

# Hepatoprotective Effect of Mountain Papaya (*Vasconcellea pubescens* A.DC.) Fruit Extract against Acetaminophen-Induced Acute Liver Damage

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Abstract: The liver is a main organ of drugs metabolism in humans. Many studies have reported that acetaminophen has an effect on liver injury. Targeting mitochondrial oxidant stress is a promising therapeutic option for acetaminophen hepatotoxicity. Previous study has shown that mountain papaya (*Vasconcellea pubescens* A.DC.) fruit has an effect against lipid peroxidation activity. This research aims to know the hepatoprotective effect of mountain papaya fruit ethanolic extract (MPFE) in rats after induced by acetaminophen. The rats were divided into six groups, with group I not administered with any treatment, group II administered with suspension of 0.25% CMC-Na as negative control, group III was administered with suspension of silymarin as positive control. Groups IV, V, and VI were given MPFE with variation doses 120; 240 and 480 mg/kg body weight for 14 days. At day 14th, all groups except the normal group were induced with acetaminophen in toxic dose (2 g/kg body weight). The liver injury was measured by ALT, AST, bilirubin, ALP value and liver histology profile. The results showed that MPFE could significantly decrease liver cell injury ( $p < 0.05$ ) by ALT, AST and liver histology profile parameters in which each dose has the same ability.

## 1 INTRODUCTION

The liver is the largest gland of the carbohydrates, lipids, proteins and xenobiotic and drug detoxification (Kumar *et al.*, 2013). As the center of metabolism in the body, liver is vulnerable to chemicals exposure which makes this organ susceptible to injury (Muriel, 2017). Acetaminophen overdose is the most frequent cause of liver injury and acute liver failure in many countries (Jaeschke and Ramachandran, 2018; Larsen and Wendon, 2014). The formation of a reactive metabolite and its binding to cellular proteins was initially thought to be responsible for cell death. A competing hypothesis was introduced that questioned the relevance of protein binding and instead suggested that P450-derived oxidant stress and lipid peroxidation causes acetaminophen-induced liver injury (Jaeschke and Ramachandran, 2018). Hepatotoxicity can occur because the metabolite n-

acetyl-p-benzoquinoneamine (NAPQI) is reactive (Brune *et al.*, 2015), and interact with covalent liver macromolecule in the cysteine resulting in the onset of oxidative stress (Vakiloddin *et al.*, 2015). Acetaminophen is activated by the enzyme cytochrome P450 become metabolite N-acetyl-p-benzoquinone imine (NAPQI) that suppress reactive glutathione covalent bonds and liver with protein (Walubo *et al.*, 2004). This bond-related to the toxicity of acetaminophen which causes liver injury (Salhanick *et al.*, 2006).

The liver injury can occur by structural damage and seen from the histological profile of the liver microscopically. Biochemical parameters in the blood serum also can be used as an indicator when the liver injury by an enzyme released from the hepatic cell organelle into the blood (Amin *et al.*, 2010). Specific enzymes that indicate liver damage is ALT (alanine aminotransferase), AST (aspartate transaminase), TB (total bilirubin), ALP (alkaline phosphatase) (Gowda *et al.*, 2009; Limdi and Hyde,

2003). Liver damage can be treated with a hepatoprotector compound (Pradhan and Girish, 2006). Many studies associate the effects of antioxidants with hepatoprotection consequence (Hsiao *et al.*, 2003; Huang *et al.*, 2010; Tzankova *et al.*, 2017).

Mountain papaya (*Vasconcellea pubescens* A.DC.), also called *Carica pubescens*, are commonly found in the Dieng plateau, Central Java (Sasongko *et al.*, 2016). Mountain papayas contain flavonoid and phenolic compounds that have antioxidant activity (Simirgiotis *et al.*, 2009; Uribe *et al.*, 2015). It has antioxidant activity with IC50 value of 8,843 to 0,983 mg/100 mL (Laily *et al.*, 2012). This research aims to know the hepatoprotective effect of mountain papaya fruit extract against acetaminophen-induced acute liver damage.

