LAPORAN AKHIR PENELITIAN TAHUN TUNGGAL

ID Proposal: ba5d39ec-8fff-43ff-b00f-afd51fb881f8
Laporan Akhir Penelitian: tahun ke-2 dari 2 tahun

1. IDENTITAS PENELITIAN

A. JUDUL PENELITIAN

UJI PREKLINIK EKSTRAK DAUN PALIASA (Kleinhovia hospita Linn.) SEBAGAI PROTEKTOR KERUSAKAN HATI DAN GINJAL YANG DIINDUKSI OBAT ANTITUBERKULOSIS PADA TIKUS PUTIH

B. BIDANG, TEMA, TOPIK, DAN RUMPUN BIDANG ILMU

Bidang Fokus RIRN / Bidang Unggulan Perguruan Tinggi	Tema	Topik (jika ada)	Rumpun Bidang Ilmu
Penyakit Infeksi dan non infeksi dengan segala permasalahannya	-	Obat, Kosmetik, dan Food Suplements.	Farmakologi dan Farmasi Klinik

C. KATEGORI, SKEMA, SBK, TARGET TKT DAN LAMA PENELITIAN

Kategori (Kompetitif Nasional/ Desentralisasi/ Penugasan)	Skema Penelitian	Strata (Dasar/ Terapan/ Pengembangan)	SBK (Dasar, Terapan, Pengembangan)	Target Akhir TKT	Lama Penelitian (Tahun)
Penelitian Desentralisasi	Penelitian Dasar Unggulan Perguruan Tinggi	SBK Riset Dasar	SBK Riset Dasar	3	2

2. IDENTITAS PENGUSUL

Nama, Peran	Perguruan Tinggi/ Institusi	Program Studi/ Bagian	Bidang Tugas	ID Sinta	H-Index
YULIA YUSRINI DJABIR Ketua Pengusul	Universitas Hasanuddin	Farmasi		6018543	3
dr. M. ARYADI ARSYAD S.Ked, M.Bmd Anggota Pengusul 2	Universitas Hasanuddin	Ilmu Kebidanan		6005397	3
Dr. Dra MUFIDAH M.Si	Universitas Hasanuddin	Ilmu Farmasi		0	0

Anggota Pengusul 1					

3. MITRA KERJASAMA PENELITIAN (JIKA ADA)

Pelaksanaan penelitian dapat melibatkan mitra kerjasama, yaitu mitra kerjasama dalam melaksanakan penelitian, mitra sebagai calon pengguna hasil penelitian, atau mitra investor

Mitra	Nama Mitra

4. LUARAN DAN TARGET CAPAIAN

Luaran Wajib

Tahun Luaran	Jenis Luaran	Status target capaian (*accepted, published, terdaftar atau granted, atau status lainnya*)	Keterangan (*url dan nama jurnal, penerbit, url paten, keterangan sejenis lainnya*)
2	Publikasi Ilmiah Jurnal Internasional	accepted/published	Pharmacognosy Journal

Luaran Tambahan

Tahun Luaran	Jenis Luaran	Status target capaian (*accepted, published, terdaftar atau granted, atau status lainnya*)	Keterangan (*url dan nama jurnal, penerbit, url paten, keterangan sejenis lainnya*)
2	Keikutsertaan dalam Seminar Internasional	sudah dilaksanakan	International Seminar on Pharmaceutical Sciences
2	Publikasi Ilmiah Jurnal Nasional Tidak Terakreditasi	accepted/published	Majalah Farmasi dan Farmakologi

5. ANGGARAN

Rencana anggaran biaya penelitian mengacu pada PMK yang berlaku dengan besaran minimum dan maksimum sebagaimana diatur pada buku Panduan Penelitian dan Pengabdian kepada Masyarakat Edisi 12.

Total RAB 2 Tahun Rp. 189,135,000

Tahun 1 Total Rp. 0

Tahun 2 Total Rp. 189,135,000

Jenis Pembelanjaan	Item	Satuan	Vol.	Biaya Satuan	Total
Analisis Data	Biaya analisis sampel	Unit	1	600,000	600,000
Bahan	ATK	Paket	1	3,200,000	3,200,000
Bahan	Bahan Penelitian (Habis Pakai)	Unit	1	112,995,000	112,995,000
Bahan	Barang Persediaan	Unit	1	3,000,000	3,000,000
Pelaporan, Luaran Wajib, dan Luaran Tambahan	Biaya seminar internasional	Paket	1	15,000,000	15,000,000
Pelaporan, Luaran Wajib, dan Luaran Tambahan	Biaya Publikasi artikel di Jurnal Nasional	Paket	1	3,000,000	3,000,000
Pelaporan, Luaran Wajib, dan Luaran Tambahan	Publikasi artikel di Jurnal Internasional	Paket	1	12,000,000	12,000,000

Jenis Pembelanjaan	Item	Satuan	Vol.	Biaya Satuan	Total
Pengumpulan Data	HR Pembantu Peneliti	OJ	1	2,400,000	2,400,000
Pengumpulan Data	HR Sekretariat/Administrasi Peneliti	OB	1	1,440,000	1,440,000
Pengumpulan Data	HR Pembantu Lapangan	OH	1	2,400,000	2,400,000
Sewa Peralatan	Peralatan penelitian	Unit	1	28,600,000	28,600,000
Sewa Peralatan	Ruang penunjang penelitian	Unit	3	500,000	1,500,000
Sewa Peralatan	Transport penelitian	OK (kali)	3	1,000,000	3,000,000

6. HASIL PENELITIAN

A. RINGKASAN: Tuliskan secara ringkas latar belakang penelitian, tujuan dan tahapan metode penelitian, luaran yang ditargetkan, serta uraian TKT penelitian.

Indonesia merupakan negara kedua dengan insiden tuberkulosis (TB) tertinggi di dunia setelah India, dengan jumlah penderita lebih dari 324.000 orang. Untuk mengatasi dan mengendalikan penyebaran infeksi TB, WHO merekomendasikan penggunaan obat antituberkulosis dalam sediaan fixed dose combination (OAT FDC) selama 6 bulan. Namun, penggunaan OAT FDC secara kronik oleh penderita TB memiliki resiko berupa efek toksik pada organ hati pasien, bahkan pada populasi tertentu OAT FDC beresiko mengganggu fungsi ginjal. Saat ini upaya yang dapat dilakukan untuk melindungi fungsi hati pasien dari toksisitas obat adalah dengan pemanfaatan ekstrak bahan alam yang memiliki efek hepatoprotektif. Sedangkan, untuk gangguan ginjal, belum terdapat terapi standar yang bersifat nefroprotektif. Tanaman Paliasa merupakan salah satu tanaman asli Sulawesi Selatan yang banyak digunakan oleh masyarakat sebagai obat penyakit liver dan hepatitis. Tanaman ini mengandung senyawa-senyawa antioksidan yang dapat melindungi sel hati, dan kemungkinan besar juga bermanfaat melindungi sel ginjal. Oleh karena itu, tujuan jangka panjang dari penelitian ini adalah untuk menghasilkan produk herbal yang mengandung ekstrak daun Paliasa yang merupakan tanaman asli Sulawesi Selatan, yang dapat digunakan untuk melindungi fungsi hati dan ginjal pasien TB selama mengkonsumsi obat antituberkulosis.

Penelitian ini berfokus pada tahap preklinik sebelum lanjut ke tahap klinik. Penelitian preklinik telah dilakukan selama 2 tahun. Pada tahun I telah dilakukan penentuan dosis OAT yang menimbulkan hepatotoksisitas dan nefrotoksisitas secara subkronik pada tikus putih. Pada tahun I juga ditemukan adanya potensi efek hepatoprotektif dan nefroprotektif ekstrak etanol daun paliasa terhadap kerusakan hati dan ginjal akibat penggunaan 28 hari obat antituberkulosis pada tikus putih. Hasil tersebut diperoleh dari pemeriksaan biomarker dan didukung pula dengan pemeriksaan histopatologi (TKT 2). Tahun II (skrining efek samping preklinik) berfokus pada skrining efek samping penggunaan ekstrak paliasa dosis tinggi (250-500 mg/kg) selama 30 hari pada tikus putih. Parameter yang diskrining termasuk: pemeriksaan darah rutin (hematologi), keseimbangan elektrolit, profil lipid, biomarker kejadian kolestasis, asam urat, kadar oksidan dan antioksidan hati dan ginjal (TKT 3). Penelitian ini akan melengkapi data untuk bisa masuk dalam tahap terapan (TKT 4). Apabila terbukti bahwa penggunaan Paliasa bersama dengan penggunaan OAT dalam jangka panjang tidak menimbulkan efek samping yang merugikan, selanjutnya akan dibuat penelitian terapan yang akan memfokuskan pada pengujian secara klinik penggunaan ekstrak daun Paliasa pada pasien TB yang sedang mengkonsumsi OAT. Dengan demikian, nantinya diharapkan penelitian ini mampu menghasilkan produk unggulan berupa sediaan ekstrak paliasa dengan dosis yang sesuai untuk melindungi hati dan ginjal pasien TB selama

menjalani pengobatan tuberkulosis dengan efek samping minimal.

Skrining efek samping penggunaan ekstrak paliasa dosis terapi bersama dengan OA T-FDC dilakukan dengan membagi tikus coba dalam 6 kelompok perlakuan

- Kelompok I: Tikus diberi suspensi NaCMC 1% (pembawa ekstrak) sebanyak 1 ml/hari setiap hari selama 30 hari
- Kelompok II: Tikus diberi NaCMC 1% peroral 1 ml/hari dan suspensi OAT-FDC 89 mg/kg setiap hari selama 30 hari. Pemberian NaCMC dan suspensi obat diberi selang waktu 4 jam.
- Kelompok III: diberikan ekstrak daun Paliasa dosis 250 mg/kgBB secara oral diikuti dengan pemberian suspensi OAT-FDC 89 mg/kg setiap hari selama 30 hari. Pemberian ekstrak dan suspensi obat diberi selang waktu 4 jam.
- Kelompok IV: diberikan ekstrak daun Paliasa dosis 500 mg/kgBB secara oral diikuti dengan pemberian suspensi OAT-FDC 89 mg/kg setiap hari selama 30 hari. Pemberian ekstrak dan suspensi obat diberi selang waktu 4 jam.
- Kelompok V: diberikan ekstrak daun Paliasa dosis 250 mg/kg secara oral setiap hari selama 30 hari diikuti dengan NaCMC 1% peroral 1 ml/hari.
- Kelompok VI: diberikan ekstrak daun Paliasa dosis 500 mg/kgBB secara oral setiap hari selama 30 hari.

