The Combined Effect of An Extracts of *Morinda citrifolia* L. Fruits and *Carica papaya* L. Leaves of Serum Transaminase and Bilirubin in Antituberculosis–induced Rats

Titik Sunarni [1*], Rini Prastiwi, Mardiyono, Yudi Rinanto, Nurul Jannah H, and Wening Wahyu Wardani

[1]*Faculty of Pharmacy, Universitas Setia Budi*

**Corresponding author : Solo, Indonesian, titiksunarni@yahoo.co.id*

Abstract

The activity testing from ethanolic exracts of Morinda citrifolia *L fruits and* Carica papaya *L. leaves as a drug that prevents the liver damage (hepatoprotector) in antituberculosis- induced rats had been performed. The fourteen rats were devide into eigth groups. The normal control without a treatment, negatif control were given INH 10mg/200g BW and rifampicin 10mg/200g (bw), positif control were given INH-Rifampicin and methicol®, and the treatment group were given INH-Rifampicin with papaya and morinda extracts with with some variation of doses. Each group were given every day for 28 days. The measuring to monitor bilirubin serum,* Alanine transaminase (*ALT) and aspartate transaminase (AST) levels on 0th, 14th, 21th and 28th day. The treatment group that given combination extract of* C. papaya *120mg/200g and* M. citrifolia *fruit 20mg/200g BW showed serum* Alanine transaminase (*ALT),* Aspartate transaminase *(AST) and serum bilirubin level were diminished significantly.*

Key Words : Morinda citrifolia*,* Carica papaya*, transaminase, bilirubin, antituberculosis*

INTRODUCTION

Commonly tuberculosis therapy used drugs such as Isoniazid, Ethambutol, Pirazinamid, Rifampicin, and streptomycin (Katzung and Bertram, 2004; Gun, 2007). The antituberculosis combined 3 or 4 kinds of drugs are useful as a treatment as well as to prevent resistance prevent of resistance. Based on the results of clinical trials that isoniazid and rifampicin are known to be most active in treating pulmonary tuberculosis (Sulaiman, 1990).

One of the side effects that may be incurred due to the use of anti tuberculosis is impaired liver function, from the mild to the severe form of liver tissue necrosis (Arsyad, 1989). The effect of clinical study based on the hepatotoxicity was caused by isoniazid. Experimental animals were given rifampicin with high dose not happen hepatotoxicity. The hepatotoxicity effect will appear when the two drugs are isoniazid and rifampicin were combined. This is due to rifampicin have the effect as an inducer of enzyme microsomes oxidase, so when combined with isoniazid may cause hepatotoxicity isoniazid to increase heavy. The study of side effects antituberculosis showed if the administering a dose 50mg/kg isoniazid and 100mg/kg rifampicin in the pigs for 21 days can cause liver damage (Adhvaryu et al 2007). Tuberculosis therapy usually in a long time that it would enlarge the risks of occurred hepatotoxicity.

Carica papaya L. leaves and *Morindra citrifolia* L. fruits could been primarily proved to be an useful hepatoprotective (Hembing, 2008; Hariana,

2008). The juice of *M. citrifolia* fruits may decrease the levels of enzymes GOT (Glutamate Oxaloacetat Transaminase) and GPT (Glutamate Pyruvate Transaminase) in rats were given a high-fat diet (Marsono, 2007). The other studies have reported if the extract M. citrifolia can reduce of hepatic damaged is induced by CCl_4 (Irfianti, 2007) and can reduce necrosis kidney cells of rats (Zain, 2006). The etanolic extract of *M.citrifolia* fruits can reduce the activity of an enzyme ALT dan AST (Rendi, 2009), serum bilirubin levels (Ridwan, 2009) and can reduce hepatic necrosis due to isoniazid and rifampisin (Roni, 2009).

The research was conducted to determine the effectiveness of the use of a combination of *M.citrifolia* fruit and *Carica papaya* leaves extract against the side effects hepatic damage that often appear in trt of tuberculosis. The parameter of this research liver damage was serum transaminase activity (AST and ALT) and bilirubin levels.

