LAPORAN AKHIR PENELITIAN TAHUN TUNGGAL

ID Proposal: 1b27adf2-1f47-4317-995a-a69c7dba66dc
Laporan Akhir Penelitian: tahun ke-1 dari 1 tahun

1. IDENTITAS PENELITIAN

A. JUDUL PENELITIAN

Uji Efektivitas Minyak Jintan Hitam (Nigella sativa) dalam Melindungi Fungsi Hati dan Ginjal Tikus yang diberi Levofloksasin secara Subkronik

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

Bidang Fokus RIRN / Bidang Unggulan Perguruan Tinggi	Tema	Topik (jika ada)	Rumpun Bidang Ilmu
Kesehatan	Teknologi kemandirian bahan baku obat	Saintifikasi jamu & herbal, teknologi produksi pigmen alami	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 Kompetitif Nasional	Penelitian Tesis Magister	SBK Riset Dasar	SBK Riset Dasar	2	1

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
Nurfadilah Mahasiswa Bimbingan 1	Mahasiswa Pasca Sarjana Farmasi	-	Melakukan ekstraksi, perlakuan hewan coba dan pengumpulan data	0	0
dr. ARIF SANTOSO S.Ked, Sp.P, Ph.D.Med.Sc. Dosen	Universitas Hasanuddin	Ilmu Bedah	Terlibat dalam pengolahan dan interpretasi data	6007106	3

Pembimbing Anggota 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*)
1	Artikel di Jurnal Internasional Terindeks di Pengindeks Bereputasi	Accepted	Journal of HerbMed Pharmacology

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*)

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 1 Tahun Rp. 25,650,000

Tahun 1 Total Rp. 25,650,000

Jenis Pembelanjaan	Item	Satuan	Vol.	Biaya Satuan	Total
Analisis Data	HR Sekretariat/Administrasi Peneliti	OB	1	250,000	250,000
Analisis Data	Biaya analisis sampel	Unit	100	15,000	1,500,000
Bahan	ATK	Paket	1	900,000	900,000
Bahan	Bahan Penelitian (Habis Pakai)	Unit	1	11,450,000	11,450,000
Pelaporan, Luaran Wajib, dan Luaran Tambahan	Publikasi artikel di Jurnal Internasional	Paket	1	4,500,000	4,500,000
Pengumpulan Data	HR Pembantu Peneliti	OJ	1	1,000,000	1,000,000
Pengumpulan Data	HR Pembantu Lapangan	OH	1	1,000,000	1,000,000
Pengumpulan Data	Biaya konsumsi	OH	2	300,000	600,000
Pengumpulan Data	Transport	OK (kali)	3	150,000	450,000
Sewa Peralatan	Peralatan penelitian	Unit	1	4,000,000	4,000,000

6. HASIL PENELITIAN

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

Tuberkulosis (TB) adalah penyakit infeksi yang disebabkan oleh Mycobacterium tuberculosis. Hingga saat ini, tuberkulosis masih menjadi penyakit infeksi menular yang paling berbahaya di dunia dan Indonesia menempati posisi ke dua kasus tuberkulosis multi drug resistant (TB-MDR) tertinggi di Asia Tenggara menurut WHO. Selama ini penyakit infeksi seperti tuberkulosis diatasi dengan penggunaan antibiotik. Rifampisin (RIF), Isoniazid (INH), etambutol (EMB), streptomisin dan pirazinamid (PZA) telah dimanfaatkan selama bertahun-tahun sebagai anti-TB. Namun, banyak penderita telah menunjukkan resistensi terhadap obat lini pertama ini sehingga penderita TB semakin meningkat. Pengobatan TB-MDR menggunakan obat anti tuberkulosis lini kedua seperti levofloksasin. Obat Anti Tuberkulosis (OAT) lini kedua memiliki toksisitas melebihi obat lini pertama serta banyak menimbulkan efek samping seperti gangguan hepar, gangguan ginjal, gangguan pendengaran dan neuropati. Dengan gangguan tersebut tentunya akan mengurangi kualitas pengobatan pada pasien khususnya pasien Tuberkulosis Multi Drug Reaction (TB-MDR). Minyak Jintan hitam memiliki kandungan yaitu thymoquinone sebagai antioksidan yang memiliki efek proteksi terhadap toksisitas pada ginjal dan hati. Berdasarkan hal tersebut maka peneliti tertarik untuk melakukan penelitian lebih lanjut dengan menggunakan minyak jintan hitam dalam upaya pemulihan dan mengurangi efek samping yang ditimbulkan khususnya pada organ ginjal dan hati.

Penelitian ini bertujuan untuk mengetahui apakah minyak jintan hitam dapat memberikan efek protektif terhadap gambaran histopatologi ginjal dan hati tikus putih jantan (Rattus novergicus) yang diinduksi Obat Anti Tuberkulosis (OAT) lini kedua Levofloksasin.