Setelah 30 hari perlakuan, darah tikus diambil sebanyak 4 ml lalu disentrifugasi dan serumnya disimpan di lemari pendingin (-20oC) hingga waktu analisa. Skrining efek samping memonitor parameter hematologi (WBC, RBC, HCt, Hb, trombosit, neutrofil, eusinofil, basofil, monosit), elektrolit, parameter kolestasis (Bilirubin total, Alkali fosfatase), kerusakan otot (CK dan LDH) dan metabolisme lipid (kolesterol total, LDL, HDL). Setelah pengujian biomarker darah dilakukan pengukuran aktivitas oksidan dan antioksidan organ hati dan ginjal tikus. Aktivitas superoksid dismutase, glutation peroksidase dan konsentrasi MDA (parameter peroksidasi lipid) diukur menggunakan serangkaian assay kit dan serapannya diukur menggunakan microplate reader.

Hasil penelitian tahap I menunjukkan efek proteksi ekstrak Daun Paliasa dalam melindungi hati dan ginjal terhadap peningkatan kadar SGOT dan SGPT serta Kreatinin pada tikus yang diinduksi OAT-FDC dosis toksik. Pemeriksaan histopatologi juga memperlihatkan kerusakan hati dan ginjal yang lebih sedikit pada tikus yang diberi ekstrak daun Paliasa. Dosis yang efektif mencegah kerusakan hati dan ginjal akibat dosis toksi OAT-FDCadalah 250 mg/kg dan 500 mg/kg. Hasil penelitian tahap II menunjukkan terdapat perubahan hematologic pada penggunaan OAT dan kombinasi dengan ekstrak Paliasa dapat menyebabkan perubahan hematologi semakin terlihat. Selain itu, terjadi penurunan kadar kolesterol dan gula darah dengan penggunaan ekstrak paliasa dosis 500 mg/kg. Aktivitas antioksidan GPx dan SOD dalam hati dan ginjal meningkat dengan adanya pemberian ekstrak Paliasa yang menunjukkan mekanisme proteksi Paliasa melaui peningkatan aktivitas enzim antioksidan endogen.

Luaran yang ditargetkan adalah publikasi ilmiah di Jurnal Internasional dan diseminasi hasil penelitian melalui presentasi di seminar internasional. Luaran yang telah dilaksanakan antara lain: 1) Data penelitian terkait efek hepatoprotektif dan nefroprotektif esktrak daun Paliasa telah “ACCEPTED” untuk diterbitkan dalam “Journal of Herbmed Pharmacology” terindeks SCOPUS (Q3); 2) Data penelitian terkait efek hepatotoksik dan nefrotoksik obat OAT fixed dose combination (OAT-FDC) pada tikus telah melalui tahap “SECOND REVISION” untuk dipublikasi dalam “FABAD Journal of Pharmaceutical Sciences” terindeks Scopus (Q4); 3) Penelitian data efek samping hematologi ekstrak daun paliasa telah dipresentasikan dalam Asian Federation for Pharmaceutical Sciences (AFPS) Conference 2019 pada tanggal 23-27 Oktober 2019; 4) Data penelitian terkait efek Paliasa dan OAT terhadap kadar bilirubin tikus telah “PUBLISHED” pada Nusantara Medical Science Journal (jurnal nasional tidak terakreditasi).

B. KATA KUNCI: Tuliskan maksimal 5 kata kunci.

Obat antituberkulosis; ekstrak daun paliasa; hepatoprotektif; nefroprotektif; efek samping

Pengisian poin C sampai dengan poin H mengikuti template berikut dan tidak dibatasi jumlah kata atau halaman namun disarankan seringkas mungkin. Dilarang menghapus/memodifikasi template ataupun menghapus penjelasan di setiap poin.

C. HASIL PELAKSANAAN PENELITIAN: Tuliskan secara ringkas hasil pelaksanaan penelitian yang telah dicapai sesuai tahun pelaksanaan penelitian. Penyajian dapat berupa data, hasil analisis, dan capaian luaran (wajib dan atau tambahan). Seluruh hasil atau capaian yang dilaporkan harus berkaitan dengan tahapan pelaksanaan penelitian sebagaimana direncanakan pada proposal. Penyajian data dapat berupa gambar, tabel, grafik, dan sejenisnya, serta analisis didukung dengan sumber pustaka primer yang relevan dan terkini.

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Efek pemberian ekstrak paliasa dan OAT terhadap sel darah merah

Hasil pengukuran parameter hematologi sel darah merah berupa RBC, HG, HCT, MCV, MCH, MCHC dan PLT (Tabel 1). Dari parameter tersebut, diperoleh danya perbedaan yang signifikan pada parameter mean corpuscular volume (MCV) yang merupakan indikator ukuran (volume) sel darah merah. Setelah dilakukan uji lanjutan menggunakan post hoc Tukey's HSD menunjukkan bahwa kombinasi pemberian OAT dan paliasa baik dengan dosis sedang (250 mg/kg) maupun dosis tinggi (500 mg/kg) dapat menstimulasi perubahan ukuran sel darah merah ($P<0.05$). Sedangkan, bila ekstrak Paliasa diberikan secara tunggal (tanpa OAT) tidak menyebabkan terjadinya perubahan MCV yang signifikan dibandingkan placebo maupun kontrol.

Efek pemberian ekstrak paliasa dan OAT terhadap sel darah putih

Hasil pengukuran parameter hematologi sel darah putih berupa WBC, neutrophil, limfosit, monosit, eusinofil dan basophil (Tabel 2). Dari parameter tersebut, diperoleh adanya perbedaan yang signifikan pada parameter White Blood Cells (WBC) yang merupakan indikator jumlah sel darah putih dalam satuan volume mikroliter. Menariknya, yang berbeda signifikan adalah antara kontrol sehat (yang tidak disonde) dengan grup placebo yang diberi suspensi pembawa tanpa ekstrak melalui sonde (oral gavage) ($p<0.05$). Hal ini mungkin menunjukkan adanya proses inflamasi maupun infeksi selama proses perlakuan pada grup placebo, namun tidak terjadi pada tikus yang diberi Paliasa maupun OAT. OAT merupakan obat antibakteri spektrum luas sehingga bisa mencegah terjadinya infeksi pada tikus. Efek antibakteri Paliasa sudah pernah dibuktikan oleh penelitian yang lain (REF). Selain jumlah WBC, komponen-komponen sel darah putih tidak mengalami perbedaan yang signifikan antara control sehat, plasebo dan kelompok perlakuan ekstrak maupun OAT.

Efek pemberian ekstrak paliasa dan OAT terhadap keseimbangan elektrolit

Keseimbangan elektrolit sangat penting untuk menjaga homeostasis. Pada penelitian ini kadar elektrolit plasma tikus diukur setelah pemberian Paliasa dan OAT untuk melihat apakah ekstrak Paliasa maupun OAT dapat menyebabkan ketidakseimbangan elektrolit pada tikus. Diperoleh data seperti tercantum pada tabel 3. Terlihat bahwa tikus yang diberikan daun Paliasa dengan dosis 250 dan 500 mg/kg tidak memberikan perbedaan yang signifikan dibandingkan tikus control maupun placebo. Begitu pula dengan pemberian OAT yang dikombinasi dengan ekstrak Paliasa, tidak ditemukan perbedaan yang signifikan dengan plasebo.

Tabel 1. Parameter hematologi sel darah merah tikus kontrol, placebo dan tikus yang diberi perlakuan berupa OAT dan ekstrak Paliasa selama 30 hari

Perlakuan	Kontrol		Plasebo		OAT		Ekstrak Paliasa 250		Ekstrak Paliasa 250 + OAT		Ekstrak Paliasa 500		Ekstrak Paliasa 500 + OAT		P value
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
RBC (10^6/µL)	7.88	0.55	8.05	0.31	8.38	0.14	8.80	0.24	8.28	0.43	8.89	0.29	8.83	0.13	0.16
HGB (g/dl)	13.74	0.97	14.30	0.40	14.36	0.21	14.69	0.63	13.69	0.68	15.22	0.48	14.86	0.29	0.47
HCT (%)	41.18	2.81	43.77	0.96	41.69	0.61	43.63	2.44	38.69	1.90	44.52	1.33	41.34	0.71	0.70
MCV (fL)	52.43	1.60	54.64	1.38	49.79	0.92	49.41	1.92	46.96	1.58	50.13	0.63	46.83	0.87	0.008**
MCH (pg)	17.44	0.17	17.84	0.41	17.13	0.17	16.83	0.45	16.59	0.45	17.12	0.18	16.83	0.29	0.197
MCHC (g/dl)	33.39	0.76	32.64	0.23	34.44	0.33	34.16	0.55	35.36	0.38	34.18	0.09	35.93	0.24	0.125
PLT(10^3/µL)	796.43	98.12	756.86	82.92	826.43	36.49	789.29	26.55	712.57	35.52	795.50	40.44	819.57	29.29	0.622

** menunjukkan perbedaan sangat signifikan antara tikus yang diberikan placebo dengan tikus yang diberi ekstrak paliasa 250 + OAT serta Paliasa 500 + OAT

Tabel 2. Profil sel darah putih tikus kontrol, placebo dan tikus yang diberi perlakuan berupa OAT dan ekstrak Paliasa selama 30 hari

Perlakuan	Kontrol		Plasebo		OAT		Ekstrak Paliasa 250		Ekstrak Paliasa 250 + OAT		Ekstrak Paliasa 500		Ekstrak Paliasa 500 + OAT		P value
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
WBC (10^3/uL)	13.31	1.26	20.67	3.42	16.50	2.01	12.55	2.91	16.54	3.83	12.74	2.34	18.44	2.68	0.01*
NEUT (%)	19.80	1.25	16.85	2.65	18.13	1.01	17.20	3.60	13.22	0.76	15.23	1.83	11.88	1.31	0.24
LYMPH (%)	70.18	1.82	72.05	0.25	69.50	1.50	71.00	5.12	77.60	0.93	75.23	2.53	77.70	2.46	0.03
MONO (%)	6.93	0.93	8.75	0.25	4.98	0.55	10.76	0.90	6.48	0.48	6.86	0.89	7.70	1.43	0.769
EO (%)	3.35	0.84	3.97	1.13	2.76	0.14	2.87	0.34	2.49	0.40	3.22	0.43	2.11	0.38	0.511
BASO (%)	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.438

*menunjukkan perbedaan signifikan antara kelompok tikus kontrol dan tikus yang diberikan placebo

Tabel 3. Kadar elektrolit plasma tikus kontrol, placebo dan tikus yang diberi perlakuan berupa OAT dan ekstrak Paliasa selama 30 hari

Perlakuan	Kontrol		Plasebo		OAT		Ekstrak Paliasa 250		Ekstrak Paliasa 250 + OAT		Ekstrak Paliasa 500		Ekstrak Paliasa 500 + OAT		P value
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
Na+ (mmol/L)	140.00	0.45	140.67	0.56	140.71	0.68	139.80	1.39	141.14	0.67	141.33	0.49	138.29	0.57	0.38
K+ (mmol/L)	4.10	0.19	0.19	0.06	4.03	0.06	4.16	0.14	4.39	0.22	4.07	0.12	4.26	0.09	0.786
Cl- (mmol/L)	103.00	0.45	102.67	0.76	101.57	0.30	102.60	0.75	0.22	0.36	103.33	0.21	100.57	0.30	0.632