MATERIALS AND METHODS

Material and equitments

The main samples are ethanolic extracts of *Morinda citrifolia* L. fruits and *Carica papaya* L. leaves that have been drying. *M. citrifolia* L. fruits and *C. papaya* L. leaves were extracted by soxhlet with ethanol 70%. This plants were colleted from Surakarta, Indonesia, have been identified and the extracts have been standardized by Departement of Pharmaceutical Biology, Faculty of Pharmacy, Setia Budi University, Indonesia. Identification for chemical content of plants have been done in Thin layer chromatography (TLC). Identification of the flavonoid, alkaloid, saponin, tannin, and antrakinon, according to the usual way done.

Chemical and reagents were Isoniazid and Rifampicin (PT. Kimia Farma), ALT assay Kit, AST assay Kit and Bilirubin assay Kit (Diagnostic System International (Diasys) Germany), CMC-Na (PT. Bratachem), metichol® (PT. Otto), silika gel GF 254 (E-Merck Darmstadt Germany). Animals were male wistar rats of age 2,5-3,5 months weighing initially about 180-220 g, were kept in the animal house of Faculty of Pharmacy, Setia Budi University, Indonesia. The animals were randomly divided into groups of five each and maintained under standart conditions. All animals were fed the standart rodent pellet and water *ad libitum*. The equipments used in the research was a fotometry *stardust* and microsentrifuge.

The Dose and Preparation of Materials Test

A dose of *M. citrifolia* leaves and *C. papaya* fruit extract have been counted of a dose of empirical which is converted in dose of an extract. The dose of Isoniazid (INH) and Rifampicin were each 10 mg/200 g BW rats (Pal et al 2006) and methicol® 4.5mg/200 g BW. All test materials were suspended in the CMC 1% on a particular concentration.

Experimental Protocol

Total 40 rats were divided into eight different groups, five in each by per oral treatment. Group I (K1) rats were on normal diet and served as controls. Group II (K2) were given isoniazid 10 mg/g BW and rifampicin 10 mg/g BW. Group III (K3) rats were given methicol® (anti-hepatoxin). Group IV (P1) were given INH-Rifampicin and extract of *C. papaya* L. leaves 120 mg/200 g BW. Group V (P2) were given INH-Rifampicin and extract of *M. citrifolia* L. fruits 20 mg/kg BW. Group VI (P3) were given INH-Rifampicin and the combined extract of of *C. papaya* L. leaves 120 mg/200g BW and *M. citrifolia* L. fruits 20 mg/200g BW. Group VII (P4) were given INH-Rifampicin and the combined extract of *C. papaya* L. leaves 120 mg/200g BW and *M. citrifolia* L. fruits 10 mg/200g BW. Group VIII (P5) were given INH-Rifampicin and the combined extract of of *C. papaya* L. leaves 60 mg/200g BW and *M. citrifolia* L. fruits 20 mg/200g BW. Each group were given the treatment every day for 28 days. Each

rat taken his blood for to monitor bilirubin serum, ALT and AST level. The measuring to monitor serum *Alanine transaminase (*ALT), *aspartate transaminase* (AST) and total bilirubin levels on 0th, 14th, 21th and 28 th day.

Determination Enzyme Activity of ALT, AST and Bilirubin Levels

The activity of ALT, AST and level of bilirubin serum were measured using standard kits from Diasys Germany. The blood of the test animals were taken through the eye vein, then was cetrifuged at 3500 rpm for 15 min at 37˚C, and the supernatant was used the level of ALT, AST and total bilirubin serum. One hundred microliter of serum was added to 1000 µl AST kit or ALT kit, and total bilirubin serum was determined by mixing 25 µl serum to 1000 µl reagent kit of total bilirubin serum. The level of AST, ALT and total bilirubin was determined by photometry *Stardust* at 546 nm.

Data Analysis

The resuts were reported as mean $\pm$ SEM, then presented in graphical form the relation of ALT, AST activities and bilirubin levels as a function of the dose with treatment groups. Statistical significance was analysis by using the normality test of Kolmogorov-Smirnov, then analyzed statistically by the Kruskal-Wallis test and Mann Whitney test. P values lower than 0,05 were considered statistically significant.

