Hepatoprotector Effect of Papaya (*Carica Papaya L.*) Ethanol Extract on Male *Wistar* Rats Induced by Acetaminophen

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Abstract **---Hepatoprotector compound can provide liver protection to the liver from damage caused by drugs, chemical compounds and viruses. This study was aim to determine the hepatoprotector effect of several doses of papaya (*Carica papaya L.*) ethanol extract in acetamnophen-induced rats. This research was an experimental research. 25 rats were randomly divided into five treatment groups: normal group, negative control (acetaminophen 250 mg / kg body weight), dose 1 (6.95 mg / 200 gram rats weight), dose 2 (13.9 mg / 200 gram rats weight), and dose 3 (27.8 mg / 200 gram rats weight). Papaya (*Carica papaya L.*) ethanol extract was given for 7 days. 2 hours after the 7th day of the research, the male white rats were induced by acetaminophen. SGOT and SGPT levels measurement was performed on day 8. Data were analyzed using One-way anova followed by LSD. SGOT and SGPT parameters, showed significant differences between negative control compared normal group, dose 2 and dose 3. SGOT and SGPT levels found to be decreasing nearing normal at dose 2. Based on this data, papaya (*Carica papaya L.*) has the most effective on dose 2 (13.9 mg / 200gram BB).**

Keywords: ethanol extract of papaya **(Carica papaya L.)*, hepatoprotector, acetaminophen, SGOT, SGPT***

I. INTRODUCTION

Based on WHO data [1], chronic liver disease and liver cirrhosis were the 12th leading cause of death in 2007 in the United States in 29,165 (1.2%). In 2007 the prevalence of liver cirrhosis in Australia was 2% and in Japan was 2.7%, while the prevalence of liver cirrhosis in Indonesia in 2007 was 1.7%. Hepatic disease in Indonesia is generally still high. Data by Ministry of Health [2], in Indonesia. Hepatic disease ranks third after infectious diseases and lung disease. One reason is from the use of hepatotoxic drugs. The result of Riskesdas in 2013, the prevalence of Hepatitis in Indonesia is 1.2%. The five provinces with the highest prevalence of hepatitis are East Nusa Tenggara (4.3%), Papua (2.9%), South Sulawesi (2.5%), Central Sulawesi (2.3%) and Maluku (2.3%). The type of hepatitis that infects many Indonesians is hepatitis B (21.8%).

Drug-induced hepatotoxicity is a potential complication that is almost always present because the liver is the metabolic center of all drugs and foreign substances that enter the body [2]. Hepatotoxic agents include paracetamol. Paracetamol is an effective analgesic drug used as an independent treatment to relieve fever, headaches and mild to moderate pain. Paracetamol is metabolized to N-acetyl-p-benzo-quinonimine toxic metabolite (NAPQI) which is a dangerous active metabolite. The reported hepatotoxic effects on humans occurred in a single dose of 10-15 grams after toxic dose intake [4].

Damage to hepatic cells causes intracellular hepatic enzymes to enter the blood vessels so that the levels of the intracellular enzyme in the blood increase. This increase in hepatic intracellular enzymes can be measured as a parameter of hepatic cell damage [5]. Increased enzymes in case of liver damage include Serum Glutamate Piruvat Transaminase (SGPT) and Serum Glutamate Oxosacetate Transaminase (SGOT) [6].

Previous research has shown that papaya fruit juice (*Carica papaya* L.) is reported to have a hepatoprotective effect. The content of antioxidants in papaya fruit juice (*Carica papaya* L.) can protect the paracetamol-induced mouse with a dose of 678.24 mg / 200 grams of rat weight. When converted to dose for humans, the dose obtained is 37.68 grams / 70 kg of human weight [7].

Based on the description above, it is expected that the preparation of papaya (*Carica papaya* L.) ethanol extract still has a hepatoprotector effect with smaller doses marked by a parameter of decreased serum SGPT and SGOT levels.

II. MATERIALS AND METHODS

The tools used are analytical balance, waterbath, centrifuge (Hettich EBA), a set of glasses, a set of

maceration, a mortar, a blender, photometer (Intherma 168). The samples used in this study were papaya fruit obtained from Pancatengah Sub-district, Tasikmalaya District in February 2018. The materials used are SGOT and SGPT (Dyasis®) reagents, paracetamol tablets, 96% ethanol, 10% ammonia, chloroform, HCL 1N, NaOH, reactant anisaldehyde-sulfuric acid or vanillin-sulfuric acid, magnesium powder, 1% FeCl3, 1% gelatin solution, ether, Lieberman-burchard reagent. The test animals used are 2-3 months old 25 wistar strain male white rats with average weight 200 gram.