Efek pemberian ekstrak paliasa dan OAT terhadap parameter kolestasis

Tabel 4. Kadar bilirubin total dalam serum tikus yang diberi placebo, OAT dan ekstrak Paliasa sebelum dan sesudah perlakuan 30 hari

	Bilirubin (mg/dl)	
Perlakuan	**Hari 0**	**Hari 30**
Plasebo	0.298	0.348
OAT	0.386	0.648
Ekstrak Paliasa 250	0.195	0.459
Ekstrak Paliasa 250 + OAT	0.403	0.356
Ekstrak Paliasa 500	0.502	0.575
Ekstrak Paliasa 500 + OAT	0.439	0.182

Tabel 5. Kadar enzim alkali fosfatase (ALP) dalam serum tikus yang diberi placebo, OAT dan ekstrak Paliasa sebelum dan sesudah perlakuan 30 hari

	ALP (mg/dl)	
Perlakuan	**Hari 0**	**Hari 30**
Plasebo	218.98	60.89
OAT	112.13	244.81
Ekstrak Paliasa 250	111.69	70.15
Ekstrak Paliasa 250 + OAT	57.82	322.83
Ekstrak Paliasa 500	106.23	179.01
Ekstrak Paliasa 500 + OAT	122.04	106.97

Tabel 4 menunjukkan kadar bilirubin sebelum dan sesudah pemberian perlakuan selama 30 hari. Pemberian OAT ditemukan meningkatkan kadar rata-rata bilirubin total dari 0.386 menjadi 0.648, yang berarti peningkatannya hamper 2 kali lipat. Peningkatan tersebut terlihat pada 2 tikus yang memiliki kadar bilirubin yang meningkat setelah perlakuan >1,2 mg/dl, dimana nilai awal kadar bilirubin total pada serum adalah antara 0,03-1,2 mg/dl. Pemberian ekstrak Paliasa bersama OAT terlihat tidak meningkatkan bahkan menurunkan kadar rata-rata bilirubin tikus. Namun, terdapat 1 tikus pada kelompok Paliasa 250 yang mengalami peningkatan bilirubin hingga mencapai kadar lebih dari 2,1 mg/dl.

Table 5 menunjukkan kadar ALP yang merupakan indicator terjadinya penyumbatan pada saluran empedu. Diperoleh kadar awal ALP untuk tikus (sebelum perlakuan) berkisar antara 10,02-273,3 mg/dl. Dari hasil penelitian diperoleh bahwa terjadi peningkatan ALP setelah pemberian OAT dari 112,13 menjadi 244,81. Begitu pula dengan kelompok yang diberi OAT bersama dengan Paliasa 250 mg/kg. Tetapi, pemberian ekstrak paliasa 500 dan OAT tidak menyebabkan tikus mengalami kenaikan ALP. Hal ini mengindikasikan bahwa ekstrak Paliasa dosis 500 mg/kg kemungkinan memiliki efek protektif terhadap terjadinya penyumbatan asam empedu akibat penggunaan OAT.

Efek pemberian ekstrak paliasa dan OAT terhadap profil lipid

Tabel 6. Kadar Low Density Lipoprotein (LDL) dalam serum tikus yang diberi placebo, OAT dan ekstrak Paliasa sebelum dan sesudah perlakuan 30 hari

	LDL (mg/dl)	
Perlakuan	**Hari 0**	**Hari 30**
Plasebo	84.66	102.93
OAT	108.25	130.82
Ekstrak Paliasa 250	124.67	102.50
Ekstrak Paliasa 250 + OAT	73.79	133.77
Ekstrak Paliasa 500	92.85	147.21
Ekstrak Paliasa 500 + OAT	84.56	129.31

Tabel 6 menunjukkan kadar LDL tikus yang diberi OAT dan ekstrak Paliasa selama 30 hari dan perbandingannya dengan tikus yang hanya diberi pembawa (placebo). Terlihat bahwa hampir semua kelompok tikus mengalami sedikit peningkatan LDL setelah 30 hari perlakuan, termasuk tikus placebo. Tidak terdapat perbedaan yang signifikan antarkelompok, sehingga diasumsikan bahwa baik OAT maupun ekstrak paliasa tidak memberikan pengaruh yang signifikan pada kadar LDL tikus. Kemungkinan besar peningkatan LDL disebabkan oleh makanan yang dikonsumsi tikus selama perlakuan.

Tabel 7. Kadar kolesterol total dalam serum tikus yang diberi placebo, OAT dan ekstrak Paliasa sebelum dan sesudah perlakuan 30 hari

	Kolesterol (mg/dl)	
Perlakuan	**Hari 0**	**Hari 30**
Plasebo	51.15	53.27
OAT	72.45	52.16
Ekstrak Paliasa 250	81.18	50.96
Ekstrak Paliasa 250 + OAT	66.27	39.94*
Ekstrak Paliasa 500	53.77	38.00*
Ekstrak Paliasa 500 + OAT	60.14	65.04

Tabel 7 menunjukkan kadar kolesterol total tikus yang diberi OAT dan ekstrak Paliasa selama 30 hari dan perbandingannya dengan tikus yang hanya diberi pembawa (placebo). Terlihat bahwa setelah 30 hari perlakuan, tidak terjadi peningkatan kolesterol pada tikus placebo. Tikus yang diberi OAT dan Paliasa mengalami penurunan kolesterol total, tetapi penurunan kolesterol yang signifikan lebih terlihat pada kelompok ekstrak paliasa konsentrasi 500 dan ekstrak paliasa 250 ditambah OAT. Sehingga diasumsikan ekstrak paliasa dapat menurunkan kadar kolesterol total tikus, dan efek ini semakin terlihat ketika ekstrak paliasa ditambah dengan OAT.

Tabel 8. Kadar Trigliserida dalam serum tikus yang diberi placebo, OAT dan ekstrak Paliasa sebelum dan sesudah perlakuan 30 hari

	Trigliserida (mg/dl)	
Perlakuan	**Hari 0**	**Hari 30**
Plasebo	100.17	123.91
OAT	95.77	113.32
Ekstrak Paliasa 250	95.14	108.50
Ekstrak Paliasa 250 + OAT	94.08	98.20
Ekstrak Paliasa 500	100.17	91.95
Ekstrak Paliasa 500 + OAT	92.63	80.69

Terilhat dari table 9, baik OAT maupun ekstrak paliasa tidak memberikan pengaruh yang signifikan terhadap kadar trigliserida tikus.

Efek pemberian ekstrak paliasa dan OAT terhadap glukosa darah dan insulin

Tabel 9. Kadar glukosa darah sewaktu tikus yang diberi placebo, OAT dan ekstrak Paliasa sebelum dan sesudah perlakuan 30 hari

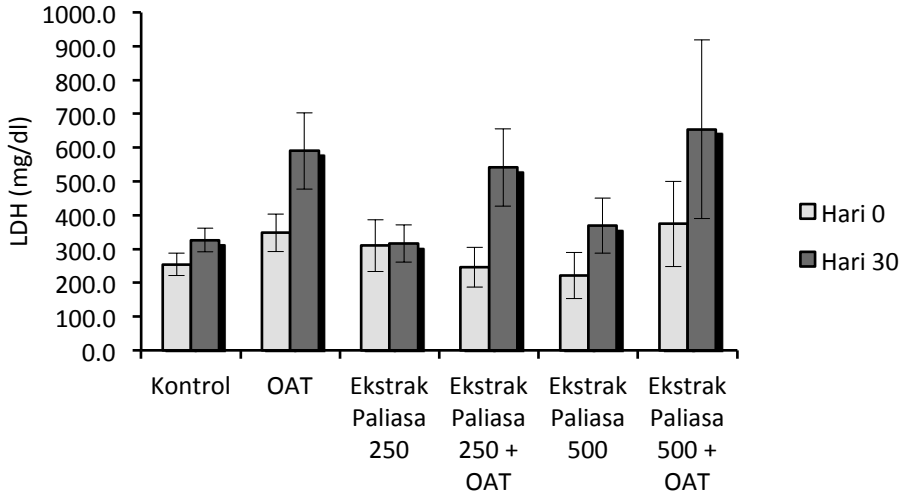
	Glukosa darah (mg/dl)	
Perlakuan	**Hari 0**	**Hari 30**
Plasebo	85.83	147.94
OAT	82.71	144.39
Ekstrak Paliasa 250	85.09	96.95
Ekstrak Paliasa 250 + OAT	82.13	107.81
Ekstrak Paliasa 500	85.83	58.82
Ekstrak Paliasa 500 + OAT	73.41	115.11

Tabel 9 menunjukkan kadar glukosa tikus yang diberi OAT dan ekstrak Paliasa selama 30 hari dan perbandingannya dengan tikus yang hanya diberi pembawa (placebo). Terlihat bahwa hampir semua kelompok tikus mengalami peningkatan kadar glukosa darah setelah 30 hari perlakuan, namun masih masuk dalam normal range. Kecuali tikus yang diberi ekstrak Paliasa 500 mg/kg, terlihat penurunan GDS hingga 60 mg/dl. Hal ini menimbulkan perhatian bagi masyarakat yang akan mengkonsumsi ekstrak paliasa dengan jangka panjang dengan dosis tinggi. Di satu sisi, hasil tersebut menunjukkan adanya efek hipoglikemik ekstrak 500 mg/kg yang dapat dimanfaatkan untuk pasien hiperglikemia.