RESULTS AND DISCUSSION

In the present study, the negative control was hepatotoxicity rats model was induced by administration of Isoniazid 10 mg/kg BW, Rifampicin 10 mg/kg BB orally with force feeding for 28 days. and whereas the positif control by administration of isoniazid 10 mg/kg BW, Rifampicin 10 mg/kg BB and methicol® 4,5mg/200g BW orally. Methicol® is a hepatoprotective and methicol® contains important substances that are useful for protecting the liver such as methionine, choline, vitamin B complex and vitamin E. The substance in the methicol can press liver damage, through the mechanism against free radicals.

Results of determination enzyme activity of ALT and AST to each groups were presented in the histogram as shown in Figure 1 and Figure 2. The statistical significance of difference (P<0,05) was evaluated *Kruskal-Wallis* test and showed significant difference from each groups treatment, whereas for the variable time (day) treatment showed no significant different. The groups treatment further was evaluated *Mann and Whitney* test which can be used to compare the ability of group treatment with other group. The results of analysis data ALT activity was interpreted if the combination extract of *C. papaya* leaves 120/200g BW and M. citrifolia fruits 20mg/200g BW (P3 group) indicated a decrease in the activity of the ALT enzyme better if compared to all the other treatment.

The results of the analysis by *Mann-Whitney* test for AST activity, showed the group treatment with a dose of combination *C. papaya* leaves extract 120mg/200 g BW and *M citrifolia* fruit 20mg/200 g BW more effective in lowering levels of ALT and AST in white rats male strain wistar induced by INH and Rifampisin.

Results of the examination serum total bilirubin each group were presented in the histogram as shown in Figure 3. Based on a statistical analysis of the non parametric *Kruskal-Wallis* of total bilirubin show significant difference from each group treatment, whereas for the variable time (day) treatment show no significant different. The groups treatment further was evaluated *Mann and Whitney* test and the results of analysis data ALT activity was interpreted if the combination extract of *C. papaya* leaves 120/200g BW and *M. citrifolia* fruits 20mg/200g BW (P3 group) indicated more effective in lowering levels of serum total bilirubin in white rats male strain wistar induced by INH and Rifampicin.

