



Kleinhovia hospita extract alleviates experimental hepatic and renal toxicities induced by a combination of antituberculosis drugs

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ABSTRACT

Introduction: Antituberculosis drugs are associated with hepatic and renal toxicities due to drug's radical metabolites. *Kleinhovia hospita* L extract possesses a potent antioxidant capacity that can be beneficial in eradication of oxidative-induced cell damage. This study aimed to evaluate the effects of *K. hospita* hydro-alcoholic extract on biomarkers and structure changes in liver and kidney induced by a combination of antituberculosis drugs (CAD), comprising isoniazid, rifampicin, pyrazinamide and ethambutol in Wistar rats.

Methods: Thirty-five male Wistar rats were assigned into one of the five groups: control, CAD, and CAD with *K. hospita* extract in three different doses (125, 250 and 500 mg/kg). The extract was administered three hours prior to CAD and all treatments were carried out for 28 days. Following the last day of treatment, blood samples and organs were collected for biomarker analysis and histopathological examinations.

Results: Twenty-eight days of CAD treatment in rats induced marked elevation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), serum creatinine and urea levels compared to controls. *K. hospita* extract at higher doses (250 mg/kg and 500 mg/kg) significantly improved ALT, urea and creatinine levels in the rats treated with CAD ($P < 0.05$), although it did not significantly reduce AST. Furthermore, liver and renal tissue damages induced by CAD were restored with *K. hospita* extract treatment, especially at higher doses.

Conclusion: *Kleinhovia hospita* extract treatment has the potential to protect liver and renal damage induced by toxic doses of CAD.

Implication for health policy/practice/research/medical education:

The incidence of organ toxicities has been a major problem experienced by tuberculosis (TB) patients during the course of antituberculosis treatment. The antioxidant activity of *K. hospita* extract potentially provides a therapeutical effect to alleviate the hepatotoxicity and nephrotoxicity induced by antituberculosis drugs. Hence, the use of this extract might be beneficial in patients who use these antituberculosis drugs.

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Introduction

Tuberculosis (TB) is one of the top ten causes of death worldwide, with around 10 million new TB cases found in 2017 alone (1). Most TB cases are effectively treated by taking the core antituberculosis (AT) regimen comprising isoniazid, rifampicin, pyrazinamide, and ethambutol, for six months (2). The success of TB treatment essentially relies on the adequate dose and patients' adherence to their

medication regimen (3). Thus, the use of combination of antituberculosis drugs (CAD) is recommended to simplify TB regimen and consequently improve drug adherence (4). However, serious side effects of AT may also discourage patient's compliance with medication (3). Isoniazid, rifampicin and pyrazinamide regimens potentially induce hepatotoxicity, and their concomitant use might increase the risk (5,6). Previous studies have

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reported 12-32% patients who are treated with AT agents experience hepatotoxicity indicated by elevation of ALT more than 3-5 times upper normal level (ULN) (6-8). In addition to hepatotoxicity, the use of AT treatment, in particular rifampicin, has been reported to cause acute renal failure (9-11). The incidence of rifampicin-induced nephrotoxicity is around 7%, wherein 27% of the cases lead to permanent renal damage (11). These serious side effects obviously challenge the completion of the lengthy TB treatment protocol.

Kleinhovia hospita, belonging to the family of Sterculiaceae, is a native plant from Indonesia, but may also be found in Asia Pacific region. *K. hospita* has been empirically employed in Indonesia to treat hepatitis from time to time. The chemical compounds contained in *K. hospita* extract, such as cycloartane triterpenoids (12,13), eleutherol and kaempferol 3-O- β -D-glucoside, are known to possess antioxidant activities (14). The hepatoprotective effect of *K. hospita* has been demonstrated in chronic hepatic patients as well as against experimental paracetamol overdose (15). More recently, the protective effect of *K. hospita* extract has also been shown against doxorubicin-induced cardiac, liver and renal dysfunctions (16). Based on that, the present study aimed to investigate the effects of *K. hospita* hydro-alcoholic extract on biomarkers and structure changes in liver and kidney induced by CAD comprising isoniazid, rifampicin, pyrazinamide and ethambutol in Wistar rats.

