

# Hepatoprotective activity of Abelmoschus manihot L. Medik leaves extract and its nanoparticle against carbon tetrachloride-induced hepatotoxicity in rats

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## HEPATOPROTECTIVE ACTIVITY OF *Abelmoschus manihot* L. Medik LEAVES EXTRACT AND ITS NANOPARTICLE AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN RATS

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### ABSTRACT

*Abelmoschus manihot* L. Medik leaves used as food and traditional medicine. Leaves decoction water is used to treat various diseases. Flavonoids content in the leaf such as hyperoside has the antiviral, antiinflammatory, cardioprotective and hepatoprotective effect. This study aimed to determine the hepatoprotective activity of *Abelmoschus manihot* L. Medik leaves extract and its nanoparticles against carbon tetrachloride induced hepatotoxicity in rats.

This was an experimental research. *Abelmoschus manihot* L. Medik fresh leaves dried and extracted with maceration method using 95% ethanol. The macerate concentrated to obtain a viscous extract. Some extract made into test solutions with a concentration of 8 mg/mL, and some extracts were prepared into nanoparticles with emulsion solvent evaporation method using 0.1% PLGA solution in ethyl acetate and 2.5% PVA solution with a concentration of 0.5 mg/mL. Test animals were 20 male white rats divided into 4 treatment groups ie positive control (Curcuma), negative control (1 % sodium CMC solution), extract group (dose 20 mg/200 g BW) and nanoparticle group (dose 0.25 mg/200 g BW). The rats were treated for 11 days, on the 8th day the rats were induced by CCl<sub>4</sub> intraperitoneally with a dose of 1000 mg/1000 g BW. On the 12th day, rats blood were taken and tested for SGOT and SGPT levels and histopathologic observations were performed. Data were analyzed statistically with Anava one way.

The results showed that *Abelmoschus manihot* L. Medik leaves extract and its nanoparticles lowering the SGOT and SGPT levels. Statistical analysis result showed a significant result ( $p < 0.05$ ) which means that the extract and its nanoparticles have the hepatoprotective activity. The activity of nanoparticles with small dose equals with extract with a larger dose. Rat liver in all treatment groups experienced necrosis, but showed regeration except on negative control group.

**Key words : *Abelmoschus manihot* L. Medik leaves, nanoparticle, hepatoprotective activity**

### INTRODUCTION

*Abelmoschus manihot* L. Medik is a tropical annual plant that is widely found in the area of North Sulawesi. In addition to food, it is also used as a traditional medicine. Empirically, people consume leaves decoction water to treat various diseases such as cholesterol, kidney and ulcer disease, improve glomerular filtration function, reduce proteinuria,

messenger hyperplasia that can reduce kidney tissue damage (Mamahit and Sukamto, 2010; Shao-Yu et al., 2006). Its leaf has been tested to prevent ovariectomy-induced femoral osteopenia (bone mineral density conditions lower than normal limits on joints due to surgical removal of the uterus/ovary) (Lin-lin et al., 2007; Jain et al., 2009).

Abelmoschus manihot L. Medik plants contain flavonoids that are quercetin-3-o-robinobiosid, hyperin, isoquercetin, gossipetin-8-o-glucuronide, and myricetin (Li<sup>2</sup> et al., 2006). The flowers contain quercetin-3-robinoside, quercetin-3'-glycoside, hyperin, myrecetin, anthocyanin, and hyperoside. Hyperoside has the antiviral, antinociceptic, antiinflammatory, cardioprotective, hepatoprotective, and protective effects against gastrimucosal (mucous membrane layer of the stomach) (Lin-lin et al., 2007; Jain et al., 2009). Research conducted by Pine et al (2013) showed that 96% of ethanol is a solvent that can extract most flavonoids on Abelmoschus manihot L. Medik leaves.

Flavonoid compounds have various important functions for health, among others in reducing the risk of cardiovascular disease, blood pressure, atherosclerosis, and as an antioxidant (Hodgson et al., 2006). Antioxidants can protect biomolecules against oxidative stress so as to reduce the risk of cardiovascular disease as well as certain types of cancer (Abubakar, 2010). In addition to being an antioxidant, flavonoids can also modulate cell signal pathways and their effects can be marked on cell function by altering protein and fat phosphorylation and modulation of gene expression (Číž et al., 2010).

Compounds with large molecules such as flavonoids can be difficult to absorbed in the body, this problem can be overcome by making nanoparticle preparations. Small particle size can facilitate drug absorption, thus accelerating the therapeutic effects and decreasing the amount of drug used in the preparation. This study aimed to determine the effect of hepatoprotector Abelmoschus manihot L. Medik leaves ethanolic extract and its nanoparticle against carbon tetrachloride induced hepatotoxicity in rats..

## METHODS

This was an experimental research.

Tools: blender, analytical scales, maseration masks, separating funnel, rotary evaporator, vortex, rat sonde, micropipette, injection syringe.

Material: 96% ethanol, aquadest, ethyl acetate (pa), chloroform (pa), n-butanol (pa), n-hexane, olive oil, Curcuma tablets.

Animals Test : Wistar male white rats aged 3 months.

