from Group III
Note: H=normal hepatocyte, N=necrotic area

solution. The liver tissues were processed for paraffin embedding and sections (5 µm thick) were taken in a microtome. After staining with hematoxylin and eosin, slides were examined under the microscope (400x) for histopathological changes and counted for the average of the normal cells in each group The existence of a necrotic area on hepatocytes, was characterized by rupture of the cell membrane, vacuolization of the cytoplasm, karyolisis, pyknosis, and karyorrhexis. Moreover, the quantitative data were statistically analyzed using the One Way Analysis of Varian (ANOVA) followed by the Tukey test using the Statistical Product and Service Solutions (SPSS) for Windows version 13.0. A p-value of less than 0.05 was considered as statistically significant.

Results

The data analyzed by the one way ANOVA statistical test showed p = 0.00; it means, the difference of the average number of normal hepatocytes between groups was significant.

The Tukey test also showed the difference of the average number of normal hepatocytes between groups were significant one by another. The result from group I showed the liver contained normal cells, without significant injury of the membrane, round and complete nucleus. The average number of liver cell in this group was 464 ± 9.59281 cells / field (Figure 1); group II underwent necrosis and the average of the cells was 146 ± 7.56885 cells / field (Figure 2); group III showed some normal liver cells, and some necrotic area with the normal liver cells average was 263 ± 14.10860 cells / field (Figure 3).

Discussion

The effect of CCl_4 in 24 hour acute exposure in the control positive group was marked by the existence of necrotic area on hepatocytes, characterized by the rupture of cell membrane, vacuolization of the cytoplasm, karyolisis, pyknosis, and karyorrhexis. These findings are further supported by earlier reports by Girish et al.[8] that CCl_4 produced various histological changes in the hepatocytes after 24 hours of administration.[8,9] The analytic study of the average number of normal hepatocytes showed the difference between the control negative and control positive group were significantly different (p=0.00). It could be concluded that CCl_4 that was given to group II made significant injury to the liver cell. This process began from CCl4 turned into CCl3 and reacted with the oxygen forming more reactive substance CCl_3O_2 and then, it reacted with membrane cell phospholipid and initiated an extensive intrahepatic peroxidation reaction chain. In the end, there was necrosis of the hepatocytes.[4]

Group III had more normal hepatocytes compared to group II. The data were significant after being analyzed statistically. The protective effect of this propolis was about 37%. According to Chanchao et al.[10], the propolis itself is non-toxic to the normal cells. The condition of oxidative stress is established due to insufficient defense capacities against reactive oxygen species (ROS). The ROS also affect the antioxidant defense mechanism, and reduce intracellular concentration of antioxidant produced by the body. Although our body has its own antioxidant and regulation to this substance capacity physiologically, other source of antioxidant is important as well for scavenging free radicals that are formed in the body. Additionally, propolis consists of more than 300 different compounds including flavonoids, phenolics, wax, vitamins, and minerals.[5] It has been proved that bees do not change its chemical composition. In propolis, the major antioxidant is flavonoids. It could give protection to the liver cells by decreasing formation of CCl_3 and CCl_3O_2.[11] It has already been studied that Trigona spp. bee propolis has a high level of antioxidant, especially flavonoid 4% which is higher than in another propolis that is produced by Apis mellifera bee is 1.5%.[5,6] Besides, the propolis contains another substance that is known for its antioxidant and antiinflammatory activities such as caffeic acid phenethyl ester (CAPE) where it showed a free radical scavenging effect.[12]

It can be concluded that *Trigona spp.* bee propolis has a hepatoprotective effect against CCl4-induced liver injury in male Wistar rats histopathologically.

References

1. Kementerian Kesehatan Republik Indonesia. Masyarakat Dunia Peringati Hari Hepatitis. [cited 28 July 2010]. Available from: www.depkes.go.id/index.php?vw=2&id=1156.
2. Muriel P. Role of free radicals in liver diseases. Hepatol Int. 2009;3(4):526–36.
3. Kumar V, K.Abbas A, Fausto N, Aster JC. Cellular Responses to Stress and Toxic Insults: Adaptation, Injury, and Death. In: Schmitt W, Gruliow R, editors. Robbins and Cotran Pathologic Basis of Disease. 7th ed.

Philadelphia: Elsevier Saunders; 2010. p. 20–2.
4. U.S Environmental Protection Agency Washington D. Toxicological review of carbon tetrachloride. Washington DC: United States Environmental Protection Agency; 2010.
5. Surendra NS, Bhushanam M, Ravikumar H. Antimicrobial Activity of Propolis of Trigona sp. and Apis mellifera of Karnataka, India. Prime Journal of Microbiology Research. 2012;2(2):80–5.
6. Mahani, Karim R, Nurjanah N. Keajaiban propolis trigona. Jakarta: Pustaka Bunda Grup Puspa Swara; 2011.
7. Umthong S, Puthong† S, Chanchao C. Trigona laeviceps Propolis from Thailand: antimicrobial, antiproliferative and cytotoxic activities. Am J Chin Med. 2009;37(05):855–65.
8. Girish C, Koner BC, Jayanthi S, Rao KR, Rajesh B, Pradhan SC. Hepatoprotective activity of six polyherbal formulation in CCl4-induced liver toxicity in mice. Indian J Exp Biol. 2009;47(4):257–63.
9. Xu J-Y, Su Y-Y, Cheng H-S, li S-X, Liu R, Li W-X, et al. Protective effects of fullerenol on carbon tetrachloride-induced acute hepatotoxicity and nephrotoxicity in rats. Carbon. 2010;48(5):1388–96.
10. Chanchao C, Umthong S, Phuwapraisirisan P, Puthong S. Trigona laeviceps propolis: chemical compositions and antiproliferative activity on cancer cell lines. Planta Med. 2011;77:49.
11. Shukla S, Bhadauria M, Jadon A. Effect of propolis extract on acute carbon tetrachloride induce hepatotoxicity. Indian J Exp Biol. 2004;42(10):993–7.
12. Wu W-M, Lu L, Long Y, Wang T, Liu L, Chen Q, et al. Free radical scavenging and antioxidative activities of caffeic acid phenetyl ester (CAPE) and its related compounds in solution and membranes: a structure-activity insight. Food Chem. 2007;105(1):107–15.