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Toxicity Study of *Aloe vera* Extract for Multi Drugs Resistant (MDR) of Tuberculosis

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ABSTRACT

Several recent studies have shown that *Aloe vera* extract is very benefit to help the treatment of patients with TB MDR, but for the dose of toxicity still have many differences of arguments. We studied the effects of *A. vera* extract on mice to identify potential toxic and to assess the normal dose to hepatoprotective. This study used a post-test-only control group design with twenty-one of mice were randomly selected as sample. All the samples was divided by six groups and in each groups had three mices. Group 1 (control) was orally administered with 1 ml/day of distilled water and groups 2 - 5 received graded levels; 200, 300, 400, 500, 1000 and 2000 mg/kg of *A. vera* etanol leaf extract respectively. The amount of mice mortality was observed once daily, performed for 14 days. Haematological tests were conducted for sub-acute and chronic tests. Acute and sub-acute toxicity test results for 2 x 24 hours, 14 days and 60 days using 7 doses of stages (200,300,400, 500, 1000 and 2000 mg / kg BW), no mice experienced death. For chronic toxicity, was shown from SGOT and SGPT for 60 days which showed no significant increase in all doses. It's with a range of SGOT 40 to 87 U/L levels, and SGPT 0-35 U/L values. The ethanol extract of *A. vera* do not produce significant toxic effect during sub-acute and chronic treatment in mices. Hence, the extract can be utilized for formulations.
KEYWORDS: Toxicity, *A. vera*, Leaf Extract, Tuberculosis Disease

1. INTRODUCTION

Tuberculosis is an infectious disease remains a problem worldwide, especially in developing countries [1]. Tuberculosis was the world's health problems were serious and must be addressed because of the emergence of resistance to first and second line drugs, particularly with regard to multidrug resistant (MDR) and extensively drug resistant (XDR) against Mycobacterium tuberculosis [2]. Increased prevalence of Mycobacterium tuberculosis (MTB) which is resistant to many drugs would increase the morbidity and mortality of the disease [2]. In addition, three first-line drugs are pyrazinamide, isoniazid and rifampicin have hepatotoxic effects. Long-term treatment with antituberculosis drug use can lead to drug-induced liver injury (DILI) [3].

The mechanism of DILI was includes cell stress, mitochondrial damage, and specific immune response [4]. The liver as the organ that serves to detoxify cell will experience prolonged stress. Stress on the cell will trigger an increase in inflamatory cytokines. As a result, the liver cells become more susceptible to apoptotic effects of TNF-α, Fas ligand and IFN-γ [5]. These effects can be inhibited by inhibitors of apoptosis proteins (IAPs) or Bcl-2 [6].

Extracts of the *A. vera* plant have long been used as herbal remedies and are also now promoted as a dietary supplement, in liquid tonics, powders or tablets, as a laxative and to prevent a variety of illnesses [7]. *A. vera* can be used to reduce drug resistance as an anti-tuberculosis drug, and has been shown in previous studies to inhibit bacteria Mycobacterium Tuberculosis H37Rv and MDR TB strains HE (resistant to INH & Ethambutol) and SR (Streptomycin & Rifampicin Resistance) [8]. *A. vera* extract has been shown to contain antioxidants that can act as hepatoprotective. *A. vera* has potential as an immunomodulator and proven that *A. vera* extract could inhibit the increase of percentage of NK cells, Th-17 cells, TNFα, IL-1. It also has potential as a hepatoprotective as evidenced by inhibiting MDA, SGOT, SGPT, hepatic cell apoptosis and PXR expression. Administration of *A. vera* can suppress the production of TNF-a and the percentage of Th17 cells as a result of antituberculosis drug administration. *A. vera* can be a useful alternative to natural materials in the successful treatment of tuberculosis through the inhibition of side effect [9].

Many studies *A. vera* extract could no toxic, but some studies it's could cause toxic effects in mice. The dose of toxicity still have many differences of arguments. The results of previous research of LD50 value of *A. vera* extract expressed with cumulative mortality in male and female mice was 35 g / kg BW and equal to dose 24,5 g / kg BW in mice. In the acute toxicity test, *A. vera* leaf extract treatment by oral route at doses up to 2560 mg/kg produced death in 20% (2/10) of chicks during 24 h or 14 days of observation. The therapeutic use of the hydroalcoholic extracts of *A. vera* had very low toxicity in oral acute high dose administration [2]. In another study, *A. vera* had induced acute liver damage. Acute hepatitis *A. vera* associated with the use of *A. vera* was reported in 2015 in an old women [10]. Therefore, based on the reason above is important to study the effects of *A. vera* extract on mice to identify potential toxic and to assess the normal dose to hepatoprotective.