The Way of Research.

1. Test Animal Preparation

The test animals used were wistar strain male white rats that were 2-3 months old with an average weight of 200-250 grams. There are 25 rats used in the test that has been divided into 5 groups with various treatments, each group consisting of 5 rats.

2. Plant Determination

Determination of the plant was conducted to determine the identity of the plants that will be used during the study. Plant determination was done at Herbarium Jatinangor Laboratory of Plant Taxonomy Jatinangor West Java.

3. Making of papaya (*Carica papaya* L.) ethanol extract

Preparation of the extract was done by maceration i.e. 550gram papaya simplicia powder inserted in the vessel, soaked in 96% ethanol for 4 days while stirred occasionally, and performs solvent replacement every 24 hours. The maseration result (maserate) is filtered using filter paper. Then the filtrate (extract) obtained was concentrated by using a water bath until the entire solvent wasted.

4. Phytochemical Screening.

Phytochemical screening performed includes examination of saponins, flavonoids, alkaloids, tannins, steroidal quinones, monoterpene terpenoids and sesquiterpenes.

5. Determination of Papaya (*Carica papaya* L.) Dose.

Based on research [6] it is known that papaya fruit juice can protect paracetamol-induced mouse with a dose of 678.24 mg / 200 grams of rat weights.

Dose of Papaya extract:

Dose I = 6.95 mg/ 200gram rat weight

Dose II = 13.9 mg/ 200gram rat weight

Dose III = 27.8 mg/ 200gram rat weight

6. Preparation of Suspension and Determination Paracetamol Dosage

Toxic dose of paracetamol in humans occurs at a single dose of 10-15 grams (200-250 mg / kg) [3]. The dose given at 250 mg / kg. The paracetamol suspension in 1% CMC is prepared by dissolving a certain grams of paracetamol that have been weighed into 1% CMC to a predetermined concentration, i.e. the hepatotoxic dose.

7. Grouping of Test Animal and Effect Testing of Hepatoprotector

Before the trial began, rats adapted to the cage for 6 days, Adaptation is done to avoid the risk of stress that can affect the content of the blood serum. During the adaptation period, the rats were given a standard feed and not given any treatment.

The study was performed following a completely randomized design, using 25 male white rats divided into 5 groups equally. Group I rats were not given any treatment (normal controls). Group II rats were given 1% CMC for 7 consecutive days and followed by administration of paracetamol dose 250 mg / kg rat weight 2 hours after 7 days of CMC administration (negative control). Rats group III to V given fruit ethanol extract of papaya (*Carica papaya* L.) respectively at a dose of 6.75 mg / 200gram rat wieght; 13.9 mg / 200gram rat weight; 27.8 mg / 200gram rat weight; for 7 consecutive days and on day 7 were given paracetamol dose of 250 mg / kg. Blood sampling performed after 24 hours of administration of toxic doses of paracetamol. Blood collection is done in the vein of the tail.

8. Measurement of SGOT and SGPT Enzymes

Blood biochemical tests on Serum Glutamate Oxosaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT) were performed by centrifugating the rat blood at 2000 rpm for 15 minutes to obtain serum. A total of 100 mL of serum obtained, plus a 1000 mL reagent kit.

Data Analysis.
SGPT and SGOT data of the rats were analyzed by Analysis of Variance (ANOVA) using SPSS program version 22.

III. RESULTS

1. Extraction

The result of maceration simplicia of papaya fruit using 96% ethanol solvent produce liquid extract as much as 2 L and after evaporation done in waterbath, obtained a thick brownish colored extract as much as 256,20gram with the value of yield at 46,58%.

2. Pythochemical Screening

Phytochemical screening was carried out on the powder based simplicia and papaya extract (*Carica papaya* L.). The purpose of the oral phytochemical screening is to determine the secondary metabolite content found in papaya fruit (*Carica papaya* L.). The phytochemical screening of simplicia powder and papaya extract (*Carica papaya* L.) has negative positive results on alkaloid compounds, flavonoids, phenols and monoterpenoids and sesquiterpenoids.

