

Comparison of the effectiveness of honey and vitamin C in inhibiting the occurrence of liver fibrosis and lipid peroxidation in biliary obstruction of Wistar Rats

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ABSTRACT

Introduction: Jaundice comes from the Greek, a clinical condition with skin color and mucosa to become yellowish. The cases number of obstructive jaundice with choledocholithiasis at Hasan Sadikin Hospital Bandung-Indonesia from 2014 to 2016 were 132 cases. On the other hand, honey and vitamin C, which are supplements that are easy to locate in Indonesia, have antioxidant and hepatoprotective abilities to inhibit liver fibrosis and reduce MDA levels effectively, which is one indicator of lipid peroxidation. Therefore, this study tried to compare the role of honey with vitamin C in inhibiting the occurrence of liver fibrosis and lipid peroxidation.

Methods: This study was an experimental study with a parallel design that aimed to determine the formation of liver fibrosis and plasma malondialdehyde (MDA) values as an indicator of lipid peroxidation in biliary obstruction conditions given honey and vitamin C (treatment group) and without honey and vitamin C (control group) in Wistar rats.

Results: Giving honey 3 mg and vitamin C 225 mg in biliary obstruction for 14 days gave an inhibitory effect on liver fibrosis and lower plasma MDA levels compared to the control group. Also, honey had an inhibitory effect on fibrosis and lower plasma MDA levels compared to vitamin C.

Conclusions: Oral administration of 3 mg honey in biliary obstruction of Wistar rats had better results than 225 mg of vitamin C in inhibiting liver fibrosis and lipid peroxidation.

Keywords: jaundice, honey and vitamin C, biliary obstruction, liver fibrosis, lipid peroxidation.

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INTRODUCTION

Jaundice is a clinical condition that occurs due to increased bilirubin levels in the plasma whose levels reach > 3 mg/dl. Jaundice has symptoms in the form of discoloration of the skin and mucosa to be yellowish. The prevalence of jaundice cases in Indonesia is still not known with certainty, but there was a prevalence of several diseases that cause clinical manifestations of jaundice.^{1,2} In the United States, about 10-15% of the adult population suffered from gallstones, while in Asia the figure was the incidence ranges from 3-10%.³ The prevalence of gallstones in China was about 10.7%, Japan was 3.2%.⁴ Patients with jaundice due to extrahepatic biliary obstruction have a high potential for complications

that can even lead to death. The main difficulties of obstructive jaundice are cholangitis, coagulation disorders, and liver damage that progresses to liver fibrosis and cirrhosis.² The number of choledocholithiasis patients at dr. Hasan Sadikin Hospital in Bandung in 2014-2016 was 132 cases of obstructive jaundice with choledocholithiasis and most of them occurred at the age of >40 years (68%) and in women (60%) with the manifestation of cases were upper abdominal pain and jaundice all over the body, and an increase in serum alkaline phosphatase was found in all patients which indicated a decrease in liver function.¹

On the other hand, honey and vitamin C are two things that are easy to find in Indonesia and long been trusted as

ingredients that can be beneficial for health. Honey is a by-product of flower nectar and the upper digestive tract of honey bees, which is concentrated through the dehydration process in the beehive.⁵ Honey has bactericidal, bacteriostatic, antifungal, antiviral, scolicidal, antioxidant, antitumoral, hepatoprotective and anti-inflammatory effects. The phenolic compounds of honey are phenolic acids and flavonoids, which act as antioxidants and hepatoprotective.⁶ Giving honey 10 mg/kgBW per day can prevent liver cell damage caused by obstructive jaundice.^{7,8} Apart from honey, vitamin C is also a strong reducing agent and free radical scavenger in biological systems. As an effective reducing agent, vitamin C also functions as a powerful

antioxidant, capturing reactive oxygen and nitrogen species in the body. Reactive species are produced by normal cellular processes as well as environmental stressors and can cause oxidative damage to lipids, cellular proteins, and nucleic acids in DNA.⁴