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2. MATERIALS AND METHODS

Animal

Male mice (20-30 g) were obtained from the Biosains Laboratory Animal Center, Brawijaya University. All animals were kept in the room, maintained under environmentally controlled conditions of 25 ± 1^0C and 12 h light-12 h dark cycle. Food and water were available freely. All experimental protocols were approved by the Animal Ethics Committee from the Health Research Ethics Committee, Health Polytechnical of Malang, Malang, East Java, Indonesia

A. vera **Extraction**

A. vera plants are cleaned and aerated to dry, with a moisture content of 5%. Plants that have been dried cut and ground to a form a powder. A total of 300 g of powder put in a flask (2000 ml) for macerated in 96% ethanol. Maceration was performed for 6 hours while shaken using a shaker with a speed of 40 RPM. *A. vera* powder marinade was refluxed for 3 hours and filtered using Whatman filter paper (No. 42). Pulp filtration was refluxed again with 96% ethanol, repeated 2 times. Ethanol in the filtrate was removed by evaporated using a vacuum evaporator at 40°C, in order to obtain a crude extract.

Acute and Chronic toxicity test

Toxicity test procedures were carried out [11,12]. All the samples of mice was divided by six groups and in each groups had three mices. Group 1 (control) was orally administered with 1 ml/day of distilled water and groups 2 - 5 received graded levels; 200, 300, 400, 500, 1000 and 2000 mg/kg of *A. vera* etanol leaf extract respectively. All of the samples were fasted for 24 hours before treatment is given.

Sub Acute toxicity test

The sign of toxicity and death were observed over 2 weeks. Observed once daily for 14 days. Observed sign of toxic and death, body weight and pathological examination. During the experiment, food and water were available freely.

Chronic toxicity test

All of groups was administered orally with the extract *A. vera* daily for 60 days after the treatment in order to detect a delayed occurrence of toxic effect. Food and water were available freely during the experiment. The appearance of signs of toxicity, behavioral alterations, and mortality was observed and recorded. The body weight of rat was measured each week

Liver Function Test

Transaminase serum aspartate amino transferase (AST) and alanine amino transferase (ALT)] activities were examined with automated analyzer (Hitachi 912)

Data analysis

The data was presented in mean ± SD. Then all data were analyzed using One-Way ANOVA test. One-Way ANOVA statistic test followed by Tuckey test to know the difference of each group. If the One-Way ANOVA test does not meet the requirements then data transformation is performed and if it does not meet the requirements of the Kruskal-Wallis test, if significant is continued with the Mann-Whitney test.

3. RESULTS AND DISCUSION

The popularity and the use of herbal medicine products gradually increasing among healthy individuals. The one of them is *A. vera* extract. The use of *A. vera* extract has been used widely in the world as an herbal medicine. The hepatic toxicity is a potential complication of these compounds that may lead the hepatic insufficiency [10]. Many studies had showed the beneficial of *A. vera* and its effect to hepatoprotective. But in another hand some studies had showed that *A. vera* had produced hepatotoxite.

In 37-years old previously healthy man was hospitalized with signs and symptoms of hepatic toxicity. He had no previous history of liver disease, alcohol consumption, and blood Transfusion. He had taken *A. vera* capsule 500 mg of the extract *A. vera* (1 capsule/day) for 3 weeks. Laboratory test had showed increasing of bilirubin, asparta aminotransferase, and alanine aminotransferase. When *A. vera* was immediately discontinued and the patient resolved completely within 7 days. Laboratory findings gradually returned to normal ranges within 7 weeks [10]. The livers of rat treated with propolis showed protection against the toxic effect of carbon tetrachloride, but *A. vera* extract failed to restore the normal appearance of hepatocytes [13]. Hepatitis in a 57-year old female could be linked to the ingestion of Aloe barbadensis miller compounds. The patient´s hepatitis resolved completely after discontinuing this medication [14].

The results of previous research of LD50 value of *A. vera* extract expressed with cumulative mortality in male and female mice was 35 g / kg BW and equal to dose 24,5 g / kg BW in mice. In the acute toxicity test, *A. vera* leaf extract treatment by oral route at doses up to 2560 mg/kg produced death in 20% (2/10) of chicks during 24 h or 14 days of observation. The therapeutic use of the hydroalcoholic extracts of *A. vera* had very low toxicity in oral acute high dose

administration [15]. *A. vera* polysaccharides (AVGP) exerts a potent protective effect against chronic alcohol-induced liver injury. Its hepatoprotective effect appears to be associated with its antioxidant capacity and its ability to accelerate lipolysis and inhibit inflammatory response [16].