Materials and Methods

Preparation of chemicals and drugs

CAD tablets (Rifastar[®], Indofarma) were purchased in a registered pharmacy in Makassar, Indonesia. Each tablet contained 150 mg rifampicin, 75 mg isoniazid, 400 mg pyrazinamide, and 275 mg ethambutol HCl. The tablets were pulverized and suspended with Na salt of carboxyl methylcellulose 1% (NaCMC) immediately before administration. The daily dose given to animals was 712 mg/kg of rat body weight. Biomarker assay kits, including GOT (ASAT) IFCC mod.liquiUV, GST (ALAT) IFCC mod.liquiUV, urea liquicolor and creatinine liquicolor, were obtained from Human Diagnostics Worldwide (Germany).

Preparation of *Kleinhovia hospita* extract

Kleinhovia hospita extract was obtained with maceration extraction method by submerging 400 g dried ground *K. hospita* leaves in 70% ethanol aqueous solution in maceration chambers for three days at room temperature. The 70% ethanol was chosen as the solvent because of its semipolar nature; hence more compounds would be extracted out of the leaves. The *K. hospita* extract was then concentrated using a rotary evaporator (Heidolph[®]). The concentrated extract was kept in desiccator at room temperature (25°C) to remove excess solvent or moisture.

The extract was prepared in a 1% NaCMC suspension immediately before administration to the animals.

Preparation of animals

Thirty-five male Wistar rats (150-200 g) were placed in the animal house of Pharmacology and Toxicology Laboratory, Hasanuddin University (Makassar, Indonesia). The rats were housed in well-aerated cages with wood-based bedding at room temperature with 12:12 h light/dark cycle. All animals were provided with standard pellets and water *ad libitum*. The rats were accustomed to the laboratory environment for seven days before the experiment commenced.

Experimental protocols

Rats (n = 35) were divided into five groups. Group I only received 1% NaCMC as the placebo (controls), group II received CAD in 1% NaCMC suspension with the dose of 712 mg/kg of rat body weight, group III received CAD + *K. hospita* extract in low dose (125 mg/kg BW), group IV received CAD + *K. hospita* extract in medium dose (250 mg/kg BW), group V received CAD + *K. hospita* extract in high dose (500 mg/kg BW). The daily dose of CAD was 712 mg/kg of rat body weight. Animals were daily weighed to accordingly adjust the amount of CAD and extract given to each animal. *K. hospita* extract was administered daily three-hours prior to CAD treatment. All treatments administered via oral gavage for 28 days. The collection of serum was carried out prior to treatment (day 0) and 24 hours after the experiment ended (day 28). At day 30, the rats were sacrificed to harvest their livers and kidneys for further examination.

Biochemistry analysis

Blood samples were centrifuged at 2500 rpm for 20 minutes or until serum separation from blood cells was thorough. The serum was kept in -20°C until analyzed. The ALT, AST, serum creatinine and urea levels were measured using diagnostic kits for Humalyzer 3000 (Human[®]) based on the kit instructions.

Histopathological examination

After removed, rat livers and kidneys were directly cleansed in ice-cold phosphate-buffered saline (PBS) before fixed in 10% buffered formalin solution. After 48 hours, the organs were trimmed and prepared using a tissue processor (Thermo Scientific[®]). The processed tissues were then embedded in paraffin wax, sectioned into 4-5 μ m of thickness with a microtome (Sakura[®]), and stained with Hematoxylin and Eosin (H&E). The tissue sections were analyzed using a light microscope (Nikon[®]) connected to a computer screen.

Statistical analysis

All data was analyzed using SPSS 22 software. Data

was examined using the Kolmogorov-Smirnov test to determine normal distribution before analyzed, using one-way ANOVA. Differences between groups were identified using Duncan post hoc test. Statistical significance was reached if $P < 0.05$. The data that was not distributed normally was analyzed using the Kruskal-Wallis test. Numerical data is presented in mean \pm standard error mean (SEM).