In Indonesia, Vitamin C can be found either in the form of supplements or raw materials, or food, while most honey production in Indonesia still depends on natural products (forests). Honey and vitamin C both have antioxidant abilities so that they can effectively reduce MDA levels which is one of the indicators of lipid peroxidation.^{8,9,10} Thus, it can be identified that honey and vitamin C each have antioxidant and hepatoprotective effects. Therefore, researchers need to conduct a study that analyzes the comparison of

the role of honey and vitamin C in the occurrence of lipid peroxidation and liver fibrosis by using rats treated with biliary duct ligation so that it causes the effect of biliary obstruction.

METHOD

This study was an experimental study with a parallel design that aimed to determine the formation of liver fibrosis and plasma malondialdehyde (MDA) values as an indicator of lipid peroxidation in biliary obstruction conditions given honey and vitamin C (treatment group) and without honey and vitamin C (control group) in Wistar rats. In this study, 28 rats were used which were divided into 4 treatment groups (groups A-D). The sample inclusion criteria used in this study were Wistar rats

aged 4-6 months, Rattus strain rats, male, body weight 300 g, and in good condition. Group A was the sham surgery group (placebo), which received laparotomy without BDL (bile duct ligation). Group B was the group with BDL action without honey and vitamin C (control). Group C was the group with BDL action and given 3 mg of honey.¹¹ Group D was the group with BDL action and 225 mg of vitamin C. The administration of honey and vitamin C was oral.

Histopathological examination of liver tissue was carried out using a laparotomy procedure to perform a liver incision biopsy. Lipid peroxidation in this study was examined using the spectrophotometric method of 2-thiobarbituric acid reactive substances (TBARS). From the histopathological assessment of the liver

Table 1. Results of calculation of liver fibrosis semiquantitative severity score.

Variable	Groups				p
	A	B	C	D	
Central Vein					
Normal	7 (100.0%)	4 (57.1%)	8 (85.7%)	5 (71.4%)	0.265
Moderate Thickening	0 (0.0%)	3 (42.9%)	1 (14.3%)	1 (14.3%)	
Clear Thickening	0 (0.0%)	0 (0.0%)	0(0.0%)	1 (14.3%)	
Fibrosis					
Perisinusoidal					
Normal	7 (100.0%)	2 (28.6%)	7 (100.0%)	5 (71.4%)	0.006*
Localized fibrosis	0 (0.0%)	5 (71.4%)	0 (0.0%)	2 (28.6%)	
Diffuse Fibrosis	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Portal Track					
Normal	7 (100.0%)	1 (14.3%)	4 (57.1%)	3 (42.9%)	0.007*
Without Septa	0 (0.0%)	1 (14.3%)	3 (42.9%)	0 (0.0%)	
With Septa	0 (0.0%)	6 (71.4%)	0 (0.0%)	4 (57.1%)	
Cirrhosis	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Number of Septa					
None	7 (100.0%)	2 (28.6%)	7 (100.0%)	3 (42.9%)	0.005*
≤6/10 mm	0 (0.0%)	3 (42.9%)	0 (0.0%)	3 (42.9%)	
>6/10 mm	0 (0.0%)	2 (28.6%)	0 (0.0%)	1 (14.3%)	
Nodular organization	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Septa Width					
Thin	7 (100.0%)	2 (28.6%)	7 (100.0%)	3 (42.9%)	0.005*
Thick	0 (0.0%)	4 (57.1%)	0 (0.0%)	3 (42.9%)	
Very thick	0 (0.0%)	1 (14.3%)	0 (0.0%)	1 (14.3%)	
>2/3 biopsy area	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	

*Significant (p<0.05)

Table 2. Malondialdehyde (MDA) level comparison between all groups.