In our study (Table 1 and Table 2), the result of observations on 2 x 24 hours for14 days and 60 days that using 7 doses of stages (200, 300, 400, 500, 1000 and 2000 mg / kg BW), no mice experienced death. The result SGOT and SGPT examinations within 60 days had showed a significant increased in all doses. The increasing of SGOT and SGPT levels with a range of SGOT 40 to 87 U / L levels, and SGPT levels SGOT Normal SGR 3-45 I / L and SGPT 0-35 U / L values. The increasing of it was not significant.

Table 1: Effect of *A. vera* extract on body weight of male mice

	Dose (mg/kg)	Body Weight (g)		
		Day 0	Day 30	Day 60
Control		26.5±1.9	26.7±2.2	30.5±1.7
Group 1	200	25.3±3.7	25.2±3.8	31,3±2.6
Group 2	300	24.7±2.0	24.9±1.9	29.2±0.5
Group 3	400	24.7±1.8	25.3±1.9	29.2±1.3
Group 4	500	24.7±2.5	24.5±1.8	29.7±0.4
Group 5	1000	26.3±2.0	26.3±2.0	30.4±0.8
Group 6	2000	24.6±2.6	24.6±2.6	28.9±0.7

Values are expressed as mean ± SD, n = 3 p>0.05

Table 2: Effect of *A. vera* extract on SGPT an SGOT Level of male mice

	Dose (mg/kg)	SGOT (U/L)	SGPT (U/L)
Control		95,3±4.04	44±2.3
Group 1	200	119±0.3	70±43.9
Group 2	300	134±2.5	66±48.9
Group 3	400	204±0.7	52±1.4
Group 4	500	186±1.3	81±0.7
Group 5	1000	101±6.3	47±65.8
Group 6	2000	85±4.04	75±6.5

Values are expressed as mean ± SD, n = 3 p>0.05

We support that *A. vera* extract has a low toxic effect. But to use it must perform with the appropriate dose and the liver function should be monitored. In our study (figure 1) had showed the content of *A. vera* extract which has high potency as hepatoprotective substance includies; Rutin, 6-Hydroxytricetin 6.7.3'.5'-tetramethyl eter 5-robinobioside, Aloeresin E, Isoaloeresin D, Isorabaichromone. We suggest for the next study not using extract but using the smaller particulate in liposome or nano particles. We suspect that smaller particles can reduce or even eliminate the effects of hepatotoxite and certainly have more effective of drug effects.

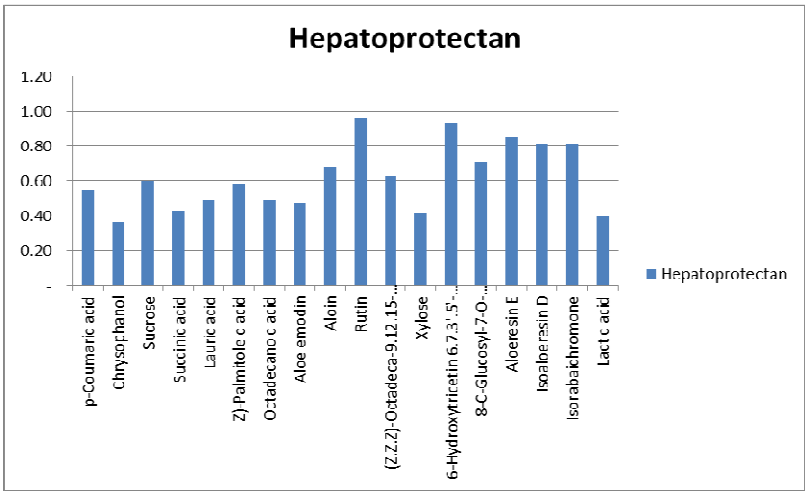

Figure 1. The results of hepatoprotectic test on the active ingredient content of *A. vera* extract using insilico test

4. CONCLUSION

It was concluded that administration of *A. vera* with 7 doses of stages (200,300,400, 500, 1000 and 2000 mg / kg BW), do not produce significant toxic effect during acute, sub-acute and chronic administration in mices. Hence, the extract can be utilized for formulations. *A. vera* was safe and can be a useful alternative to natural materials in the successful treatment of tuberculosis.

Confict of interest:
All authors declare that no conflict of interest in the research and publication of this study.

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