TABLE I. Pythochemical Screening Results

Pengujian	pereaksi	Warna	Test Results	
			Simplisia	Extract
Alkaloids	Mayer	White Sedimen	+	+
	Dragendroff	Orange Sedimen	+	+
Flavonoids	Concentrated Zn + Hcl	Yellow	+	+
Saponins	+ Strongly shaked Distilled Water	Foam	-	-
Quinone	NaOH	Yellow or Red	-	-
Phenol	FeCl3	Dark Green, Black	+	+
Tannin	Gelatin	White Sedimen	-	-
Triterpenoid and steroid	Liberman buchardt	Triterpene (red) Steroid (blue)	-	-
Monoterpenoid and Sesquiterpenes	Vanilline sulphate	Black	+	+

3. SGOT and SGPT Parameter

In this study, paracetamol (acetaminophen) used as an inducer of liver damage. If hepatic cells induced by paracetamol toxic doses, hepatic cells may become injured through some oxidative stress processes due to the accumulation of NAPQ1 (N-Acetyl p-benzoquinone) metabolites that damage mitochondria and inhibit hepatocyte cell formation [8]. Mitochondrial cell membrane damage causes the liver to secrete SGOT and SGPT enzymes.

From the results of the study, the data of SGOT activity can be seen in table 2.

TABLE II. Hasil data SGOT (*Serum Glutamate Oksaloasetat Transaminase)*

No	Group				
	Normal	Negative Control	dose 1	dose 2	dose 3
1	70.8	153.2	157.7	66.7	91
2	57.9	159.6	136.8	78.8	93.4
3	58.1	146.7	154.9	61.9	96.8
4	47.3	108.1	154.7	47.9	99.3
5	50.4	154.4	117.1	75.4	89.4
Average	56.9*	144.4	144.24	66.14*	93.98*
Deviation Standard	9.08	20.80	17.29	12.21	4.07

Remarks:
Normal = No Treatment
Negative Control = CMC 1% and Paracetamol Induction
Dosis 1 = papaya ethanol extract 6.95 mg/200gram rats weight.
Dosis 2 = papaya ethanol extract 13.9 mg/200gram rats weight.
Dosis 3 = papaya ethanol extract 27.8 mg/200gram rats weight.
(*) = Different significance from the negative control.

From the conducted research, SGOT showed an average negative control group 144.4 ± 20.8 U / L and normal control group 9:08 ± 56.9 U / L. This suggests that paracetamol used as an inductor may cause liver damage in which high levels of SGOT are present in the negative control group given paracetamol compared with SGOT levels in the normal control group. SGOT levels in normal white blood serum serum ranged from 19.3 to 68.9 U / L [9].

IV. DISCUSSION

The Average levels of SGOT on dose 1 6.95 mg / 200gram rats weight, dose 2 13.9 mg / 200 gram rats weight, and dose 3 27.8 mg / 200 gram rats weight respectively 144.24 ± 17, 29 U / L, 66.14 ± 12.22 U / L and 93.98 ± 4.07 U / L. From these results it can be seen that dose 2 i.e. 13.9 mg / 200 grams of rat BB found to be the most effective in decreasing levels of SGOT compared with a dose of 3 27.8 mg / 200 grams of rat weight.

Increased levels of the SGOT enzyme after administration of toxic doses of paracetamol may induce necrosis primarily through a NAPQI metabolite characterized by elevated levels of the SGOT enzyme. Increased levels of the SGOT enzyme can not be ascertained any damage to the liver. This is because the SGOT enzyme is one of the enzymes found in the heart muscle and liver. This enzyme is found in moderate concentrations in skeletal, renal and pancreatic muscles. In the event of injury, especially in the liver cells and heart muscle, this enzyme will be released into the blood [10].

The results of this study were analyzed using ANOVA test. The result of anova analysis showed that sig (0.000) ($p < 0.05$) mean that there is significant difference by induction of papaya ethanol extract (*Carica papaya L.*) to serum SGOT enzyme level. From the results of a further test LSD (Least Significant Difference), dosing of the activity of SGOT. The research of papaya (*Carica papaya* L.) ethanol extract showed that there was a different effect on the activity of SGOT. Highest reduction activity is at dose 2 i.e. 13.9 mg / 200 grams of rat weight with AST activity values on average 66.14 ± 12.22 U /L.

Other parameters used in this study are the SGPT parameters. Normal levels of SGPT in normal white blood serum serum ranged between 29,8 – 77,0 U/L [9]. The results obtained are SGPT activity data which can be seen in the table 3.