Results

Liver biomarkers

As shown in Figure 1, the control rats that did not receive CAD experienced no significant increase in levels of liver and renal biomarkers on day 28 compared to day 0. In fact, AST level in control rats significantly decreased. This may emerge as a result of a better adaptation of the rats with their environment after 28 days in the laboratory. The ranges of AST and ALT levels on day 28 in control rats were 60-90 UI/L and 38-54 UI/L, respectively, while serum creatinine and urea were 0.228-0.538 mg/dL and 25-58 mg/dL, respectively.

In contrast, administration of CAD at the given dose for 28 days significantly increased the liver biomarkers (AST, ALT) as well as the renal biomarkers (serum creatinine and urea) of treated rats compared to their baseline values ($P < 0.05$). When compared with control rats on day 28, the AST and ALT levels of the group treated with CAD only were almost doubled, indicating liver dysfunction. Unlike CAD group, all groups that were treated with *K. hospita* extract prior to CAD showed slightly lower AST

level (Figure 1A); however, it did not reach statistical difference from that of CAD group due to high variability in AST levels among animals within the group. The ALT levels of groups receiving *K. hospita* extract were also lower compared to CAD treatment only; but only those treated with extract in mid-dose (250 mg/kg) showed a significant decrease in ALT level compared to that of CAD group ($P < 0.05$).

In this study, the elevation of biomarkers was calculated to provide information about the average of biomarker changes occurred in animals from day 0 to day 28 (Table 1). It is shown that the highest elevation of AST and ALT occurred in the CAD group ($P < 0.05$ compared to controls), and more than half of the animals experienced $>50\%$ elevation of AST and ALT after treatment (see Table 1). On the contrary, only 2 out of 7 (29%) animals that received *K. hospita* extract either 250 mg/kg or 500 mg/kg, encountered $>50\%$ increase in AST level. Furthermore, at those doses, *K. hospita* treatments significantly halted the elevation of ALT level with fewer number of animals experienced $>50\%$ elevation of ALT.

Renal biomarkers

The serum creatinine and urea levels of CAD increased significantly on day 28 compared to their baseline levels (day 0). Those renal biomarker levels were also significantly higher compared to the control group on day 28 (Figure 1). The elevations of creatinine and urea levels in CAD were 0.16 ± 0.022 (increased 60% from day 0) and 30.8 ± 5.09 mg/dL (increased 120% from day 0),

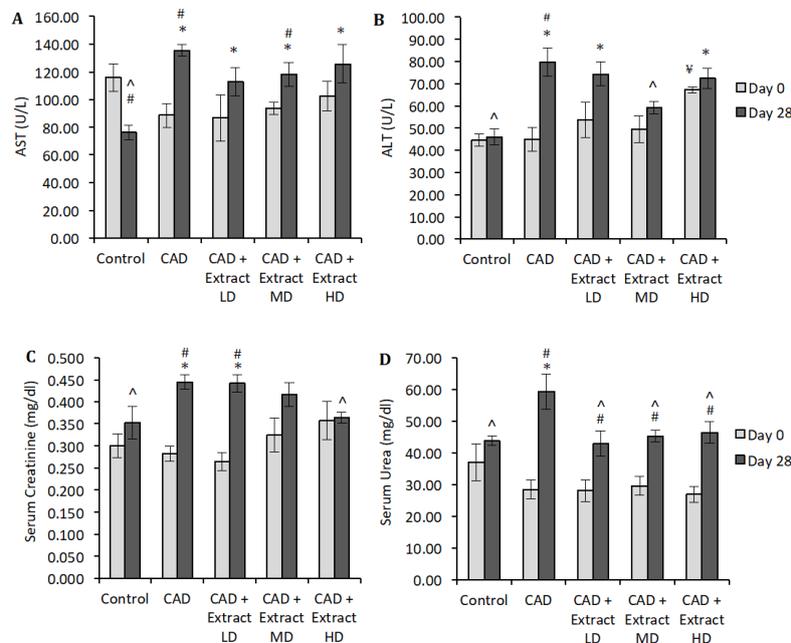


Figure 1. Liver and renal biomarker levels before (day 0) and following treatments (day 28). Data is presented in mean \pm SEM. Controls: placebo, CAD: combination of antituberculosis drugs. Extract LD (low dose): 125 mg/kg, Extract MD (mid dose): 250 mg/kg, Extract HD (high dose): 500 mg/kg. * $P < 0.05$ from control group on day 28. # $P < 0.05$ from baseline level at day 0. ^ $P < 0.05$ from the CAD group on day 28. ¥ $P < 0.05$ from all other groups on day 0.