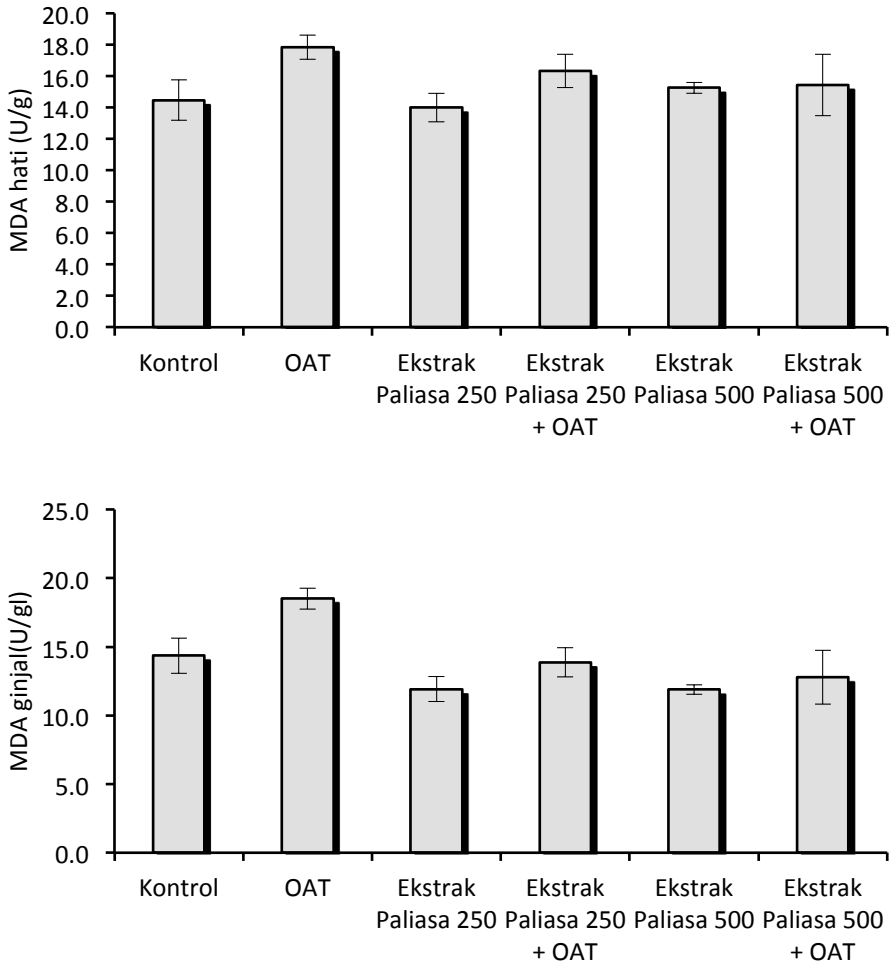
Tabel 10. Kadar insulin dalam plasma tikus yang diberi placebo, OAT dan ekstrak Paliasa sebelum dan sesudah perlakuan 30 hari

	Insulin (pg/dl)	
Perlakuan	**Hari 0**	**Hari 30**
Plasebo	1.23	1.42
OAT	2.71	2.39
Ekstrak Paliasa 250	2.09	2.75
Ekstrak Paliasa 250 + OAT	2.13	2.11
Ekstrak Paliasa 500	1.38	1.22
Ekstrak Paliasa 500 + OAT	2.21	2.18

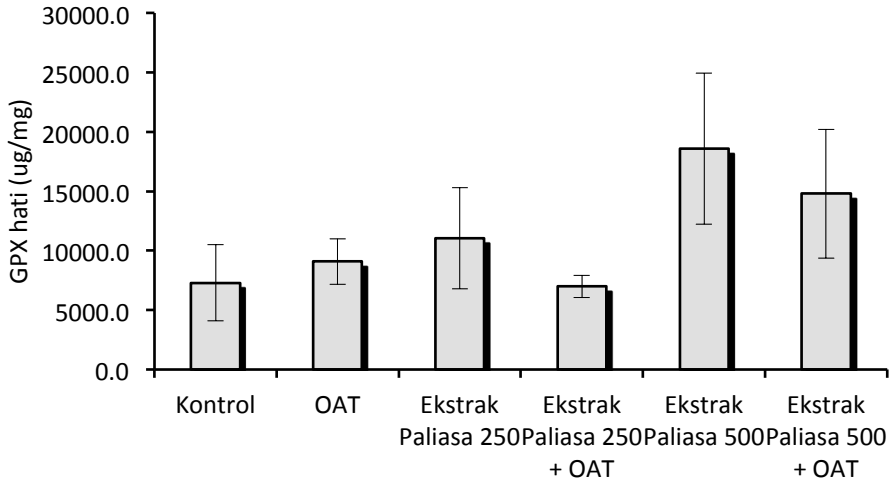
Efek pemberian ekstrak paliasa dan OAT terhadap kadar LDH dan peroksidasi lipid hati dan ginjal

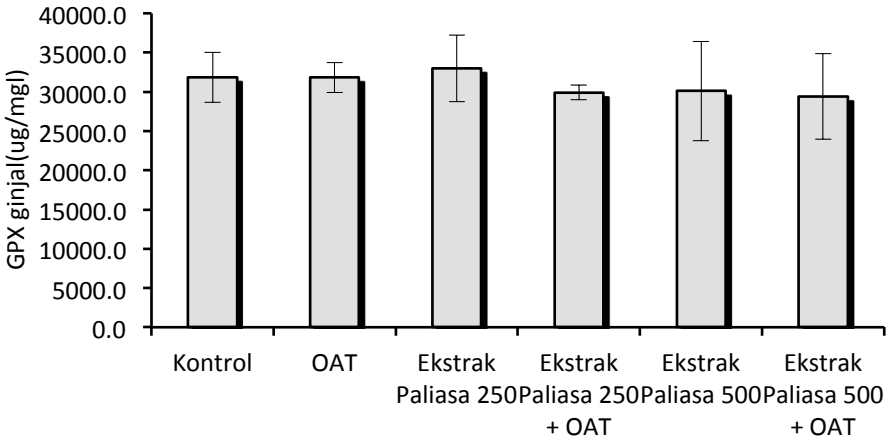


Gambar 4. Kadar laktat dehydrogenase (LDH) plasma tikus setelah 30 hari perlakuan.

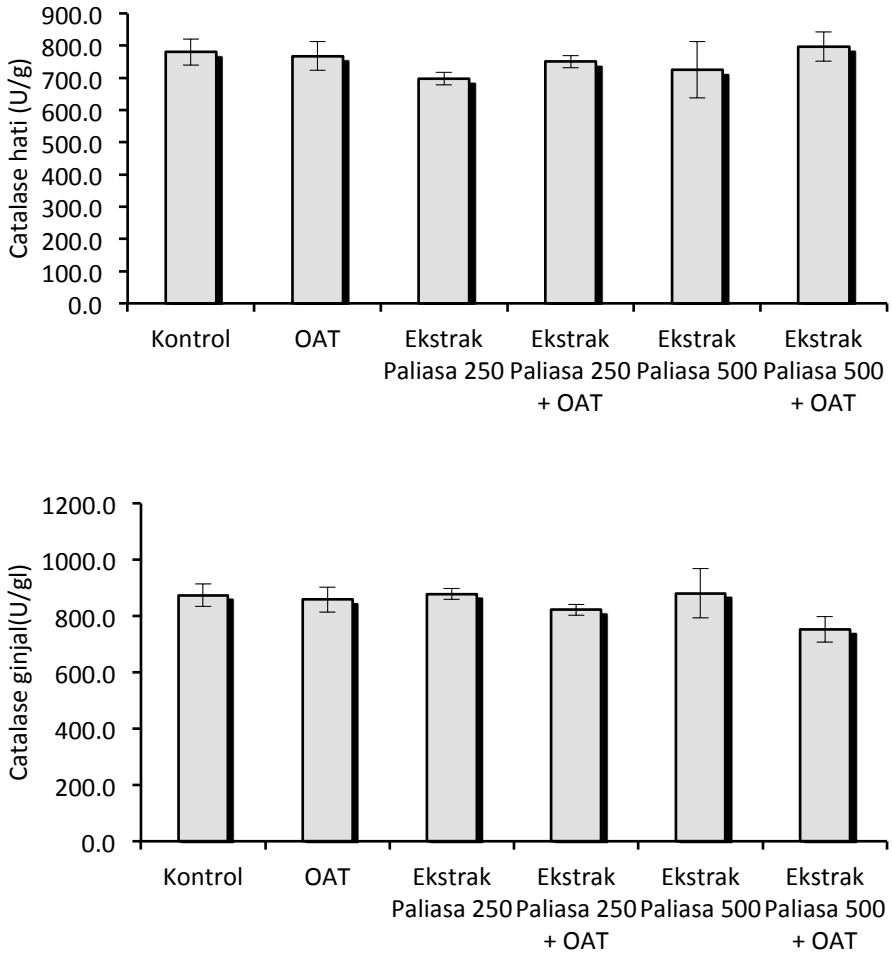


Gambar 5. Kadar Malondialdehid (MDA) orhan hati (A) dan ginjal (B) tikus setelah 30 hari perlakuan.

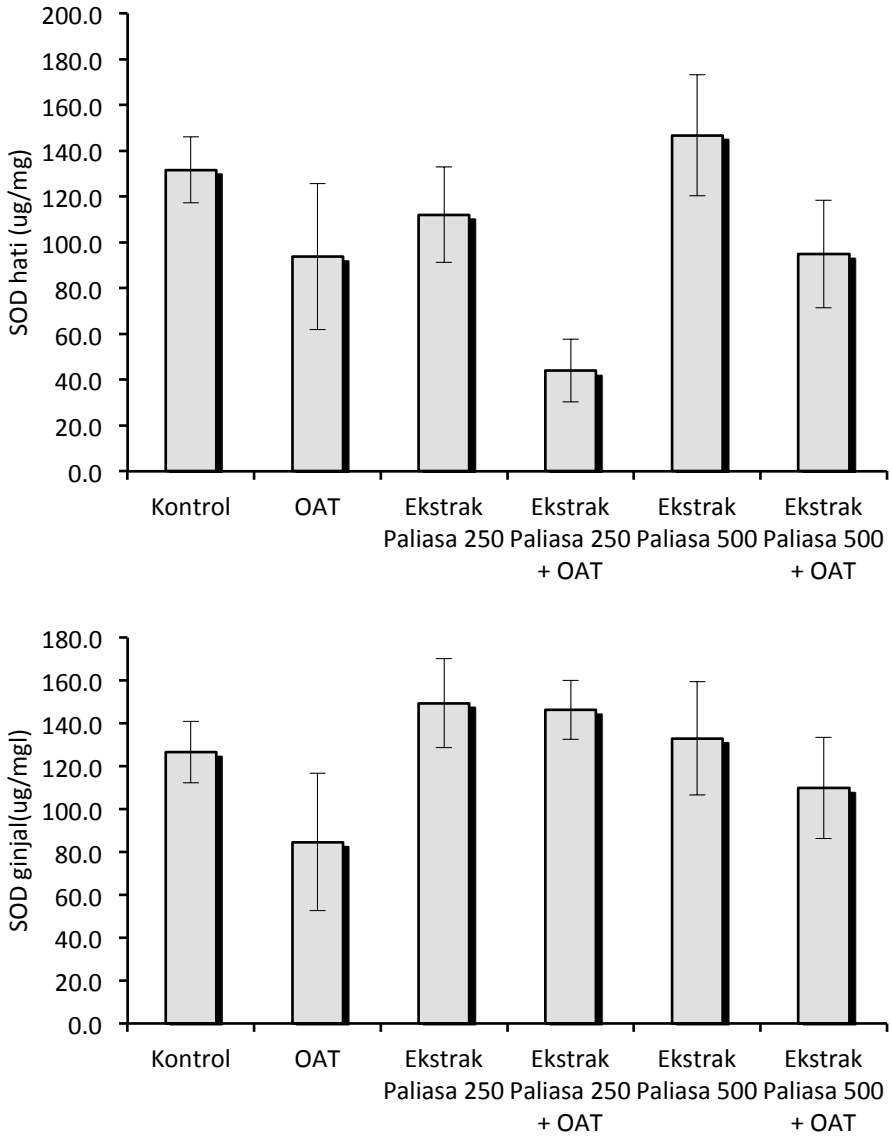




Gambar 6. Aktivitas glutation peroksidase (GPx) organ hati (A) dan ginjal (B) tikus setelah 30 hari perlakuan.



