



**KEMENTERIAN RISET, TEKNOLOGI DAN PENDIDIKAN TINGGI
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FAKULTAS KEDOKTERAN
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RSUP Dr. WAHIDIN SUDIROHUSODO MAKASSAR
KOMITE ETIK PENELITIAN KESEHATAN**



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REKOMENDASI PERSETUJUAN ETIK
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Tanggal: 26 Februari 2019

Dengan ini Menyatakan bahwa Protokol dan Dokumen yang Berhubungan Dengan Protokol berikut ini telah mendapatkan Persetujuan Etik :

No Protokol	UH19010022	No Sponsor	
Peneliti Utama	Imelda Dhea Meylin B	Protokol	
Judul Peneliti	Pengaruh Pemberian Ekstrak Etanol Daun Paliasa (kleinhovia hospita L) Terhadap Bilirubin Total Tikus (rattus norvegicus) Yang Diinduksi Obat Antituberkulosis Kombinasi Dosis Tetap		
No Versi Protokol	2	Tanggal Versi	20 Februari 2019
No Versi PSP		Tanggal Versi	
Tempat Penelitian	Laboratorium Fakultas Farmasi Universitas Hasanuddin Makassar		
Jenis Review	<input type="checkbox"/> Exempted <input checked="" type="checkbox"/> Expedited <input type="checkbox"/> Fullboard Tanggal	Masa Berlaku 26 Februari 2019 sampai 26 Februari 2020	Frekuensi review lanjutan
Wakil Ketua Komisi Etik Penelitian	Nama Prof.Dr.dr. Suryani As'ad, M.Sc.,Sp.GK (K)	Tanda tangan	
Sekretaris Komisi Etik Penelitian	Nama dr. Agussalim Bukhari, M.Med.,Ph.D.,Sp.GK (K)	Tanda tangan	

Kewajiban Peneliti Utama:

- Menyerahkan Amandemen Protokol untuk persetujuan sebelum di implementasikan
- Menyerahkan Laporan SAE ke Komisi Etik dalam 24 Jam dan dilengkapi dalam 7 hari dan Laporan SUSAR dalam 72 Jam setelah Peneliti Utama menerima laporan
- Menyerahkan Laporan Kemajuan (progress report) setiap 6 bulan untuk penelitian resiko tinggi dan setiap setahun untuk penelitian resiko rendah
- Menyerahkan laporan akhir setelah Penelitian berakhir
- Melaporkan penyimpangan dari prokol yang disetujui (protocol deviation / violation)
- Mematuhi semua peraturan yang ditentukan

THE PROTECTIVE EFFECT OF PALIASA (*Kleinhovia hospita* L.) LEAF EXTRACT AGAINST ELEVATED TOTAL BILIRUBIN SERUM INDUCED BY TOXIC DOSE OF ANTITUBERCULOSIS IN RATS

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Abstract

Introduction: Liver dysfunction is implicated with the use of Antituberculosis (AT) leading to low compliance of TB patients to AT regimen. One of biomarker that is important to measure liver dysfunction is total bilirubin serum. Paliassa leaves have been empirically used to improve liver function in South Sulawesi. This study aimed to determine the effect of ethanolic extract of Paliassa leaves on total bilirubin serum in rats.

Method: Twenty rats were divided into five groups: Group I served as the healthy control was only given NaCMC suspension, group II was treated with 178 mg/200g of AT suspension, while group III, group IV and group V were given Paliassa extract 125 mg/kgBB, 250mg/kgBB and 500 mg/kgBB, respectively, 4 hours prior to AT administration. Treatments were performed once a day for 28 days. Blood sampling was carried out 24 hours following the last treatment. The total bilirubin levels were measured using Humalyzer 3500.

Result: The results showed that the administrations of AT suspension for 28 days significantly increased rat total bilirubin levels. Paliassa leaf extract in all given dose was able to reduce the total bilirubin levels of rats compared to the group treated with AT only. However, statistical significance was only reached by the groups that were treated with Paliassa extract 500 mg/kg.

Conclusion: Therefore, it is concluded that Paliassa extract at a dose of 500 mg/kg has protective effect against AT-induced elevation of total bilirubin serum in rats.