Table 1. The comparison between percentage of elevation in liver and renal biomarker levels and percentage of animals with >50% elevation of biomarker level following 28-days of treatments

Treatments	AST		ALT		Serum Creatinine		Serum Urea	
	Elevation (IU/L)	% animal	Elevation (IU/L)	% animal	Elevation (mg/dL)	% animal	Elevation (mg/dL)	% animal
Controls (n=7)	-39.7 ± 10.54 [^]	0	1.4 ± 2.73 [^]	0	0.05 ± 0.02	0	6.8 ± 5.03 [^]	0
CAD (n=7)	46.7 ± 8.25*	57	34.7 ± 10.57*	57	0.16 ± 0.02*	57	30.8 ± 5.09*	71
CAD + Extract LD (n=5)	26.1 ± 15.54*	60	20.5 ± 9.80	60	0.18 ± 0.03*	60	14.8 ± 6.64 [^]	40
CAD + Extract MD (n=7)	24.6 ± 8.65*	29	9.8 ± 7.12 [^]	29	0.09 ± 0.06	43	15.6 ± 4.44 [^]	29
CAD + Extract HD (n=7)	23.2 ± 11.03*	29	5.0 ± 5.32 [^]	14	0.01 ± 0.04 [^]	14	19.5 ± 3.33	43

Elevation is calculated from the difference between biomarker level day 28 and day 0. Data elevation is presented in mean ± SEM.

% animal is the percentage of animals in the group that experienced >50% elevation of biomarker level after treatments

Controls: placebo; CAD: combination of antituberculosis drugs; Extract LD (low dose): 125 mg/kg; Extract MD (mid dose): 250 mg/kg; Extract HD (high dose): 500 mg/kg.

[^]P < 0.05 compared to CAD group. *P < 0.05 compared to control group.

respectively. These elevations were significantly higher than those seen in the control group (see Table 1). The percentage of animals experienced >50% elevation of creatinine and urea levels were 57% and 71%, respectively.

When compared to CAD group, rats that received *K. hospita* extract in mid and high doses had lower creatinine level, but only reached statistical significance with high dose extract ($P < 0.05$). All three groups treated with *K. hospita* extract showed significant reduction in urea levels compared to that in CAD group (see Figure 1). Indeed, the level of serum urea in *K. hospita* treated groups was similar to that seen in the control group on day 28. As seen in Table 1, the number of animals that experienced >50% elevation of creatinine and urea was also reduced in *K. hospita* treated groups, particularly in mid and high doses.

Liver histopathology

Control rats had normal liver histology structure as depicted in Figure 2A (100x magnification) and 2a (400x magnification). The central vein was clear with no congestion, the hepatocytes had a healthy-looking nucleus, and there was no sign of inflammation. In contrast, in CAD treated rats, there was a pool of blood congesting the central vein and increased number of inflammatory cells found in sinusoid (Figure 2B). Degeneration of hepatocytes was evident, which showed the characteristic of ballooning cytoplasm, hydropic hepatocyte, as well as vacuolization (Figure 2b). Following *K. hospita* extract treatment in low dose (125 mg/kg), liver histological damage was still observed, shown by congestion, accumulation of inflammatory cells, hepatocyte hydropic degeneration, and vacuolization (Figure 3A, 3a). In rats treated with the mid dose of *K. hospita* extract (250 mg/kg), the hepatocyte had less histopathological changes, only a few scattered inflammatory cells were found in the sinusoid; yet, vessel congestion was still evident (Figure 3B, 3b). Meanwhile, with high dose (500 mg/kg) of *K. hospita* extract, the hepatocyte structures were similar to that in

normal controls, only a few histopathological changes found, including inflammatory cells and congestion (Figure 3C, 3c).