Gambar 7. Kadar Catalase organ hati (A) dan ginjal (B) tikus setelah 30 hari perlakuan.



Gambar 8. Kadar Superoxide dismutase organ hati (A) dan ginjal (B) tikus setelah 30 hari perlakuan.

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2. Data penelitian terkait efek hepatotoksik dan nefrotoksik obat OAT fixed dose combination pada tikus melalui proses "Second revision" pada "FABAD Journal of Pharmaceutical Sciences" terindeks Scopus (Q4)
3. Penelitian data hematology "telah dipresentasikan" dalam Asian Federation for Pharmaceutical Sciences (AFPS) Conference 2019 in conjunction with The 4th International Conference on Advance Pharmacy and Pharmaceutical Sciences (ICAPPS) pada tanggal 23-27 Oktober 2019
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Raflizar, R., 2009. *Sub Chronic Toxicity Test From Alkohol Extract Paliasa Leaves (Kleinhovia Hospita Linn) To Hepar/Liver and Kidney of Experimental Mice.* Media Penelitian dan Pengembangan Kesehatan, **19**(4 Des).

Dokumen pendukung luaran Wajib #1

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DEVELOPMENT OF LIVER AND RENAL INJURY IN RATS INDUCED BY FIXED DOSE COMBINATION OF ANTITUBERCULOSIS REGIMEN

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Running title: Development of organ toxicities due to antituberculosis regimen in rats

SUMMARY

Introduction: Fixed dose combination of four antituberculosis drugs (4FDC-AT) use is recommended to eradicate tuberculosis infection. However, it may induce liver and renal dysfunctions. This study aimed to evaluate time-dependent development of liver and renal injury in rats induced by subchronic use of 4FDC-AT regimen. Materials and Methods: Male Wistar rats (150-250 g) were divided into two groups of six. Group 1 was given placebo while group 2 was treated with 4FDC-AT (89 mg/200 g) for 28 days. Blood samples were withdrawn to measure serum ALT and creatinine levels on day 0 (baseline), 7, 14 and 28 of treatment. Following the last treatment, rat liver and kidney were harvested for histological analysis. Elevation of ALT and creatinine levels were considered noteworthy if the levels are >50% from upper baseline. Results: None of the placebo rats experienced a significant increase in ALT and creatinine levels from day 7 to 28. In contrast, ALT escalated in 50% of rats in 4FDC-AT group starting at day 14; however, the 4FDC-AT treatment only increased creatinine levels in 17% of rats after 28 days. Histopathological analysis of liver and kidney in the placebo group showed normal histology structures, while in 4FDC-AT treated rats showed profound hydropic and lipid degeneration of hepatocytes. In renal tubules, infiltration of inflammatory cells and vacuolization were only evident in one rat. Conclusion: 4FDC-AT administration rapidly induces liver dysfunction and structural damage in rats while development of renal injury was slower and limited.

***Key words**: Fixed dose combination of antituberculosis, experimental hepatotoxicity, nephrotoxicity*

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INTRODUCTION

Tuberculosis (TB) is a global burden and around ten million new cases are still identified in 2017 alone. At least 60% of these cases are originated from Asian countries including India, Indonesia and China.[1] The current strategy to control TB infection emphasizes the use of Fixed Dose Combination (4-FDC) regimen of antituberculosis (AT), comprises of isoniazid, rifampicin, pyrazinamide, and ethambutol for two months, followed by the 2-FDC regimen for four months.[2] This regimen is found beneficial to lessen TB infection; however, there is a concern that FDC-AT may be associated with serious side effects, including hepatotoxicity and nephrotoxicity.

Several studies in Asia have reported around 10-30% of AT-treated patients develop liver dysfunction and hepatotoxicity [3-5]. Hepatotoxicity of AT is associated with isoniazid, rifampicin or pyrazinamide use alone, but the risk may increase if taken together due to drug-drug interactions [6]. This may negatively affect the patient's compliance with medication [7]. In some cases, AT-induced hepatotoxicity may require the patients to reduce the dose of AT used, or in more extreme cases, compel the patients to stop taking the medication [8]. This may negatively impact the success rate of the treatment and even increase the risk of AT resistance [7].

In addition to hepatotoxicity, 7% of TB patients are found to develop acute kidney injury (AKI) during the intensive phase of AT [3, 9]. Although the prevalence is low, several cases may not be recovered leading to permanent renal damage [10, 11]. Renal toxicity of AT is predominantly triggered by the use of rifampicin, yet again, when combined with isoniazid, the risk could be amplified [12].

The hepato-renal toxicity of AT is idiosyncratic in nature and widely dependent upon the characteristics and susceptibility of the host [13]. The clinical manifestations can vary from asymptomatic elevations of liver enzyme levels to profound liver failure [14]. AT-induced hepatic and renal toxicities have been implicated with some risk factors, including age, comorbidities, co-medication, and Asian race [13, 14]. Nevertheless, it is still difficult to predict whether someone would experience liver toxicity of merely an asymptomatic ALT rise during AT treatment [5].

The use of animal model might be essential to revealing the possible outcomes of subchronic use of AT especially in 4FDC dosage form. The 4FDC-AT form is of particular importance as not much data has used the regimen in animal studies. Therefore, this research aimed to study the development of liver and renal damage in rat animal model induced by subchronic use of 4FDC-AT regimen. Liver and renal damages are indicated by elevation of serum biomarker levels (<50% from upper baseline) with evident histopathological changes in liver and kidney tissue structures following 28 days of treatments.

MATERIALS AND METHODS

Chemical and drug preparation

Diagnostic kits for glutamate-pyruvic aminotransaminase (GOT (ASAT) IFCC mod.liquiUV) and creatinine (creatinine liquicolor) analysis were purchased from Human® (Makassar, Indonesia). 4FDC-AT tablets were purchased from local pharmacy (Indofarma, Makassar, Indonesia), which comprised of rifampicin (150 mg), isoniazid (75 mg), pyrazinamide (400 mg) and ethambutol HCl (275 mg).

The dose of 4-FDC used were calculated from conversion of human effective dose (HED) to animal effective dose (AED) by multiplying HED in mg/kg by 6.2, which is the conversion factor for rats [15]. The use of dose conversion factor is necessary as an animal model may have different biochemical, pharmacokinetics as well as physiological time, that may affect drug effects in different species [15]. As HED of 4FDC-AT for 60 kg human is 4 tablets (72 mg tablet/kg), hence, the dose given to rats was 446.4 mg tablet/kg or 89 mg tablet/200g rat body weight. This regimen contained rifampicin 62 mg/kg, isoniazid 31 mg/kg, pyrazinamide 164 mg/kg and ethambutol 113 mg/kg. The drugs were suspended in 1% Sodium CMC prior to administration.

Animal preparation

Male Wistar rats (150-200 g) were cared in animal house of Biopharmacy Laboratorium, Hasanuddin University (Makassar, Indonesia). Rats were kept in plastic well-aerated cages with maximum 4 rats per cage at a room temperature. The room was set up with 12 h light/dark cycle. All animals were provided with free access to water and food *ad libitum*. Rats were acclimated at least 7 days prior to use in the experiments. All procedures applied on the animals complied with institutional standard of care and have been approved by Institutional Animal Ethics Committee under Hasanuddin University No. UH 170121091.

Experimental protocols

Twelve rats were assigned into 2 groups (n=6): Group 1 received the placebo (1% NaCMC) while Group 2 was treated with AT 4-FDC 89 mg/200 g rat body weight via oral gavage for 28 days. Rats were weighed every day to adjust the amount of AT 4-FDC administered daily. Blood samples were withdrawn prior to treatment (day 0), on day 7, day 14 and 28 via lateral vein. Rats' livers and kidneys were harvested 24 hours following the final treatments. Three additional rats that did not receive any treatments (only water and food) were sacrificed to provide healthy liver and kidney histology.

Biochemical assays

Blood samples were collected in vacutainer tubes and centrifuged at 2500 rpm for 20 minutes. The sera were then analysed to measure ALT and creatinine levels using Humalyzer 3000 (Human®) based on kit instruction.

Histopathological examination

Livers and kidneys were immediately removed and washed in cold PBS before fixed in 10% formaldehyde for 48 hours. The specimens were then processed in a tissue processor (Thermo Scientific®), embedded in paraffin wax, and cut into 5 μm thick sections with a microtome (Sakura®). Tissue sections were stained with Haematoxylin and Eosin (H&E) and examined under light microscopes (Olympus®) connected to computer screen and a camera (Nikon®) by two observers blinded to the treatment given.

Statistical analysis

Numerical data are presented in mean ± standard deviation (SD). Data was analysed using SPSS 22 software and all statistical differences between the groups were analysed using paired T-test. Statistical significance was achieved if p value was <0.05.

RESULTS

Effects of 4FDC-AT on liver and renal biomarkers

This study is conducted using rat animal models that received 4FDC-AT regimen converted from human effective dose to rat dose. One daily dose contained rifampicin 62 mg/kg, isoniazid 31 mg/kg, pyrazinamide 164 mg/kg and ethambutol 113 mg/kg.