Procedure :

### 1. Sample Preparation and Extraction Process

Fresh leaves are taken and washed and dried in a way aerated. The dried leaves are discharged dry and pollinated. Ged leaf powder is extracted using maseration method. Weighed 1000 g of ged leaf powder is macerated with 96% ethanol solvent for 5x24 hours on a glass container with daily stirring. The filtrate was collected and concentrated with rotary evaporator to obtain viscous extract (Pine, et al, 2013).

### 2. Hepatoprotective Activity Test

#### a. Test Animal Setup Try

A total of 20 rats were divided into 4 groups, each group consist of 5 rats with an average weight of 150 g-200 g. The test animals were adapted to the environment for 7 days. The treatment group was positive control (Curcuma, dose 20 mg / 200 g BB), negative control (1% Na CMC solution), ethanol extract group (dose 20 mg / 200 g BB) and group of extract nanoparticles (dose 0,25 mg / 200 g BB).

#### b. Preparation of Test Solution

The extracts were prepared by dispersing of 400 mg extract in a solution of 1% sodium CMC to 50 mL. Positive controls using Curcuma solution made by dispersing 2 Curcuma 200 mg tablets in 1% sodium CMC solution to 50 mL. The nanoparticle preparations were prepared by emulsion solvent

evaporation method by weighing 5 mg of extract and dispersed using 0.1% PLGA solution in ethyl acetate and 2.5% PVA solution, then diluted with aquadest to 50 mL. As a negative control used 1% sodium CMC solution.

c. Testing

The rats were treated for 11 days. On the 8th day, the rats were induced by CCl<sub>4</sub> intraperitoneally

with a dose of 1 mL/1000 g BW. On the 12th day, blood samples were taken and tested for SGOT and SGPT levels and liver histopathologic observations were performed. Data were analyzed statistically with Anava one way.

**RESULTS**

The data of SGOT and SGPT levels can be seen on the table 1:

Table 1. SGOT and SGPT in the rats blood

Treatment Group	SGOT (IU/L)	SGPT (IU/L)
Negative Control	304,3 ± 67.1545 <sup>a</sup>	127,7 ± 32.4364 <sup>a</sup>
Positive Control	164,2 ± 45.9670 <sup>b</sup>	105,4 ± 24.1236 <sup>a</sup>
Leaves Extract	93,1 ± 27.9837 <sup>b</sup>	112,78 ± 52.3174 <sup>a</sup>
Nanoparticle of Extract	104,4 ± 27.7366 <sup>b</sup>	134,8 ± 79.3729 <sup>a</sup>

Average value, <sup>a</sup> means that there is differences between group (0.05>p) and <sup>b</sup> means that there is no differences between group (p>0,05)

Table 2. Histopathological Data of Rats Liver

Treatment Group	Necrosis	Fibrosis	Fatty Degeneration	Regeneration
Negative Control	+	-	-	-
Positive Control	+	-	-	+
Leaves Extract	+	-	-	+
Nanoparticles of Extract	+	-	-	+

**DISCUSSION**

SGOT and SGPT are serum contained in the blood which is one indicator of liver damage condition. CCl<sub>4</sub> is used to induce liver damage. The increase of SGOT and SGPT values was seen in the negative control group, where the value was significantly different with the normal SGOT and SGPT values in rats, ie 141 ± 67.4 IU / L and 12.6 ± 4.4 IU / L. The results showed that the highest mean values of SGOT were respectively in the control group negative, positive control, extract nanoparticles and leaf extract. While the highest mean values of SGPT were successively in nanoparticle group, negative control, leaf extract and positive control.

The analysis results of SGOT show a significance value of 0.05>p which

indicates a difference between the treatment groups. Statistical analysis showed that there was a difference (0.05>p) between the negative control group and the other three groups. While between the control group positive, leaf extract and leaf extract nanoparticles with p>0,05 showed no difference. The SGPT results showed that there was no difference (p>0,05) between the negative control group and the other three groups. The results showed that leaf extract and its nanoparticles had hepatoprotective activity. The dose ratio between the leaf extract and the nanoparticles is 80:1, but the resulting effect showed no difference. This suggests that the size of the nanoparticles generate the increased absorption of the active substance that is flavonoids that have large molecules. Small doses of nanoparticles

can produce the same effect as the extract solution without modification of particle size. The histopathologic of rat liver showed that in all treatment groups experienced necrosis caused by CCl<sub>4</sub> administration. But at the same time there was also regeneration process in the positive control group, leaf extract and extract nanoparticles. This results showed that the three treatment groups had the effect of regenerating rat liver cells.

The hepatoprotective activity of *Abelmoschus manihot* L. Medik leaves extract is caused by the flavonoid content of quercetin-3-o-robinobiosid, hyperin, isoquercetin, gossipetin-8-o-glucuronide, and myricetin (Liu et al., 2006). Flavonoid compounds have various important functions for health, among others in reducing the risk of cardiovascular disease, blood pressure, atherosclerosis, and as an antioxidant (Hodgson et al., 2006).

#### CONCLUSION

The results showed that *Abelmoschus manihot* L. Medik leaves extract and its nanoparticles have hepatoprotective effect. The hepatoprotective activity of the nanoparticle dosage form is equal to the larger dose of extract.

#### SUGGESTION

Further research is needed to determine the hepatoprotective activity of *Abelmoschus manihot* L. Medik leaves extracts or fractions with other solvents and the development of nanoparticle preparations.

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