Renal histopathology

Control group had normal architecture of renal tissue, composed of renal glomeruli and tubules (Figure 4A). The Bowman's capsule was well-defined and the Bowman's space did not show abnormal dilation. The distal and proximal renal tubules showed intact epithelial cell lining (Figure 4a). Conversely, the treatment of CAD led to a profound inflammation and hemorrhage in renal tissue, which occurred predominantly in the tubular and interstitial area (Figure 4B, 4b). Several glomeruli were atrophic, characterized by shrinkage of the glomeruli and

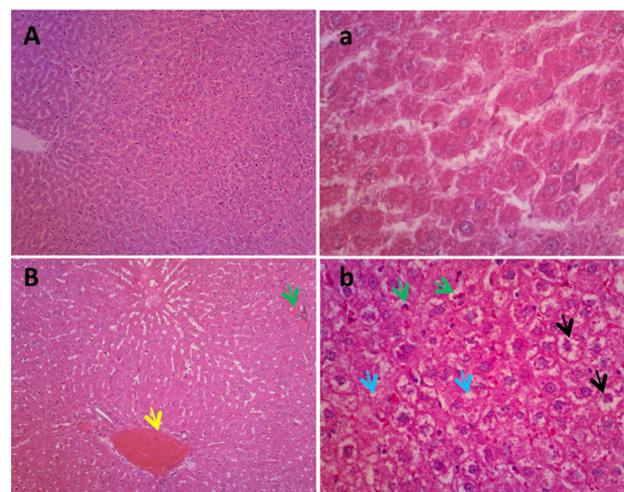


Figure 2. Light micrograph of liver tissue structures in controls (A,a) and CAD treated rats (B,b) following 28 days of treatment. H&E stain. Magnification 100x and 400x. Histopathological changes found in CAD group include congestion (yellow arrow), hepatic degeneration (black arrow), vacuolization (blue arrow) and infiltration of inflammatory cells (green arrow).

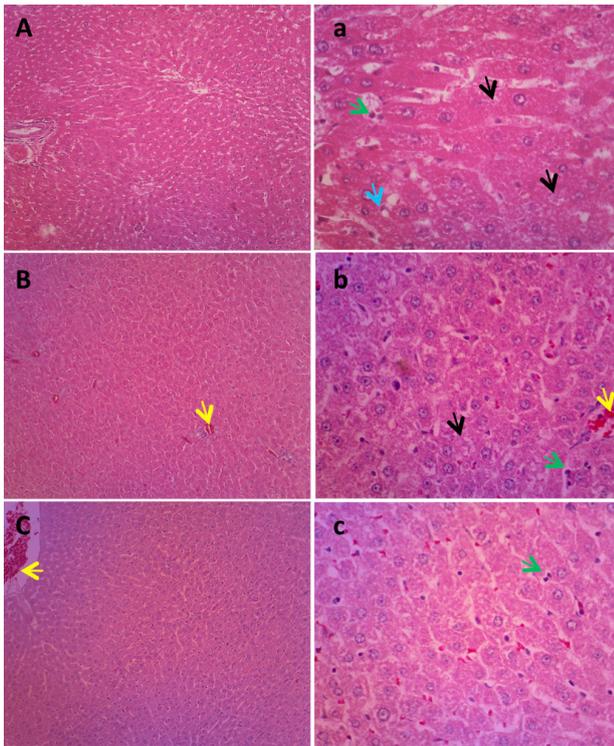


Figure 3. Light micrograph of liver tissue structures in rats treated with CAD and *K. hospita* Extract for 28 days. CAD + extract LD (A,a), CAD + extract MD (B,b), CAD + extract HD (C,c). H&E stain. Magnification 100x and 400x. In CAD + extract LD group, degeneration of hepatocyte, vacuolization and inflammatory cells were still apparent; meanwhile both CAD + extract MD and CAD + extract HD groups resembled normal hepatocyte arrangement with less inflammatory cells in the sinusoid, although congestion was still evident within blood vessels.

dilated Bowman's space (Figure 4B). *K. hospita* extract treatment in low dose (125 mg/kg) appeared to improve the capillary space in glomeruli (Figure 5A). Nevertheless, the low dose of extract did not significantly improve the renal architecture, shown by moderate inflammation and degeneration in tubular area (Figure 5a). When *K. hospita* extract dose was greater (250 mg/kg), the treated rats presented healthy renal distal and proximal tubules, with a clear lining of thick cuboidal epithelium of the lumen (Figure 5B, 5b). Comparable result was found with 500 mg/kg extract treatment, which showed a classic renal architecture in the tubular and glomerular area (Figure 5C, 5c).