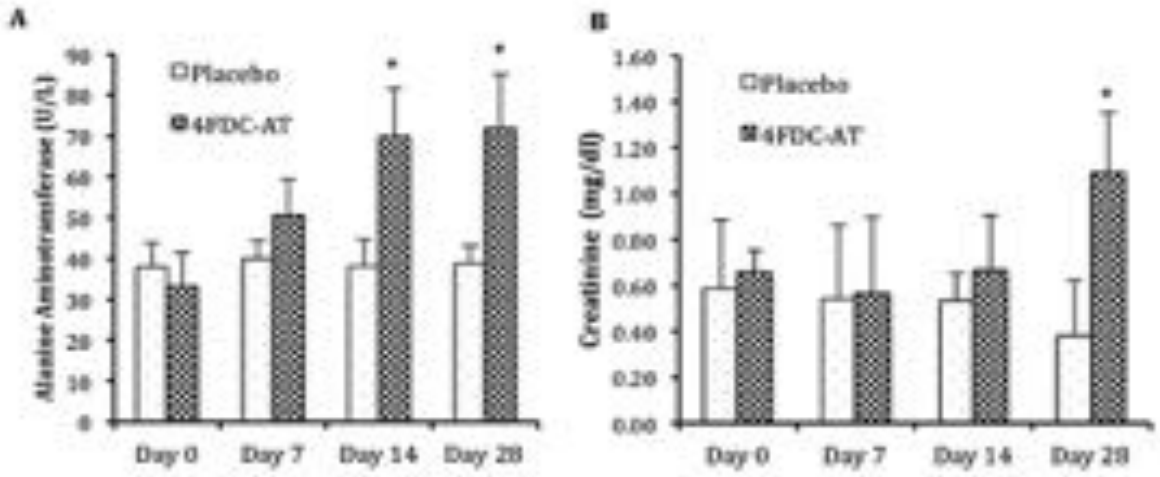

Figure 1. The serum level of alanine aminotransferase and creatinine before (day 0) and following treatments at day 7, 14 and 28 in placebo and 4FDC-AT groups

The baseline level of ALT was measured one day prior to treatment (day 0). It is found that the level of ALT in both groups was not significantly different with a range of 23-47 U/L. This range serves as the normal value in this study. Figure 1A illustrates the pattern of mean ALT level in placebo and 4FDC-AT treated rats. After 7 days of treatments, one rat experienced an increased

ALT level, but it was not considered significant (less than 50% of upper normal value). At this stage, the overall increase in mean ALT level of 4FDC-AT group was not significantly different from the placebo group. On day 14 of treatments, three out of six (50%) 4FDC-AT rats has shown an elevated ALT of >50% from normal range. Indeed, the mean ALT level of 4FDC-AT group was almost twice as high as that in the placebo group ($p<0.05$). On day 28 of treatments, the level of ALT in 4FDC-AT group did not further increase despite the continuous treatment. Yet, the mean of ALT in 4FDC-AT treated rats were still significantly higher than those treated with placebo ($p<0.05$).

The creatinine levels prior to treatments (day 0) ranged from 0.29 to 0.87 mg/dl and were not statistically different between treatment groups. Creatinine level was considered significant in this study if it reached 50% from upper normal limit of 0.87 mg/dl. Measurement of creatinine level on day 7 revealed no significant changes occurred in both placebo and 4FDC-AT groups (Figure 1B). Similar results also found after 14 days of treatments, where creatinine levels of rats from either group were still in the normal range. On that day, no significant difference was found between treatment groups. Following 28 days of treatments, one out of six (17%) rats in 4FDC-AT group have shown a significant increase in their creatinine levels. In overall, the mean creatinine level of 4FDC-AT group was significantly higher than that in placebo group ($p<0.05$).

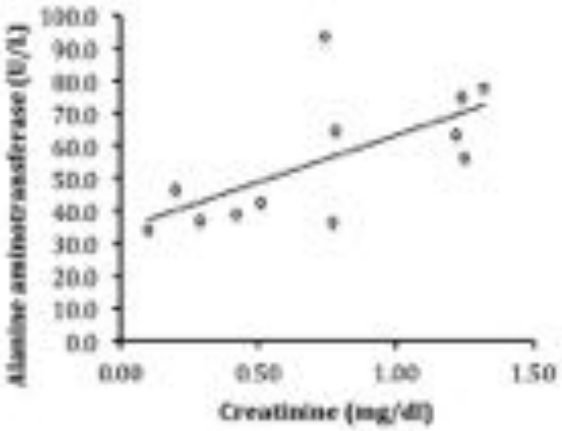

Figure 2. The scatter plots of serum creatinine and alanine aminotransferase levels in placebo and FDC-AT groups on day 28. There is moderate positive correlation found between the two biomarkers (R = 0.653)

It is apparent from figure 2 that there is a positive correlation found between elevated serum ALT levels with increased creatinine levels in rats after 28 days of treatments. The correlation is considered moderate with coefficient correlation (R) of 0.653.

Effects of 4FDC-AT on liver and renal histopathological changes

Hepatic tissue

Histopathological examination (Figure 3) showed that the liver tissue of healthy rats had normal appereance of hepatic tissue, the presence of red blood cells were depicted in some areas of sinusoid and several blood vessel were moderately congested (Figures 3A and 3B). Placebo groups did not show any lesions of pathological significance comparable to that of healthy rats (Figures 3C & 3D). In contrast, some animals in 4FDC-AT group experience abnormal liver structure, characterized by scattered hepatic degeneration with extensive hemorrhagic area (Figures 3E, 3F & 3H). Most hepatocytes showed hydropatic degeneration characterized by cytoplasm swelling which was lighter in colour (Figure 3F). Vacuolization was also found in some hepatocytes (Figure 3F) with some necrotic area (Figure 3G). The sinusoids were markedly dilated and mostly congested with blood, with increased infiltration of inflammatory cells.

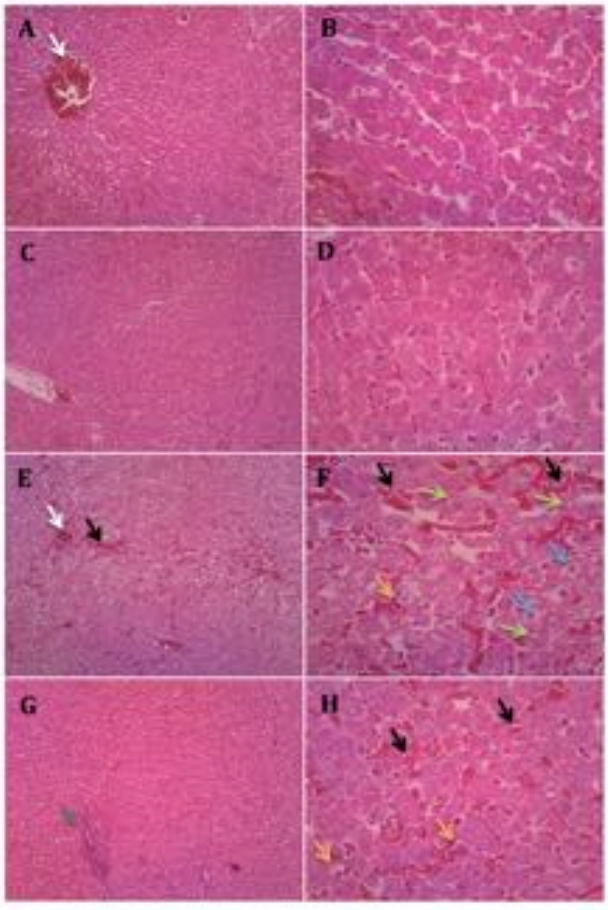

Figure 3. The liver histology in healthy rats (A, B), placebo (C, D) and 4FDC-AT-treated rats (E, F, G, H) following 28 days of treatments using H & E stain. Magnification 100x and 400x. Healthy rats and placebo group did not show significant histopathological changes. Liver structural damage found in 4FDC-AT-treated rats include extensive hemorrhage (black arrow), hepatocyte hydropic degeneration (blue arrow), vacuolization (green arrow), infiltration of inflammatory cells (yellow arrow), congestion (white arrow), and necrotic area (grey arrow)

Renal tissue

Based on examination of renal tissue (see Figure 4), the structural damage was less intense compared to that seen in liver following 28 days of 4FDC-AT treatment. However, signs of histological changes were shown, especially in renal tubules, where some haemorrhage and mild loss of tubule lining were found (Figure 4H). One rat experienced significant degeneration of tubules characterized by almost complete loss of tubule lining and profound infiltration of inflammatory cells. With 4FDC-AT dose used in this study, the integrity of glomerulus was not significantly altered; although mild histological changes such as dilated Bowman's capsule was found.

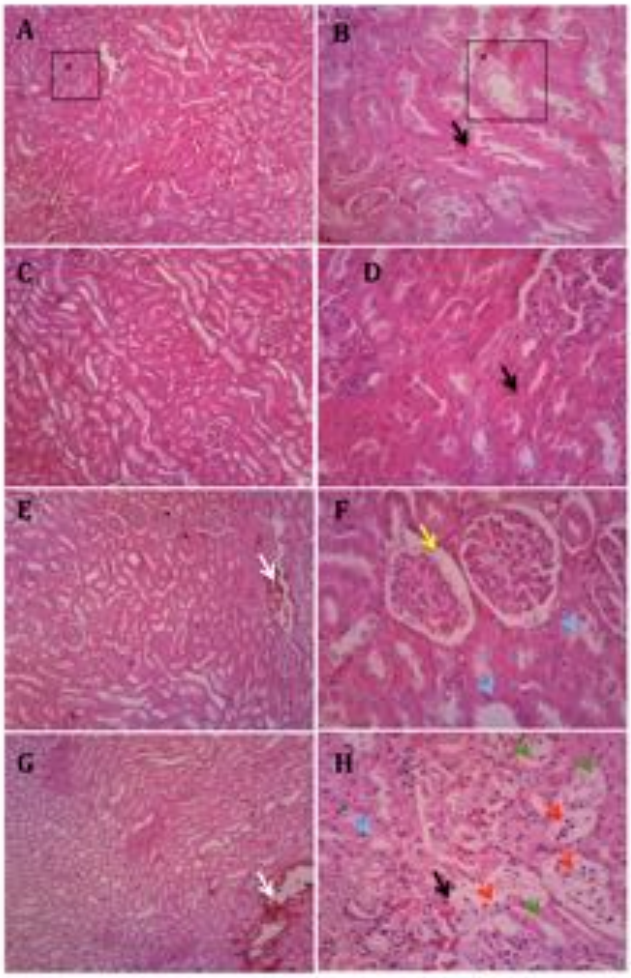

Figure 4. The renal histology in healthy controls (A, B), placebo (C, D) and 4FDC-AT-treated rats (E, F, G, H) following 28 days of treatments using H & E stain. Magnification of 100x and 400x. Healthy rats and placebo group did not show significant histopathological changes. Histopathological changes found in 4FDC-AT-treated rats include dilated Bowman capsule (yellow arrow), congestion (white arrow), mild loss of tubule lining (blue arrow), tubule degeneration (green arrow), hemorrhage (black arrow) and profound infiltration of inflammatory cells (red arrow).

DISCUSSION

The potential risk of hepatotoxicity and nephrotoxicity in TB patients with AT treatments has been recognized. However, it is difficult to study the pattern of hepatotoxicity and nephrotoxicity in humans. Although many studies have confirmed the presence of hepatotoxicity or nephrotoxicity induced by AT in animal models, but most studies only used isoniazid and rifampicin (50 mg/kg to 150 mg/kg) [16-18]. Another study has induced liver toxicity using 4FDC but the dose regimen is higher than those utilized in clinical settings [19]. This study was conducted using rat animal models that received 4FDC-AT dose calculated from AED conversion (rifampicin 62 mg/kg, isoniazid 31 mg/kg, pyrazinamide 164 mg/kg and ethambutol 113 mg/kg). This allows a direct observation of the pattern of liver and renal dysfunction during treatments, as well as their histological structures following 28 days of 4FDC-AT treatments in rats.