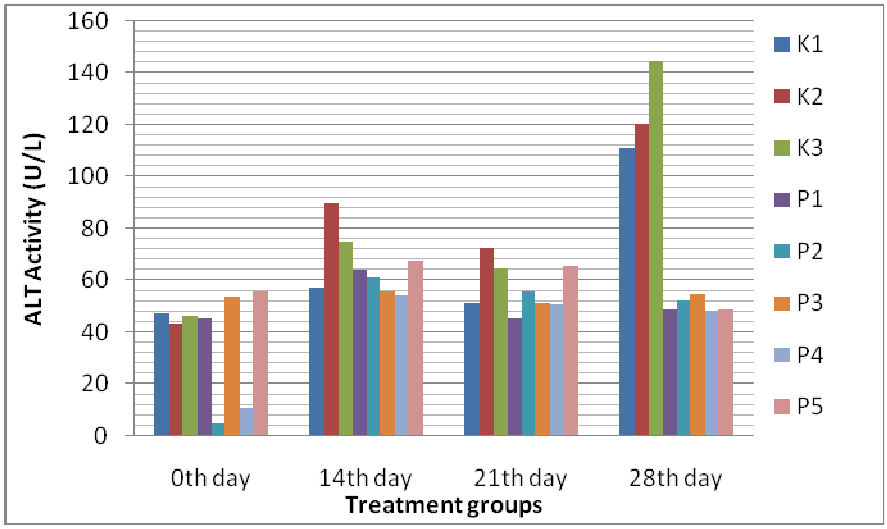

Figure 1. Histogram of mean ALT activity (U/L) in the groups of animals. **K1**: normal diet and served as controls; **K2**: negative control (INH 10mg/200g BW, RIF 10mg/200g BW); **K3**: positive control (INH 10mg/200g BW, RIF 10mg/200g BW, methicol® 4,5mg/200g BW); **P1**: INH 10mg/200g BW, RIF 10mg/200g BW, extract of *C. papaya* 120mg/200g BW; **P2**: INH 10mg/200g BW, RIF 10mg/200g BW, extract of *M. citrifolia* 20mg/200g BW; **P3**: INH 10mg/200g BW, RIF 10mg/200g BW, extract of *C. papaya* 120mg/200g BW and *M. citrifolia* 20mg/200g BW; **P4**: INH 10mg/200g BW, RIF 10mg/200g BW, extract of *C. papaya* 120mg/200g BW and *M. citrifolia* 10mg/200g BW; **P5**: INH 10mg/200g BW, RIF 10mg/200g BW, extract of *C. papaya* 60mg/200g BW and *M. citrifolia* 20mg/200g BW

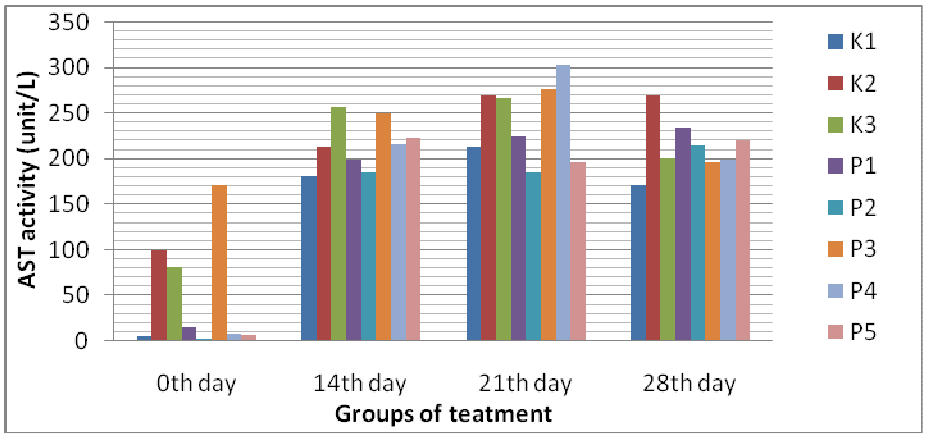

Figure 2. Histogram of average AST activity in the groups of animals. **K1:** normal diet and served as controls; **K2:** negative control (INH 10mg/200g BW, RIF 10mg/200g BW); **K3**: positive control (INH 10mg/200g BW, RIF 10mg/200g BW, methicol® 4,5mg/200g BW); **P1:** INH 10mg/200g BW, RIF 10mg/200g BW, extract of *C. papaya* 120mg/200g BW; **P2**: INH 10mg/200g BW, RIF 10mg/200g BW, extract of *M. citrifolia* 20mg/200g BW; **P3**: INH 10mg/200g BW, RIF 10mg/200g BW, extract of *C. papaya* 120mg/200g BW and *M. citrifolia* 20mg/200g BW; **P4:** INH 10mg/200g BW, RIF 10mg/200g BW, extract of *C. papaya* 120mg/200g BW and *M. citrifolia* 10mg/200g BW; **P5:** INH 10mg/200g BW, RIF 10mg/200g BW, extract of *C. papaya* 60mg/200g BW and *M. citrifolia* 20mg/200g BW.

Based on the results of the analysis of the all parameters on this research indicated that the group treatment P3 of extract combination *C. papaya* leaves 120/200g BW and *M. citrifolia* fruits 20mg/200g BW (P3 group) showed the