Discussion

Drug-induced liver injury has been one of the main concerns for using CAD treatment in TB patients. About 10% of patients who receive antituberculosis therapy may experience hepatotoxicity (17), and the incidence is higher with older age, existing comorbidities, and alcohol consumption (18). In addition, certain genetic variants implicated in drug metabolizing enzymes may carry a greater risk for hepatotoxicity (19). Another potential problem that is possibly encountered by CAD-treated

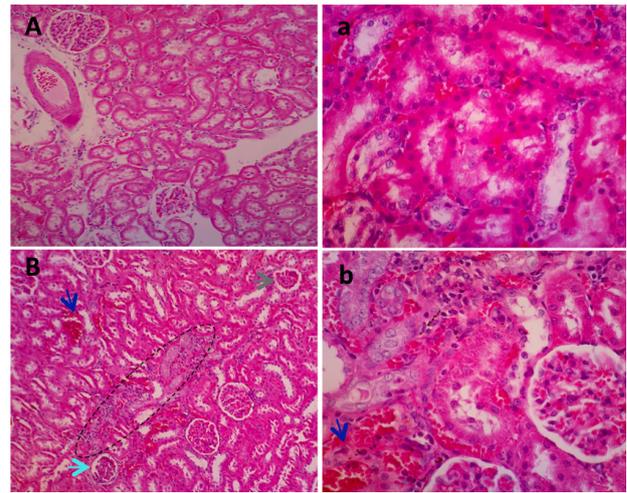


Figure 4. Light micrograph of renal tissue structure in controls (A, a) and CAD treated rats (B, b) following 28 days of treatments. H & E stain. Magnification 100x and 400x. Histopathological changes found in CAD group include hemorrhagic area (blue arrow), atrophic glomerulus (grey arrow), dilated Bowman's space (light blue arrow), and profound inflammatory infiltration (dashed line).

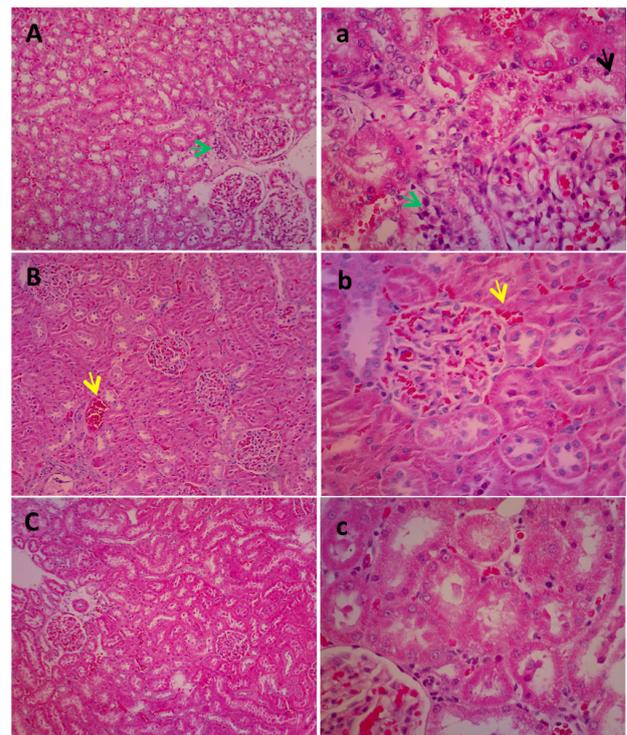


Figure 5. Light micrograph of renal tissue structure in rats treated with CAD and *K. hospita* extract for 28 days. CAD + extract LD (A,a), CAD + extract MD (B,b), CAD + extract HD (C,c). H & E stain. Magnification 100x and 400x. In CAD + extract LD group, the presence of tubule degeneration with vacuolization (black arrow) and infiltration of inflammatory cells (green arrow) was still observed. Meanwhile, improved renal tubule and glomerular structures were noticeable in CAD + extract MD and CAD + extract HD groups although blood congestion still existed (yellow arrow).

patients is acute kidney injury (20). This risk is potentially induced by rifampicin as it has been associated with idiosyncratic acute tubular or interstitial nephritis, or even glomerulonephritis in several cases (21).