The result showed that 4FDC-AT increased ALT levels starting from day 7, in which one rat experienced a 30% increase in ALT level. After 14 days of treatment, 50% of rats in 4FDC-AT group experienced >50% ALT elevation, but no further raise in ALT level after 28 days of treatment. The fact that only 50% of rats experienced ALT elevation at the end of experiment suggests not all rats are susceptible to 4FDC-AT hepatotoxicity at the particular dose given. In contrast, elevated creatinine level only appeared in 17% of 4FDC-AT rats during treatment, indicating that FDC-AT detrimental effects on renal function were not as severe as those in liver. This is also true in clinical setting. Among adverse effects associated with FDC-AT use, hepatotoxicity was more frequently reported than other unwanted effects [20-22]; whereas, the incidence of AKI due to the first-line AT was not mentioned in those studies. Apparently, the incidence of AT-induced AKI are more noticeable in geriatric patients (43 out of 61 patients), which may or may not recover after discontinuation of treatment [9].

The elevation of both ALT and creatinine levels showed a positive moderate correlation, implying that rat susceptibility to liver and renal toxicities might be influenced by similar factors. Both liver and renal toxicities of AT could be initiated by rifampicin toxic metabolites [12]. The risk increases along with reduced antioxidant activities and increased oxidant exposure in individuals [23].

The presence of hepatotoxicity is not adequately indicated by an elevation of ALT only. The histopathological evaluation would add important information about liver tissue integrity and provide more evidence of hepatotoxicity. The liver sections of rats that received placebo showed normal liver tissue histology, quite similar with those of healthy rats (Figure 3). In contrast, 28 days of 4FDC-AT treatments had led to scattered hepatic degeneration and the centrilobular region

showed a patchy appearance. Most sinusoid area was dilated and haemorrhagic, and often infiltrated by inflammatory cells (Figure 3). This finding has confirmed the presence of hepatotoxicity in rats treated with 4FDC-AT for 28 days. In fact, the rats with ALT elevation had more severe damage in their liver structures compared to those with insignificant increase of ALT. In TB patients, increased in ALT level could be asymptomatic, but may also prolonged and lead to hepatotoxicity in some cases [5]. But in rats, the raise in ALT level >50% of upper limit value was followed by a significant damage in liver histological structure.

Unlike hepatic damage, the renal histopathological changes found in this study were considered mild to moderate. Only one rat had shown extensive damage in the tubular area with profound infiltration of inflammatory cells. Previously, the presence of histological renal damage with AT drugs have been studied in Wistar rats, but the result could be vary from mild or moderate to significant alteration of rat renal histology [24, 25]. Again, different duration of treatment and the dose regimen given would greatly influence the outcomes.

CONCLUSION

In conclusion, liver toxicity of 4FDC-AT was more evident and rapidly developed than renal toxicity in rats. The elevation of ALT appeared as soon as 14 days of treatments in 50% of 4FDC-AT rats and liver histopathological damage was evident after 28 days in those rats. In contrast, renal dysfunction indicated by significant increased level of creatinine, only appeared after day 28 of 4FDC-AT treatments in 1 out of 6 rats. The 4FDC-AT treatment mostly led to mild histopathological changes in rat kidney.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

Authors do not have any conflict of interest.

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FABAD A-592 Second Revision

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Corrections have been made on the manuscript.

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Nusantara Medical Science Journal

ORIGINAL ARTICLE

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. Paliasa leaves have been emiprically used to improve liver function in South Sulawesi. This study aimed to determine the effect of ethanolic extract of Paliasa leaves on total bilirubin serum in rats.

Method: Twenty rats were devided 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 Paliasa 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. Paliasa 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 Paliasa extract 500 mg/kg.

Conclusion: Therefore, it is concluded that Paliasa 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 function[4]. As a result, high incidence of hepatotoxicity is found among tuberculosis patients treated with AT drugs around the world [5-7], 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 H_2O_2 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 (CCl_4).[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 (40ºC) 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	0.090-0.205	0.163	0.045
2	0.090			
3	0.198			
4	0.130			
5	0.157			
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).

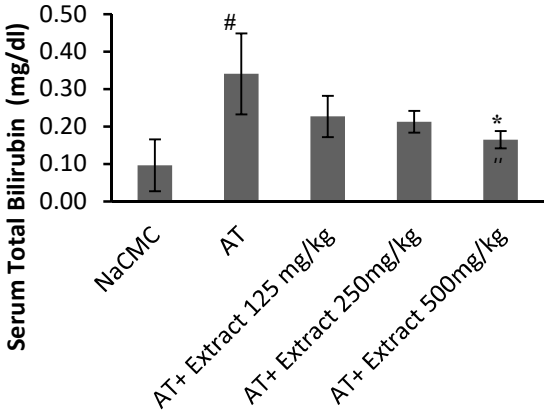

Figure 1. Serum total bilirubin in rats following 28 days of treatments.

[#]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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Kleinhovia hospita Extract Alleviates Experimental Hepatic and Renal Toxicities Induced by Combination of Antituberculosis Drugs

Running Title: *Kleinhovia hospita* improves antituberculosis-induced toxicities

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 patient adherence to their medication regimen (3). Thus, the use of combination of antituberculosis drugs (CAD) is recommended to simplify TB regimen and consequently improves 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 reported 12-32% patients who are treated with AT agents experienced hepatotoxicity indicated by elevation of ALT more than 3-5 times 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 Linn, belonging to the family of Sterculiaceae, is a native plant from Indonesia, but may also be found in Asia Pacific region. *K. hospita* sp. 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 hepatitic 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, this present study aimed to investigate 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 ethambuthol 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 contains 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 K. hospita Extract

K. 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 (Heidolp®). The concentrated extract was kept in desiccator at room temperature (25^{o}C) to remove excess solvent or moisture. The extract is 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). 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*. 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, 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^{o}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 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 μm of thickness with a microtome (Sakura®), and stained with Haematoxylin 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 ± 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 range 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, the 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 decreased in ALT level compared to that in 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 level (day 0). Those renal biomarker levels were also significantly higher compared to the control group on day 28 (Figure 1). The elevation of creatinine and urea levels in CAD was 0.16 ± 0.022 (increased 60% from day 0) and 30.8 ± 5.09 mg/dl (increased 120% from day 0), 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.

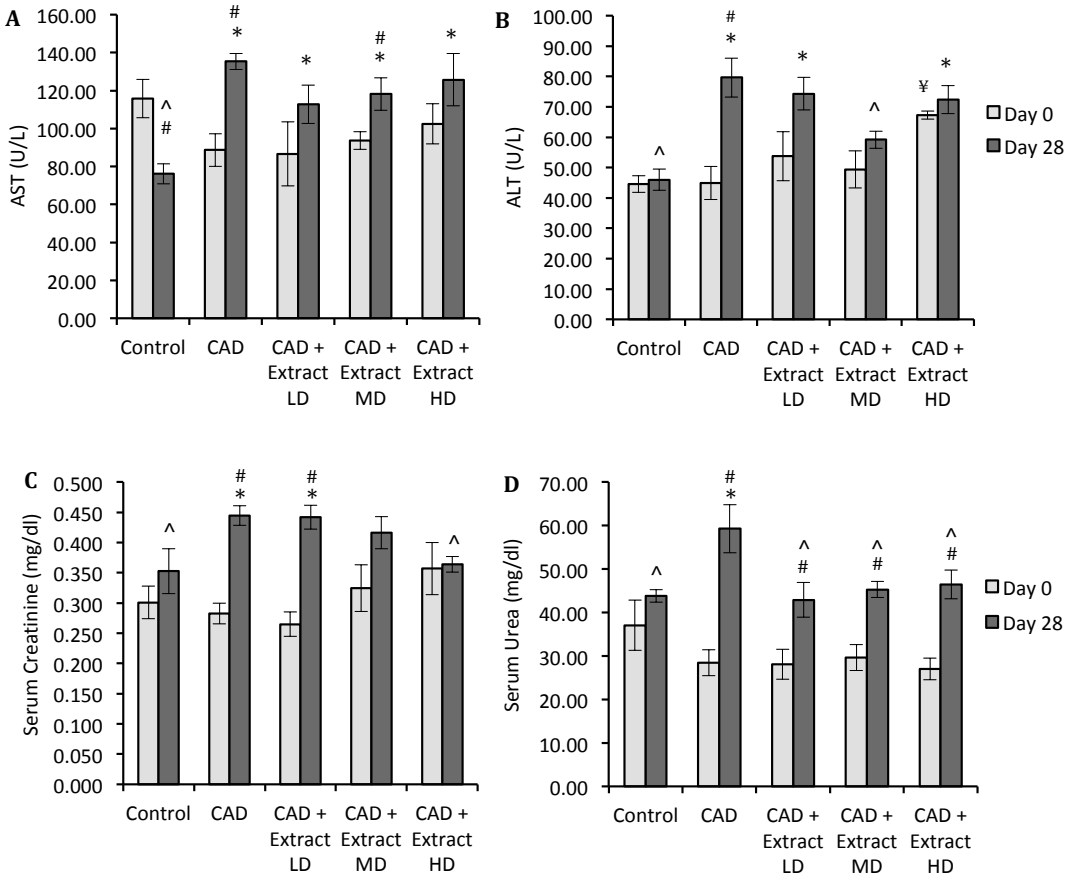

Figure 1: Liver and renal biomarker levels before (day 0) and following treatments (day 28). Data is presented in mean ± SEM. Controls: placebo, CAD: combination of antituberculosis drugs. Extract LD (low dose): 125 mg/kg, Extract MD (mid dose): 250 mg/kg, Extract HD (high dose): 500 mg/kg. *p<0.05 from 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.

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 significantly reduced 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.

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.

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).