INTRODUCTION

Liver is the main metabolic organ of the body, which has complex functions in regulating the metabolism of nutrition, drugs or toxic compounds. As most drugs are metabolized in the liver, it becomes vulnerable to drug-induced liver injury, especially when the drugs produce toxic metabolites.¹ The presence of liver injury or

damage is mainly detected by the elevation of liver enzymes in the serum, including alanine transaminase (ALT) and aspartate transaminase

(AST). In addition, serious malfunction of the liver can manifest in hyperbilirubemia due to the dysfunctional uptake, conjugation and excretion of bilirubin.² If the concentration of bilirubin serum significantly elevates, it diffuses to the surrounding tissues leading to yellowish appearance of the skin and mucous membranes, commonly called as jaundice.³

The first line of antituberculosis regimen composes of Isoniazid (INH), Rifampicin (RIF), Pyrazinamid (PZA) and Ethambutol (EMB).

Three of the four drugs (INH, RIF and PZA) are known to possess capability to harm liver function⁴. As a result, high incidence of hepatotoxicity is found among tuberculosis patients treated with AT drugs around the world⁵⁻⁷, affecting children and adult patients.⁸

Paliasa (*Kleinhovia hospita* L.) is a native plant from South Sulawesi that has been used traditionally to treat jaundice and hepatitis.⁹ Four cycloartane triterpenoid alkaloids have been isolated from *Kleinhovia Hospita*, i.e. *Kleinhospitines* A, B, C and D, and these compounds have been shown to be protective against H₂O₂ radical induced damage in hepatocyte cell culture.¹⁰ In the previous study, Paliasa leaf extract with the dose of 250 mg/kg and 500 mg/kgBB were found to have the ability to reduce liver cell damage caused by carbon tetrachloride (CCl₄).¹¹ More currently data on paliasa leaf extract also showed a superior protection against doxorubicine-induced hepatotoxicity with a dose of 250 and 500 mg/kg.¹² However, there is still limited data exploring the capacity of Paliasa leaf extract on preventing hyperbilirubinemia, especially those induced by AT drugs. Therefore, the aim of this study is to determine the effect of Paliasa leaf extract on serum total bilirubin in rats treated with toxic dose of AT drugs.

METHODS

Animal preparation

Twenty male rats weighed 150-200 g were used in the study. The animals were fed with standard pellets and drink ad libitum. The animal care protocols were carried out based on institution animal standard of care. Rats were acclimatized for 14 days prior to treatment.

Drug preparation

Antituberculosis was purchased in fixed dose combination (Rifastar®) in a local pharmacy. The tablets were pulverized and suspended in NaCMC 1% to facilitate oral gauge in rats. The AT suspension was made with 8.9 g of AT in 100 ml suspension (8.9%). The preparation of suspension was done on daily basis.

Extract Preparation

Paliasa (*Kleinhovia hospita* L.) leaves were obtained in Makassar. The samples were washed using tap water and dried in an oven (40°C) before macerated in 70% ethanol (sample: ethanol =

1:10) for 24 hours followed by re-maceration for another 24 hours. The extract obtained was evaporated using a rotary evaporator (Heidolp®) to form a thick extract. The extract was kept in a desiccator at room temperature to prevent it from excessive humidity. Immediately prior to treatment, the extract was prepared in 1% NaCMC suspension.

Experimental protocols

Animals (n=20) were assigned in 1 of 5 groups. Group I served as healthy control group was only given 1% NaCMC suspension. Group II was treated with 178 mg/kg of AT drug suspension. Group 3 was treated with Paliasa leaf extract 125 mg/kg prior to AT drug administration. Group 4 was treated with Paliasa leaf extract 250 mg/kg prior to AT drug administration. Group 5 was treated with Paliasa leaf extract 500 mg/kg prior to AT drug administration. All treatments were carried out for 28 days. Blood samples were withdrawn 24 hours following the last treatment. In addition, blood samples were randomly taken from nine healthy rats prior to treatment to measure baseline bilirubin level. The total bilirubin serum was measured using Humalyzer 3500 with Total Bilirubin Reagent® and T-Nitrite Reagent (Human®).

Statistical analysis

Data is presented in mean ± SD. Statistical analysis was performed using SPSS 24. The distribution of data was determined using Kolmogorov Smirnov analysis. Data then analyzed with One-way ANOVA. The significant difference between treatment groups was analyzed using post hoc Bonferonni test. Statistical significance is achieved if p<0.05.

RESULTS

Baseline serum total bilirubin

In this study, the level of serum total bilirubin was measured using Humalyzer 3500. For healthy wistar rats, it is found that serum total bilirubin ranged from 0.090-0.205 mg/dl with the average of 0.613 ± 0.045 (Table 1).