TABLE III. Hasil data SGPT (*Serum Glutamate Pyruvat Transaminase)*

No	kelompok				
	Normal	**Negative Control**	**dose 1**	**dose 2**	**dose 3**
1	66	105.1	90.8	50.9	79.5
2	45.6	124	88.5	70	84.7
3	57.4	138	70	71.3	77.3
4	71.1	115.3	89.3	58.3	84.9
5	52.1	139.7	85.4	67.3	70.6
average	58.44*	124.42	84.8*	63.56*	79.4*
Deviation Standard	10.29	14.78	8.50	8.70	5.92

Remarks:
Normal = No Treatment
Negative Control = CMC 1% and Paracetamol Induction
Dosis 1 = papaya ethanol extract 6.95 mg/200gram rats weight.
Dosis 2 = papaya ethanol extract 13.9 mg/200gram rats weight.
Dosis 3 = papaya ethanol extract 27.8 mg/200gram rats weight.
(*) = Different significance from the negative control

High levels of SGPT were seen in the negative control group induced by paracetamol of 124.42 ± 14.78 U / L. This value is quite high compared with the normal group with SGPT levels of 58.44 ± 10.29 U / L. This indicates the effect of liver damage induced by paracetamol as an inducer to the animal test.

In the dose group, SGPT levels were also measured with results in the dose group of 1, 6.95 mg / 200 gram rat weight, dose 2 of 13.9 mg / 200 gram rat weight and dose 3 27,8 mg / 200 gram rat weight respectively are 84.8 ± 8.50 U / L; 63.56 ± 8.70 U / L; 79.4 ± 5.92 U / L. The dose that showed the most effective decrease in SGPT levels was shown by the dose 2 i.e. 13.9 mg / 200gram rat weight.

The SGPT enzyme is a more sensitive indicator in recognizing the presence of acute liver disease. SGPT enzyme is a cytosolic or cytoplasmic enzyme so that if there is interference permeability of liver cell membrane can cause cytoplasmic components enter the blood circulation resulting in the increase of serum enzyme concentration. SGPT enzymes are easier to be found in the liver than in the heart and muscles of the body. Therefore, the increased levels of the enzyme SGPT means there is liver damage [11].

The results of this study were analyzed using ANOVA test. The result of anova analysis obtains a sig value of (0.000) ($p < 0,05$) means that there is significant difference from giving of papaya ethanol extract (*Carica papaya L.*) to serum SGPT enzyme level of rat. From the results of a further test LSD (Least Significant Difference), dosing of the activity of SGPT. The research of papaya (*Carica papaya* L.) ethanol extract showed that there was a different effect on the activity of SGOT. Highest reduction activity is at dose 2 i.e. 13.9 mg / 200 grams of rat weight with AST activity values on average 66.14 ± 12.22 U /L.

From the results of research can be seen that data levels of SGOT and SGPT did not show a linear data. This is seen from both parameters indicating that dose 2 i.e. 13.9 mg / 200gram rat weight showed the most effective level as hepatoprotector compared with dose 3 at 27.8 mg / 200gram rat weight. This is presumably because the extract of papaya fruit (*Carica papaya* L.) follows a nonlinear pharmacokinetic model that increasing the dose will be inversely proportional to the pharmacological effects caused. Nonlinearity occurs because of the difference kinetic (occurrence of saturation) on the consumption of high-dose drugs that will affect the absorption and metabolism of drugs. Drugs with high doses will cause drug absorption through the delivery system to saturate and will also experience saturation in the first-pass metabolism process in which drugs with high hepatic extraction ratios, increased doses will cause saturation in the metabolism by enzymes, thereby decreasing intrinsic clearances [12].

The occurrence of decreased levels of SGOT and SGPT enzymes in the liver or liver protection from damage caused by the administration of paracetamol toxic dose after administration of papaya ethanol extract (*Carica papaya* L.) is caused by the active compound contained in papaya fruit (*Carica papaya* L.) namely vitamin E, Vitamin A and flavonoids [13].

Vitamin E in particular plays a role in inhibiting the formation of lipid peroxide by the hydroxyl radical formed N-acetyl-p-benzo-quinonimine (NAPQI) through the mechanism of free radical arrest and metal chelation. Vitamin A is an antioxidant that has the function of protecting the body from molecules called harmful free radicals. Free radicals cause cell damage including liver cells through a process known as oxidation. Vitamin A can increase Glutathione Serum Transferase (GST) enzyme. GST enzymes can increase body glutathione levels that can be used for NAPQI conjugation [14].
Flavonoids are natural phenolic compounds that are potential as antioxidants and have bioactivity as a medicine. Flavonoids are thought to take effect in inhibiting liver damage by binding free radicals as antioxidants, thereby reducing the negative impact of free radicals by reducing the number of liver cell damage [15]. Therefore, it is

possible that flavonoid compounds contained in fruit papaya (*Carica papaya* L.) can also bind free radicals which can serve as a hepatoprotective, but further testing needs to be done about it.