Metode yang digunakan adalah eksperimental laboratorium dengan pendekatan Pre-test and Post-test Control Group Design menggunakan tikus jantan sebanyak 30 ekor yang dikelompokkan ke dalam 5 kelompok dengan masing-masing kelompok terdiri dari 6 ekor yang kemudian diberi perlakuan sebagai berikut: Kelompok I sebagai kontrol negatif yang diberi larutan Na.CMC 1%, Kelompok II sebagai kelompok positif/ kontrol patologis yang diberi suspensi levofloksasin, kelompok III, IV dan V diberi minyak Levofloksasin dan minyak jintan hitam masing-masing 0,5 ml, 1 ml, dan 2 ml. Pengukuran dilakukan setelah 28 hari masa treatment.

Penelitian ini merupakan penelitian dasar TKT 2. Hasil penelitian ini telah dipresentasikan di The 4th International Conference on Science (ICOS) tanggal 22-23 Agustus 2020 dan akan dipublikasikan di Jurnal internasional Journal of Physics: Conference Series (JPCS)(SCOPUS Q4). Selain itu, diharapkan dapat menjadi sumber informasi baru tentang manfaat minyak jintan hitam sehingga dapat dimanfaatkan secara optimal utamanya dalam terapi Tuberkulosis Multi Drug Resisten (TB-MDR).

B. KATA KUNCI: Tuliskan maksimal 5 kata kunci.

Minyak Jintan Hitam; Nigella sativa; histopatologi hati dan ginjal; Tuberkulosis, Levofloksasin

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.

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.

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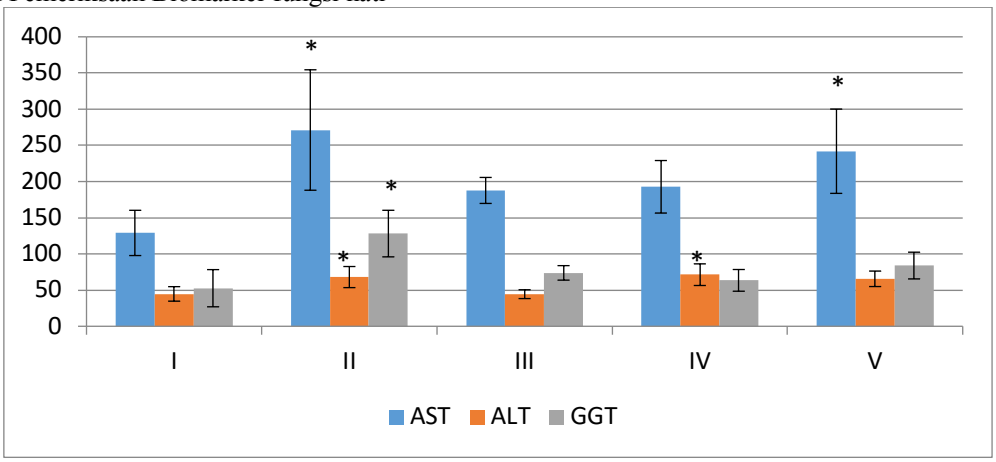
Penelitian ini bertujuan untuk berupaya mencari solusi dalam mengatasi ataupun mencegah efek samping hepatotoksik dan nefrotoksik dari obat levofloksasin dengan menggunakan minyak jintan hitam. Jintan hitam telah dikenal dan digunakan sebagai herbal dalam mengobati berbagai penyakit (1).

Dalam penelitian ini, untuk mengetahui efek hepatototoksik dan nefrotoksik levofloksasin maupun efek hepatoprotektor dan nefroprotektor dari minyak jintan hitam maka dilakukan pemeriksaan terhadap kadar aminotransferase yaitu aspartate aminotransferase (AST) atau serum glutamic oxaloacetic transaminase (SGOT) dan alanine amino transferase (ALT) atau serum glutamic pyruvic transaminase (SGPT) serta pemeriksaan kadar gamma glutamyl transferase (GGT) serta pemeriksaan kadar ureum dan kreatinin. Selain pengukuran kadar biokimia darah, juga dilakukan pengamatan histopatologi.

1. Hasil Pemeriksaan Biomarker fungsi hati



Gambar 1. Grafik perbandingan kadar AST, ALT, dan GGT setiap kelompok setelah perlakuan selama 28 hari. *p<0,05 dibandingkan dengan kelompok I setelah perlakuan. Kelompok I (NaCMC), Kelompok II (levofloksasin), kelompok III (minyak jintan hitam 1 ml/kg BB + levofloksasin), kelompok IV (minyak jintan hitam 2 ml/kgBB + levofloksasin), kelompok V (minyak jintan hitam 4 ml/kg BB + levofloksasin)

Pemberian dosis 93 mg/kg bb levofloksasin pada tikus dapat meningkatkan kadar AST, ALT dan GGT >100% (kelompok II). Nilai AST meningkat dari 64,93 U/L menjadi 271,22 U/L (378%), ALT dari 29,77 U/L menjadi 68 U/L (128%) dan GGT dari dari 25,9 U/L menjadi 128,1 U/L (393%).