Lipid Peroxidation	Groups				P
	A	B	C	D	
$\bar{X}\pm SD$	3.29±1.17	10.73±1.49	4.31±2.68	4.54±2.80	0.001
Median (min-max)	3 (1.88-5.63)	10.75 (8.50-12.88)	3.38 (1.63-9.88)	3 (1.88-9.13)	

*Significant (p<0.05)

Table 3. Post hoc analysis of advanced liver fibrosis.

Groups	A	B	C	D
Central Vein				
A		0.060	0.317	0.142
B			0.254	0.762
C				0.476
D				
Perisinusoidal				
A		0.007*	1.000	0.141
B			0.007*	0.122
C				0.141
D				
Portal Track				
A		0.002*	0.060	0.023*
B			0.015*	0.450
C				0.187
D				
Number of Septa				
A		0.009*	1.000	0.024*
B			0.009*	0.493
C				0.024
D				
Septa Width				
A		0.008*	1.000	0.024*
B			0.008*	0.674
C				0.024

*Significant (p<0.05)

Table 4. Post hoc analysis of advanced lipid peroxidation.

Groups	A	B	C	D
A		0.000*	0.385	0.289
B			0.000*	0.000*
C				0.845
D				

*Significant (p<0.05)

tissue incision biopsy and plasma MDA levels of each rat in each group, the result numbers were grouped, ranked, and analyzed. The data hereupon be tested statistically to assess its significance.

RESULT

The group treated with honey and vitamin C had mild fibrosis compared to the control group with more severe fibrosis, and there was light fibrosis of liver cells in the honey treatment group compared to the treatment group with vitamin C (Table 1).

Lipid peroxidation in this study used the MDA concentration value. Based on the results of the calculations in table 1, the average value of MDA concentration in group A was 3.29 micromolar with a standard deviation of 1.17 micromolar. The average value of MDA concentration in group B was 10.73 micromolar with a standard deviation of 1.49 micromolar. The average value of MDA concentration in group C was 4.31 micromolar with a standard deviation of 2.68 micromolar. Lastly, the average MDA concentration in group D was 4.54 micromolar with a standard deviation of 2.80 micromolar. From the data above, it can be seen that the group receiving honey and vitamin C had lower MDA results than the control group, and the group receiving honey had lower MDA results than the group receiving vitamin C (Table 2).

In the perisinusoidal parameter, portal track, number of septa and septa width in each group have p-value less than 0.05. This indicated that the perisinusoidal, portal track, number of septa and septa width in the four groups were significantly different. In other words, the treatment had a different effect on perisinusoidal, portal track, number of septa and septa width with honey can inhibit liver fibrosis

(Table 3).

Based on the results of the One Way Anova in table 3, it can be seen that lipid peroxidation using the MDA concentration value has a p-value of 0.001 where the value was smaller than 0.05. It indicated that lipid peroxidation in the four groups was significantly different. In other words, the treatment gave a different effect on lipid peroxidation that using honey and vitamin C 255 mg could inhibit lipid peroxidation better, but 3 mg honey gave the best result. Furthermore, further testing was carried out using the Post-Hoc LSD test (Table 4).

DISCUSSION

The biliary obstruction could cause some changes in the bile ducts which caused impaired liver function and more severe could lead to systemic disorders. Patients with biliary obstruction have a high risk for impaired liver function, kidney failure, cardiovascular disorders, nutritional deficiencies, bleeding problems, infections, surgical site complications and increased perioperative morbidity and mortality.^{1,9-11}

The lipid peroxidation process is one of the markers of oxidative stress which includes the conversion of ROS into TGF- β and TNF- α that are thought to be the cause of liver fibrosis.⁵ This process occurs in cell membranes composed of PUFA and includes the separation of hydrogen from carbon, then will be replaced by the entry of oxygen to produce radicals in the form of lipid peroxyl and hydroperoxide.¹²

The focus of the study was to compare the effect of oral administration of honey and vitamin C in inhibiting the process of liver fibrosis and lipid peroxidation in the biliary obstruction condition of Wistar rats. The effect of giving honey and vitamin C orally in these conditions was expected to inhibit the formation of liver fibrosis as assessed by histopathological examination and could reduce lipid peroxidation as assessed from the measurement of plasma MDA levels.