## 2 MATERIALS AND METHOD

### 2.1 Materials

The fruit of mountain papaya (*Vasconcellea pubescens* A.DC) was collected from the Dieng Plateau, Central Java, Indonesia. Studies were carried out using male Wistar albino rats (150-250 g). Rats were obtained and all handling procedures have been approved by the ethics committee of the Faculty of Medicine, Universitas Sebelas Maret with the number 421/V/HREC/2017. Chemical like Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin were analyzed using reagent kits (DiaSys Diagnostic, Holzheim, Germany). All other reagents were of analytical grade. The instruments used were freeze dryer (VirTis BenchTop®) and Spektrofotometer UV-Vis (Micro Lab 300®).

### 2.2 Sample and Extract Preparation

Mountain papaya fruit with yellowish green color was washed and cut into small pieces. The fruit was dried using a freeze dryer. After drying, the fruit was ground and sieved to a uniform particle size as sample. The sample was extracted using the maceration method with 70% ethanol solvent for seven days. The ratio of sample to solvent was 1:10.

The filtrate of the maceration was collected and concentrated with a rotary evaporator at 50°C to obtain an extract.

### 2.3 Phytochemical Screening

Phytochemical screening was carried out to screen class of phytoconstituents present in the ethanolic extract of mountain papaya stem using standard methods reported in Harborne (2012).

### 2.4 Animal Experimental Design

The experimental animals were acclimatized for one week. The rats were given aquadest drink and standard feed. The animal were divided into six groups with group I not administered with any treatment, group II administered with suspension of 0.25% CMC-Na as negative control, group III was administered with suspension of silymarin as positive control. Groups IV, V, and VI were given mountain papaya fruit ethanolic extract (MPFE) with variation doses 120; 240 and 480 mg/kg body weight for 14 days. At day 14th all groups except the normal group were induced with acetaminophen in toxic dose (2 g/kg body weight). After 48 hours induction of acetaminophen, blood sample was collected to measure ALT, AST, ALP and total bilirubin value. The liver was taken to be analysed for histology profile.

### 2.5 Histopathological Studies

Liver tissues were fixed in 10% formalin for at least 24 hours, embedded in paraffin and cut into 5 µm thick sections in a rotary microtome. The sections were stained with hematoxylin-eosin dye and observed under a microscope to detect histopathological changes in the liver (Huang *et al.*, 2010).

### 2.6 Data Analysis

The analysis was performed statistically. The normality test used was the Shapiro-Wilk test and variance test was done by Homogeneity of variance test. The normally and homogeneously distributed data were analyzed by One-way Analysis of Variance (ANOVA). To know the differences between each treatment groups and then continued with Bonferroni Post Hoc test.

Table 1: The effect of mountain papaya (*Vasconcellea pubescens* A.DC.) fruit extract against acetaminophen-induced on biochemical parameters.

Groups	ALT (mg/ dl)	AST (mg/ dl)	ALP (mg/ dl)	TB (mg/ dl)
Normal control	51.66 ± 5.25*	223.54 ± 14.67*	548,70 ± 73,72	0,502 ± 0, 01
Negative control	461.36 ± 16.60	894.72 ± 22.46	774,38 ± 25,95	0,584 ± 0,02
Silymarin 100 mg/kg B.W	76.24 ± 13.54*	216.68 ± 12.33*	564,92 ± 26,84*	0,512 ± 0,02
Extract dose 120 mg/kg B.W	284.23 ± 17.20*	355.68 ± 19.80*	612,60 ± 20,22	0,538 ± 0,02
Extract dose 240 mg/kg B.W	255.96 ± 14.10*	291.56 ± 19.26*	589,72 ± 18,76	0,534 ± 0,08
Extract dose 480 mg/kg B.W	155.7 ± 15.84*	245.24 ± 18.25*	573,80 ± 12,19	0,518 ± 0,01

Symbols represent statistical significance. \*p < 0.05, as compared to negative control group. n = 5 animals in each group

### 3 RESULT AND DISCUSSION

#### 3.1 Phytochemical Screening

The phytochemical compound of mountain papaya (*Vasconcellea pubescens* A.DC) ethanolic extract showed flavonoids, tannins and phenolic.