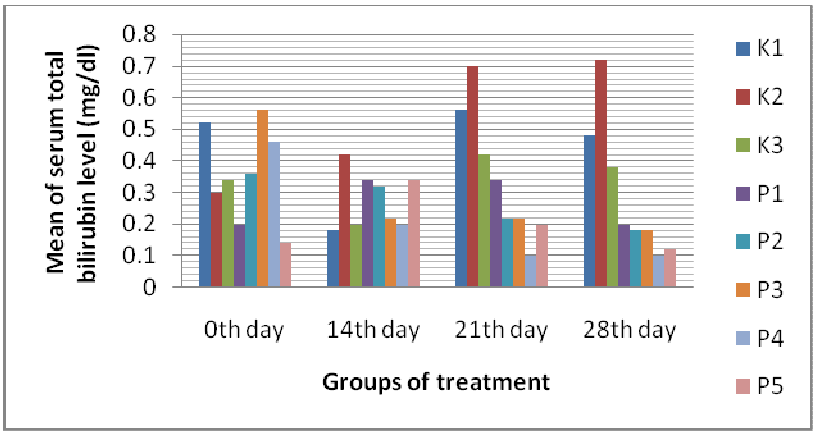

Figure 3. Serum total bilirubin serum level in the groups of animals. **K1**: normal diet and served as controls; **K2**: negative control (INH 10mg/200g BW, RIF 10mg/200g BW); **K3:** positive control (INH 10mg/200g BW, RIF 10mg/200g BW, methicol® 4,5mg/200g BW); **P1**: INH 10mg/200g BW, RIF 10mg/200g BW, extract of *C. papaya* 120mg/200g BW; **P2:** INH 10mg/200g BW, RIF 10mg/200g BW, extract of *M. citrifolia* 20mg/200g BW; **P3**: INH 10mg/200g BW, RIF 10mg/200g BW, extract of *C. papaya* 120mg/200g BW and *M. citrifolia* 20mg/200g BW; **P4:** INH 10mg/200g BW, RIF 10mg/200g BW, extract of *C. papaya* 120mg/200g BW and *M. citrifolia* 10mg/200g BW; **P5:** INH 10mg/200g BW, RIF 10mg/200g BW, extract of *C. papaya* 60mg/200g BW and *M. citrifolia* 20mg/200g BW

effects of better protection of liver damage in antituberculosis-induced rats. A compound of suspected as hepatoprotektor in *C. papaya* and *M. citrifolia* is flavonoid. The Flavonoid is a good reducing agent because it can inhibit many oxidation.

Flavonoid act as the good free radical scavenging, so given a protection of lipid membrane to destructive reactions of radical hydroxyl. Antioxidant activity of flavonoids in *M. citrifolia* fruit and *C. papaya* can inhibit oxidation reactions in the body that are caused due to the reaction of isoniazid and rifampicin. An antioxidant property is claimed to be one of the mechanisms of hepatoprotective effect of the test drug.

M. citrifolia contains proxeronin and xeronin compounds. Xeronin is one of alkaloid compounds. Proxeronine is a precursor which is required in the biosynthesis xeronine.The activity of xeronine at the molecular stage in the cells as mitochondria and microsom. Xeronine stimulated the pores dilatation of the cell membrane, that to facilitate the protein molecules intake among other cell. The cell used a protein molecules in order to maintain a balance and increase the cell activity efficiently (Christina, 2005 *cit* Solomon 1998). In addition, *M. citrifolia* fruit also contain damnachantal that is a antrakuinon compound. These compounds do to repair damaged cell structure into normal cells. Damnachantal has a mechanism to stimulate the growth structure of abnormal cells to be normal cel, so it can repair damaged cells (Anonymous, 2009).

Based on the research results have been obtained a combination of extract *C. papaya* leaves 120mg/200g BW rats and *M. citrifolia* fruit 20 mg/ 200 g BW of rats that can reduce activity of ALT, AST and total bilirubin level in male strain wistar. It is possible because the effect of synergism or complementary of active substance that can increase hepatoprotective effect.

CONCLUSION

The conclusion obtained from this research that the combination of papaya leaves extract 120 mg/ 200 g BW rats and morinda fruits extract 20 mg/ 200 g BB rats can lower levels of ALT, AST and bilirubin in liver of white rats (*Rattus norvegicus*) on the treatment of TBC with drug isoniazid and rifampicin.

ACKNOWLEDGMENT

The authors wish to thank the DP2M DIKTI, Indonesia for providing financial support at Research Grant Competition on 2010.

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