Inhibition of liver fibrosis formation process and reduction of lipid peroxidation by administration of honey and vitamin C orally occurred due to neutralization of ROS by honey and vitamin C and conversion of lipid peroxidation products to non-reactive lipid peroxidation

products.^{13,14} and with the decrease in the lipid peroxidation process, the formation of liver fibrosis will also be inhibited through the mechanism of decreasing ROS changes to TGF- β and TNF- α .¹⁵

Data from the histopathological examination of the liver incision biopsy tissue of Wistar rats showed that in the state of biliary obstruction without oral administration of honey and vitamin C, moderate to severe liver fibrosis occurred and this fibrosis process decreased with the administration of 3 mg honey and 225 mg vitamin C and this decrease was more common in rats given 3 mg of honey compared to 225 mg of vitamin C. The data from the examination of plasma MDA levels in Wistar rats showed that the average value of plasma MDA levels in the group of rats with biliary obstruction who were given 3 mg honey and 225 mg vitamin C orally was lower and closer to the average value in the sham surgery rat group, but the group of rats given 3 mg of honey decreased MDA levels better than the group of rats that received 225 mg of vitamin C per day.

Administration of honey 3 mg and vitamin C 225 mg in biliary obstruction for 14 days in our study turned out to have an effect in inhibiting liver fibrosis and these doses gave low plasma MDA levels when compared to the control group. This is statistically supported by the Kruskal Wallis test for the assessment of liver fibrosis and the One Way Anova test for the assessment of MDA levels. These two results also showed agreement with a study on the hepatoprotective effect of honey that had been previously carried out in experimental rat animals, namely Mona et al. in 2007, Ergruder et al. in 2008, Ni Cheng et al. in 2015 and Muhammad et al. in 2019 and Hamad et al. in 2020 as mentioned above. Meanwhile, the hepatoprotective effect of vitamin C in this study was also according to the research conducted in 2006 by Soylu et al., Widhitomo et al. and Reno R et al. in 2020 which concluded that vitamin C had a hepatoprotective effect.^{7,8,9,15-19}

The results of this study substantiate the findings of several previous researchers regarding the hepatoprotective effect and the effect of reducing lipid peroxidation possessed by honey and vitamin C in

experimental rats that were treated with exposure to various chemical compounds and BDL including research conducted by Ni Cheng et al. in 2015 and Muhammad et al. in 2019 proved that giving honey to rats treated with CCl₄ could prevent liver damage which was characterized by a decrease in fibrosis and MDA as well as Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT), while in the study of Ergruder et al. in 2008, it has been proven that by giving 3 mg of honey per day to Wistar rats treated with BDL shown to have a hepatoprotective effect and reduce the effect of lipid peroxidation by obtaining a decrease in fibrosis on histopathological examination and a decrease in AST and Adenosine Deaminase (ADA) from the blood serum examination.^{7,17,18}

CONCLUSIONS

Administration of 3 mg honey orally in biliary obstruction of Wistar rats had better results than vitamin C 225 mg in inhibiting liver fibrosis and lipid peroxidation.

DECLARATIONS

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Conflict of interest

There is no conflict of interest related to the publication of this research.

Ethical approval

The research was conducted at the Animal Pharmacology Laboratory of Padjadjaran University in September-October 2021. Ethical approval was obtained from the Health Research Ethics Commission, Faculty of Medicine, Universitas Padjadjaran with Number 872/UN6.KEP/EC/2021.

Author contribution

All authors had contributed to data analysis, study protocol, writing the original draft, and agreeing to the final version of the manuscript for publication.

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