#### 3.2 Hepatoprotective Effect

The rats’ biochemical parameter result like ALT, AST, ALP and total bilirubin are shown in Table I. The results demonstrated that ALT and AST were found to be significantly increased in rats treated with acetaminophen when compared with the negative control group (P<0.05) but not significantly on ALP and total bilirubin serum. The administration of mountain papaya extract for 14 days significantly decreased the activity of serum alanine aminotransferase and serum aspartate transaminase in a dose-dependent manner in acetaminophen-induced liver damage in rats compared to that of the hepatotoxic group (acetaminophen treatment) (P<0.05). The serum level of ALT and AST are largely used for determination of liver damage (Nurrochmad *et al.*, 2013). Serum glutamic pyruvic transaminase (SGPT) or also called ALT (alanine aminotransferase) is a specific enzyme that can estimate the damage of a cell especially in the liver (Gowda *et al.*, 2009; Limdi and Hyde, 2003).

The mechanism of hepatotoxic from acetaminophen is caused by the damage of hepatic cell resulting from metabolites formed at the time of reaction with cytochrome P450. In therapeutic dose, the main metabolic pathway of acetaminophen is through glucuronidation and sulfation in the liver, and only slightly metabolized by the P450 cytochrome which produces N-acetyl quinone imine

(NAPQI). NAPQI in such amounts can be detoxified by conjugation with glutathione (GSH). While paracetamol is in excessive doses, it causes saturation of the sulfate pathway, resulting in large NAPQI formation and GSH depletion (Li *et al.*, 1994). Reduced amounts of glutathione will lead the formation of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) that cause necrosis of hepatocytes. The presence of ROS will lead to a loss of mitochondrial potential membrane and loss of mitochondrial ability in synthesizing ATP. The loss of ATP will lead the present of necrosis (Hinson *et al.*, 2010).

#### 3.3 Histopathological Profile

Use the differences microscopic appearance of hepatic cells between treatment groups with ethanol extract of mountain papaya can be seen in Figure 1.

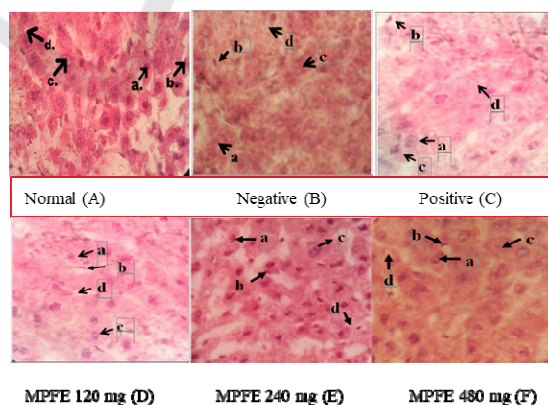


Figure 1. Histologic Profile of the Rat’s Liver. Description: (A) normal Group, (B) negative control, (C) positive control, (D) MPFE 120 mg/kg b.w, (E) MPFE 240 mg/kg b.w, (F) MPFE 480 mg/kg b.w; (a) Normal cells, (b) Picnotic, (c) Cariopsis, (d) Kariolysis

From the histological profile that showed the condition of the hepatic cell in the treatment group compared with the negative group, there were cells still in normal condition, but some cells have been damaged (necrotic). Early morphological changes include cytoplasmic edema, dilatation of the endoplasmic reticulum and polysomal disaggregation. The next process occurs triglyceride accumulation as fatty grains in the cell, progressive mitochondrial swelling with damaging crystals and complex biochemical swelling. The next stages may experience hydropic degeneration, separation of cell structures, pyknotic cell nuclei, karyorrhexis, karyolysis, breakage of the plasma membrane, and eventually necrosis (Kumar *et al.*, 2017). The dead nucleus will shrink, have irregular and dark boundaries. This process is called piknosis, and its core is called piknotik. The other possibility, the nucleus may be destroyed, leaving fragments of chromatinic substances that is dispersed in cells called kariorecidual. Finally, in a certain condition, the dead nucleus loses the ability to absorb the dye again and completely disappears, a process called karyolysis (Wilson and Price, 2006).

#### 4 CONCLUSIONS

The results showed that mountain papaya (*Vasconcellea pubescens* A.DC) could significantly decrease liver cell injury ( $p < 0.05$ ) by ALT, AST and liver histology profile parameters in which each dose has the same ability.

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