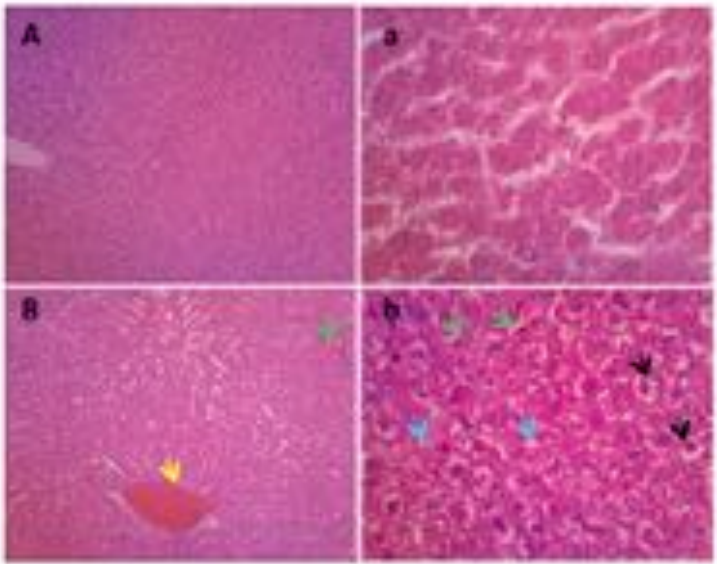

Figure 2: Light micrograph of liver tissue structures in controls (A,a) and CAD treated rats (B.b) following 28 days of treatment. H&E stain. Magnification 100 x and 400 x. Histopathological changes found in CAD group include congestion (yellow arrow), hepatic degeneration (black arrow), vacuolization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

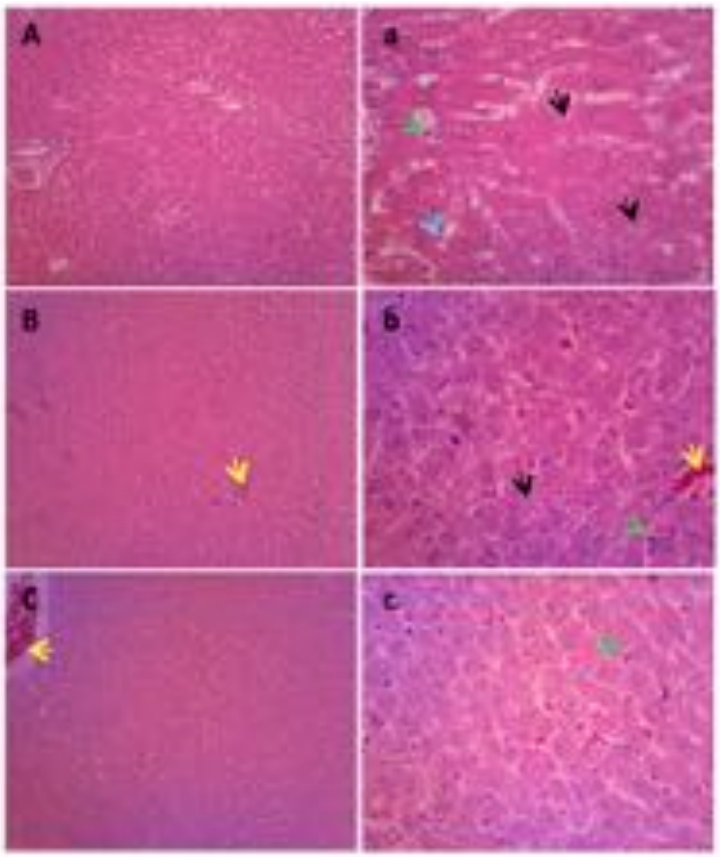

Figure 3: Light micrograph of liver tissue structures in rats treated with CAD and *K. hospita* Extract for 28 days. CAD + extract LD (A,a), CAD + extract MD (B,b), CAD + extract HD (C,c). H&E stain. Magnification 100 x and 400 x. In CAD + extract LD group, degeneration of hepatocyte, vacuolization and 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.

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 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 (Fig. 5C, 5c).

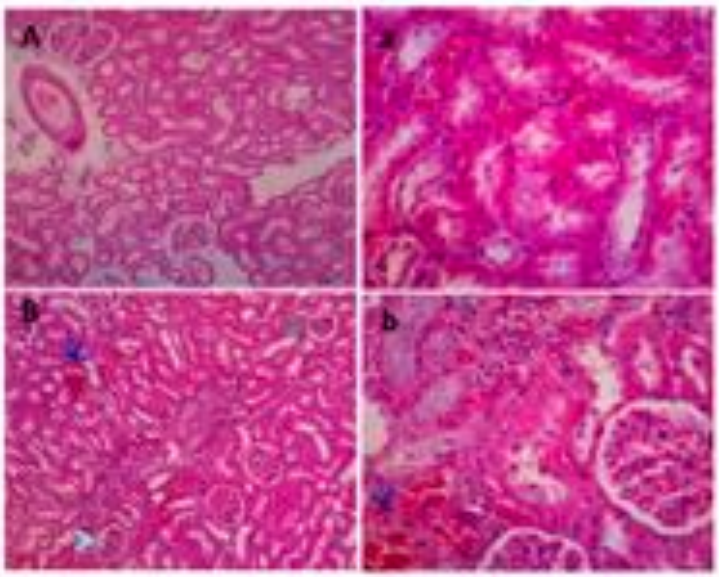

Figure 4: Light micrograph of renal tissue structure in controls (A,a) and CAD treated rats (B.b) following 28 days of treatments. H & E stain. Magnification 100x and 400x. Histopathological changes found in CAD group include hemorrhagic area (blue arrow), atrophic glomerulus (grey arrow), dilated Bowman's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

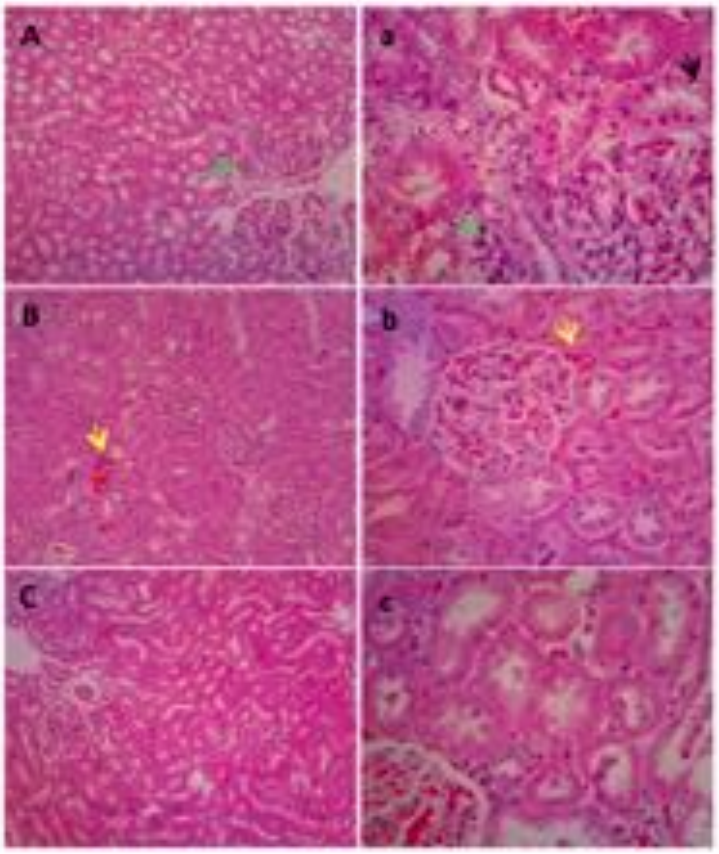

Figure 5. Light micrograph of renal tissue structure in rats treated with CAD and *K. hospita* Extract for 28 days. CAD + extract LD (A,a), CAD + extract MD (B,b), CAD + extract HD (C,c). H & E stain. Magnification 100x and 400x. In CAD + extract LD group, the presence of tubule degeneration with vacuolization (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)

Discussion

Drug-induced liver injury has been one of the main concerns for using CAD treatment in TB patients. About 10% of patients received 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 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 (2012) 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 their radical metabolites 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, rats were treated with *K. hospita* hidro-alcoholic 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 showed promising cytotoxic activities against human cancer cells *in vitro* (13, 26-28).

It is found that administration of *K. hospita* leaf extract before CAD treatment was able to inhibit the rise in ALT on day 28. This protection is 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 is 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 a lack of study that has explored *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 dose 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.

Acknowledgment

The authors would also thank Mr. Ismail for the assistance during sample collection and extract preparation.

Author's contributions

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 interest

Authors declare to have no conflict of interest

Ethical Consideration

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

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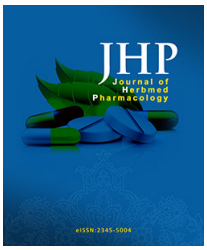

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The effect of antituberculosis and *Kleinhovia hospita* extract co-administration on hematology profiles and electrolyte balance in rats

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ABSTRACT

Background: Antituberculosis drugs (ATD) potentially cause hepatotoxicity and pre-treatment with *Kleinhovia hospita* extract has been shown to reduce ATD-induced hepatotoxicity. **Objective:** This study aimed to investigate the possible adverse effects of co-administration of ATD and Kleinhovia extract on hematology profiles and electrolyte balance in rats. **Materials and Methods:** Male Wistar rats were divided into four treatment groups. Group I only received suspending agent as placebo (n=6); group II was treated with ATD tablet 89 mg/200 g (n=7); group III received 250 mg/kg Kleinhovia extract and ATD (n=7); group IV received 500 mg/kg Kleinhovia extract and ATD (n=7). The extract was given 3 hours before ATD administration. Blood samples were taken from rat lateral veins after 28 days of treatment and analyzed to obtain complete blood counts and electrolyte levels. **Results:** ATD treatment in rats did not alter electrolyte balance but led to significantly reduced mean corpuscular volume (MCV) and increased mean corpuscular hemoglobin concentration (MCHC) compared to placebo group ($p<0.05$). ATD also lowered mean platelet volume (MPV), platelet distribution width (PDW) and platelet large cell ratio (P-LCR). Rats received ATD and Kleinhovia extract 250 mg/kg had similar alteration in their hematological indices with those treated with ATD only, meanwhile extract 500 mg/kg further reduced the PDW and P-LCR ($p<0.05$). **Conclusion:** ATD administration for 28 days in rats did not affect electrolyte level but may trigger some variations in red blood cell and platelet sizes. Co-administration with Kleinhovia extract at 500 mg/kg dose could signify the alteration of platelet size and distribution.

Key words: Antituberculosis, Kleinhovia hospita, blood cell counts, electrolyte level

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IAI Accreditation Number: 084/SK-SKP/PP.IAI/1822/I/2019

Plenary Speaker: 4.5 SKP, Invited Speaker: 4.5 SKP, Workshop Speaker: 6.5 SKP, Presenter of Oral/Poster Session: 3 SKP, Judges: 3 SKP, Committee: 2.5 SKP, Workshop Participant: 6.5 SKP, Conference Participant: 10 SKP, Moderator: 1.5 SKP

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Dr. Mahdi Jufri, M.Si., Apt.
Dean of Faculty of Pharmacy
Universitas Indonesia

Prof. Dr. Yahdiana Harahap, M.Si., Apt
President-Elect of Asian Federation for
Pharmaceutical Sciences

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Yulia Yusrini Djabir

in recognition of the valuable contribution as:

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in the 4th International Conference on Advance Pharmacy and Pharmaceutical Sciences (ICAPPS 2019)
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Dokumen pendukung luaran Tambahan #2

Luaran dijanjikan: Publikasi Ilmiah Jurnal Nasional Tidak Terakreditasi

Target: accepted/published
Dicapai: Published

Dokumen wajib diunggah:
1.

Dokumen sudah diunggah:
1. Artikel yang terbit

Dokumen belum diunggah:
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