V. CONCLUSION

Based on observations on the research that has been done, it can be concluded that the papaya (*Carica papaya* L.) ethanol extract showed activity as a hepatoprotector against paracetamol-induced toxic dose on rats, on dose 2 i.e. 13.9 mg / 200 gram rat weight has a hepatoprotective effect in mice induced with paracetamol toxic doses.

REFERENCES

[1] World Health Organization (WHO). 2007. *Angka Pengkajian Kematian*. Jakarta : WHO.

[2] Departemen Kesehatan Republik Indonesia. 2010. *Profil Kesehatan Indonesia*. Jakarta: DepKes RI.

[3] Bayupurnama, P. 2007. *Ilmu Ajar Penyakit Dalam*. Jakarta : Universitas Indonesia.

[4] Goodmam, A., dan Gilman, H. 2014. *Dasar Farmakologi Terapi Edisi 10 Vol. 2*. Jakarta : EGC.

[5] windjiati. 1995. Pengaruh Pemberian Seduhan Jamu Galian Singset Terhadap Aktivitas Sgot Dan SGPT Kelinci [Thesis]. Fakultas Farmasi Universitas Surabaya: Surabaya

[6] Akbar, N. 2007. *Diagnostik Hepatitis Akut dan Kronis*. Jakarta : Bagian Ilmu Penyakit Dalam RSCM/FKUI.

[7] Sailellah, Nining Fadlani. 2016. Uji efek hepatoprotektor jus buah pepaya (*Carica papaya L.*) pada tikus jantan (*Rattus norvegicus*) yang diinduksi parasetamol dengan parameter enzim ALT [Skripsi]. Makassar : Fakultas Kedokteran dan Ilmu Kesehatan, Universitas Islam Negeri Allaudin Makassar.

[8] Indahsari, Noer Kumala. 2017. Histopatologi hepar tikus putih (*rattus novergicus*) yang diinduksi dengan parasetamol dosis toksik pasca pemberian ekstrak etanol daun kelor (*Moringa oleifera*) : *Jurnal kimia riset*. Volume 02 No. 02. 123-130.

[9] Pilichos C, Perrea D, Demorakou M, Preza A,Donta I. 2014. *Management of carbon tetrachloride-induced acute liver injury inrats by syngeneic hepatocyte transplantation in spleen and peritonealcavity*. *World J Gastroenterol*, Volume 10 No. 14. 2099-2112.

[10] Lomanorek, Vania Y., et al. 2016. Gambaran kadar serum *Serum Glutamic Oxaloacetic Transaminase* (SGOT) pada perokok aktif usia > 40 tahun : *Jurnal e-Biomedik*. Volume 4 No. 1. 156 – 162

[11] Kahar, Hartono. 2017. Pengaruh hemolisis terhadap kadar *Serum Glutamate Pyruvate* transaminase (SGPT) sebagai salah satu parameter fungsi hati : *The Journal of Muhammadiyah Medical Laboratory Technology*. Volume 02 No. 01. 38-46 .

[12] Smith, D. 2012. *Pharmacokinetics and bioavailability of medroxyprogesterone acetat in the dog and the rat*: Vol 14 No. 04. 341-355.

[13] Ramdani, Fitria Apriliani., et al. 2013. Penentuan aktivitas antioksidan buah pepaya (*Carica papaya* L) dan produk olahannya berupa manisan pepaya: Jurnal Sains dan Teknologi Kimia. Vol 04 No.02. 115-124.

[14] Nururrahmah., et al. 2013. Analisis kadar beta-karoten kulit buah naga menggunakan spektrofotometer UV-VIS : *Jurnal Dinamika*. Volume 04. No. 01. 15-26.

[15] Fahmi, Mulya. 2015. Gambaran histopatologis hati tikus (*Rattus novergicus*) yang diinfeksi *Trypanosoma evansi* setelah pemberian ekstrak kulit batang jaloh (*Salix tetrasperma* Roxb). *Jurnal Medika Veterinaria*. Volume 09 No. 02. 141-145.