Table 1. Serum total bilirubin in healthy rats measured using Humalyzer 3500

Rat	Total bilirubin serum (mg/dl)	Range (mg/dl)	Mean (mg/dl)	Standard Deviation
1	0.237			
2	0.090			
3	0.198			
4	0.130			
5	0.157	0.090-0.205	0.163	0.045
6	0.205			
7	0.170			
8	0.159			
9	0.125			

Serum total bilirubin after treatments

Following 28 days of treatments, it is shown that rats that were only given NaCMC (healthy controls) had total bilirubin level within the baseline range (Figure 1). Meanwhile, rats that were treated with AT 178 mg/kg daily had significantly increased total bilirubin level. The mean level of total bilirubin in AT group was more than three times of those in NaCMC group. Administration of Paliasa leaf extract 125 and 250 mg/kg was shown to reduce total bilirubin level in AT-treated rats. However, the reduction of total bilirubin levels in those groups did not reach statistical difference compared to AT group. In contrast, the administration of higher dose (500 mg/kg) seemed to improve the total bilirubin level significantly. Indeed, the level of total bilirubin in rats treated with 500 mg/kg extract ranged between 0.131-0.172 mg/dl, which still falls in the baseline range (see table 1).

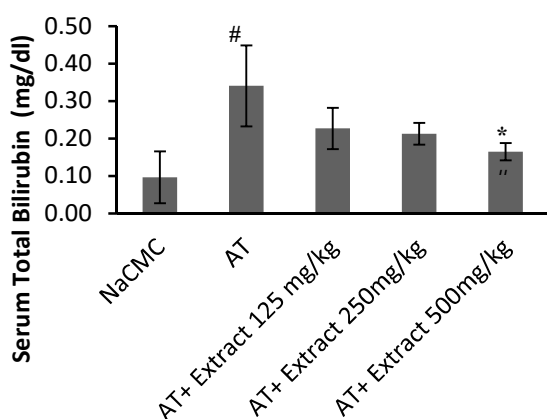


Figure 1. Serum total bilirubin in rats following 28 days of treatments.

#p<0.05 compared to NaCMC group.

*p<0.05 compared to AT group

DISCUSSION

Bilirubin is a product of heme degradation during red blood cell decomposition. The unconjugated bilirubin is carried by albumin to the liver to undergo conjugation with the enzyme glucuronosyl transferase. The conjugation process led to formation of water-soluble bilirubin, thus, it is easier to be excreted.¹³ Serum total bilirubin is the sum of conjugated and unconjugated bilirubin in the serum. From the data, it was found that rats' total bilirubin ranged from 0.090-0.205 mg/dl. This range is quite low compared to those in humans (0.3 to 1.2 mg/dl).¹³ It is necessary to perform baseline measurement prior to treatment to set a standard value of healthy rats.

In this study, rats were administered with toxic dose of AT, which is double the therapeutic dose, to induce hepatotoxicity in the rats. Figure 1 shows that the AT-treated rats experienced a significant increase in the serum total bilirubin. All animals (100%) in the AT group had elevated total bilirubin above normal range, indicating a liver dysfunction in those rats. Elevated bilirubin total in serum may result from increased conjugated and/or unconjugated bilirubin. Unfortunately, this study did not measure the direct and indirect bilirubin to give a bit of information regarding the etiology of hyperbilirubinemia in these rats. It has been shown that isoniazid can damage hepatocyte due to increased stress oxidative, which attacking the cell membrane of hepatocytes, leading to increased serum ALT and AST.¹⁴ Meanwhile, mechanism of toxicity in rifampicin-treated rats involved a damage in bilirubin transporter, leading to cholestasis and increased bilirubin in serum.¹⁵

Following 28 days of treatments, it is shown that Paliasa leaf extract was able to hold further increased in total bilirubin. Although there was an increase in serum total bilirubin in Paliasa-treated rats, it was not significantly different from NaCMC group, indicating the elevation was not severe. In fact, with higher Paliasa extract dose (500 mg/kg) the total bilirubin was still in the range of normal baseline, indicating a hepatic protection by Paliasa extract in higher dose. It has been shown that Paliasa leaf contains antioxidant compounds that may beneficial to scavenge free radicals.¹⁶ The antioxidant compounds of Paliasa leaves have been found in its ethanolic extract, i.e cycloarthane terpenoid.¹⁷ It is suggested that Paliasa leaf also have a capacity to improve

hepatotoxicity in animals induced by paracetamol, which mechanism may involve the increase in glutathione production in rat liver.¹⁸

CONCLUSION

Toxic dose of AT in rats induced liver injury that was characterized by a significant elevation of serum total bilirubin. The administration of Paliasa extract at the dose of 500 mg/kg prior to AT treatment in rats prevented the elevation of serum total bilirubin compared to those without Paliasa extract. The use of Paliasa leaf extract may find clinical significance to protect liver function from AT-induced liver damage.

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