Administration of CAD in rats using a toxic dose of 712 mg/kg in this study showed extensive liver and renal damage. The presence of CAD induced-liver damage was indicated by the elevation of liver enzyme levels in serum after 28 days of treatment, where AST increased ~60% and ALT ~100% from day 0 ($P < 0.05$). Moreover, liver damage was visualized by the profound histopathological changes in the rats' liver. The main characteristic of liver damage found in the CAD group was degeneration of hepatocytes showing a ballooning appearance of the cytoplasm. A similar finding was reported in Shabana et al study showing a disarray of swollen hepatocytes in rifampicin-treated (200 mg/kg) animals after 30 days of treatment (22). Renal damage was also noticeable following CAD treatment, where the elevation of serum creatinine was ~60% and urea was ~120% from day 0 ($P < 0.05$). In line with this, renal histopathological alteration was prominent in CAD group, especially the presence of inflammatory cells in the tubular area. Rifampicin-induced kidney damage mostly features tubular necrosis or tubulointerstitial nephritis (23); yet in this study, atrophic glomeruli were also found scattered in the renal tissue of CAD group.

Antituberculosis-induced toxicity is mostly derived from the radical metabolites of drugs that trigger oxidative stress predominantly in the liver, but may also affect the kidney at a certain degree (24). In an attempt to alleviate liver and renal toxicities, the rats were treated with *K. hospita* hydroalcoholic extract prior to CAD administration. The antioxidant compounds of *K. hospita* leaf extract have been recognized, including eleutherol and kaempferol 3-O- β -D-glucoside (25). In addition, more research currently pays attention to cycloartane triterpenoid compounds of *K. hospita* leaves as they have shown promising cytotoxic activities against human cancer cells *in vitro* (13,26-28).

Administration of *K. hospita* leaf extract before CAD treatment was able to inhibit the rise in ALT on day 28. This protection was provided by *K. hospita* extract at all given doses from 125 mg/kg to 500 mg/kg. However, *K. hospita* pre-treatment was not effective to halt the increase in AST in all groups. Different from ALT, AST may also be released from cell injuries in other organs, such as kidneys, heart or muscles, and apparently, *K. hospita* extract failed to recover this condition. A comparable result was found in our previous study exploring *K. hospita* extract effect on doxorubicin-treated animals (16). In that study, *K. hospita* treatment improved ALT levels with the dose of 100-500 mg/kg, but its effect in improving AST level was limited only to 250 mg/kg. The increase in serum creatinine and urea levels induced by CAD administration was also inhibited by *K. hospita* extract treatment at the

doses of 250 mg/kg and 500 mg/kg. In line with this, the treatment of *K. hospita* extract led to improved renal histology regardless of administration of CAD at a toxic dose. This is an important finding since there is lack of study exploring *K. hospita* extract roles in renal damage, and most of them only focus on its putative effects on hepatotoxicity or antioxidant activities (14,25,26).

Conclusion

Pre-treatment with *K. hospita* leaf extract, especially at the doses of 250 mg/kg and 500 mg/kg, alleviates the CAD-induced elevation of ALT, serum creatinine and serum urea levels in rats, indicating improved liver and kidney functions. In addition, pathological changes found in liver and renal tissues due to CAD administration were refined with *K. hospita* extract pre-treatment at higher doses. Further study is required to see whether the benefit of *K. hospita* extract in animal model can be translated in human subjects.

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Authors' contribution

YYD developed the research idea and design, data interpretation and writing the manuscript, AA organized the histological analysis, MM and RT supervised the extraction process, MNA directed the animal experimentation, FAK and NHN performed the experiment and biomarker analysis.

Conflict of interests

Authors declare to have no conflict of interest.

Ethical considerations

Animals were handled according to the International Guidelines for Care and Handling of Experimental Animals. All experimental procedures related to animals were approved by Institutional Animal Ethics Committee under Faculty of Medicine, Hasanuddin University with the ethical clearance number of UH170121085.

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