Peningkatan nilai AST pada kelompok II meningkat dua kali lipat dari sebelum perlakuan dan melebihi nilai normalnya yaitu 63 – 175 U/L. Berdasarkan hasil uji statistik anova, kelompok II dan V pada nilai AST menunjukkan signifigansi dibandingkan dengan kelompok I yaitu kelompok kontrol. Pada kelompok III dan IV menunjukkan hasil yang berbeda nyata jika dibandingkan dengan kelompok II. Hal ini dipengaruhi oleh pemberian minyak jintan hitam 1 ml dan 2 ml/kg bb. Namun pada kelompok V pemberian minyak jintan hitam 4 ml/kg bb terlihat masih belum memperlihatkan hasil yang sebaik kelompok III dan IV.

Berdasarkan hasil pemeriksaan biomarker ALT, kelompok I yang merupakan kontrol sehat, tidak menunjukkan perubahan yang signifikan setelah 28 hari diberi NaCMC. Kelompok II (pemberian levofloksasin) dan kelompok lainnya yang juga diberi levofloksasin (III, IV, V) mengalami peningkatan nilai kecuali pada kelompok III, nilai ALT nya mengalami peningkatan yang berbeda nyata dengan kelompok II. Hal ini disebabkan oleh pemberian minyak jintan hitam dengan dosis 1 ml/kg bb. Pemberian minyak jintan hitam dengan dosis 1 ml/kg BB mampu mencegah naiknya nilai ALT dan dapat mengontrol nilai ALT untuk tetap berada pada range nilai normal (19 – 48 U/L).

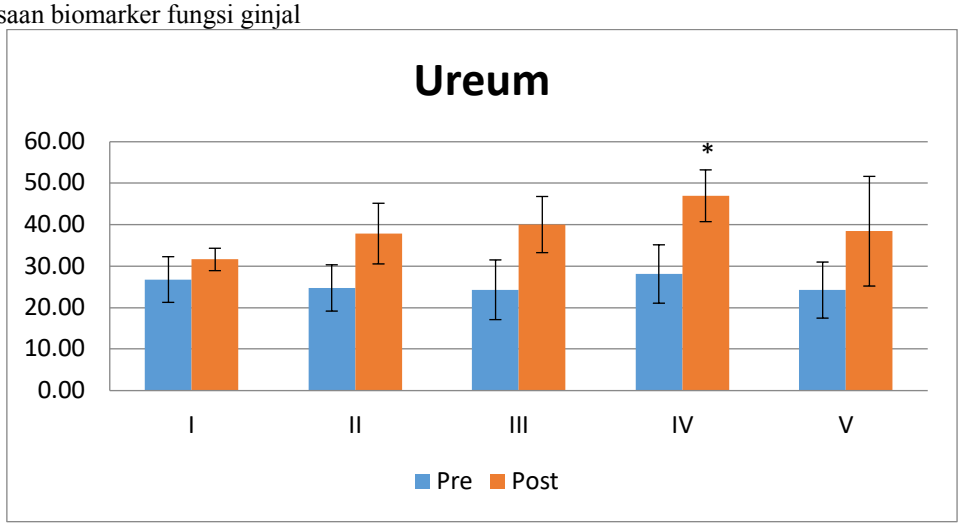
Pada hasil pemeriksaan biomarker GGT, berdasarkan uji one way anova terlihat adanya perbedaan yang signifikan pada kelompok I (NaCMC), III (Minyak jintan Hitam 1 ml/kgBB), IV Minyak jintan Hitam 2 ml/kgBB), dan V (Minyak jintan Hitam 1 ml/kgBB) terhadap kelompok II (suspensi levofloksasin). Jumlah peningkatan GGT kelompok III, IV dan V yang berbeda nyata dengan kelompok II dipengaruhi oleh pemberian

minyak jintan hitam 1, 2 dan 4 ml/kg bb. Pemberian tambahan minyak jintan hitam ini terlihat dapat mencegah naiknya kadar GGT dan dapat mengontrol nilai GGT untuk tetap berada pada nilai mendekati nilai pada kelompok normal.

AST, ALT dan GGT merupakan enzim yang umumnya digunakan untuk mendeteksi adanya kerusakan pada hati. ALT dan AST banyak ditemukan pada organ hepar, jantung, otot rangka, otak, dan ginjal. Enzim ini akan dikeluarkan ke dalam sirkulasi darah apabila terjadi kerusakan atau kematian sel, hal inilah yang menyebabkan kenaikan pada nilai AST dan ALT. ALT merupakan penanda yang paling sering digunakan dalam mendeteksi toksisitas pada hati. Pengukuran kadar ALT lebih spesifik dalam mendeteksi kelainan hepar dibandingkan dengan AST karena enzim ini lebih banyak terdapat dalam hepar (30). Gamma glutamyl transferase (GGT) juga termasuk salah satu enzim yang digunakan dalam mendeteksi adanya disfungsi hati. GGT banyak ditemukan di hati, ginjal dan pankreas. Meskipun peningkatan GGT memiliki spesifisitas yang rendah untuk penyakit hati namun merupakan salah satu prediktor terbaik dari adanya kerusakan hati (2).

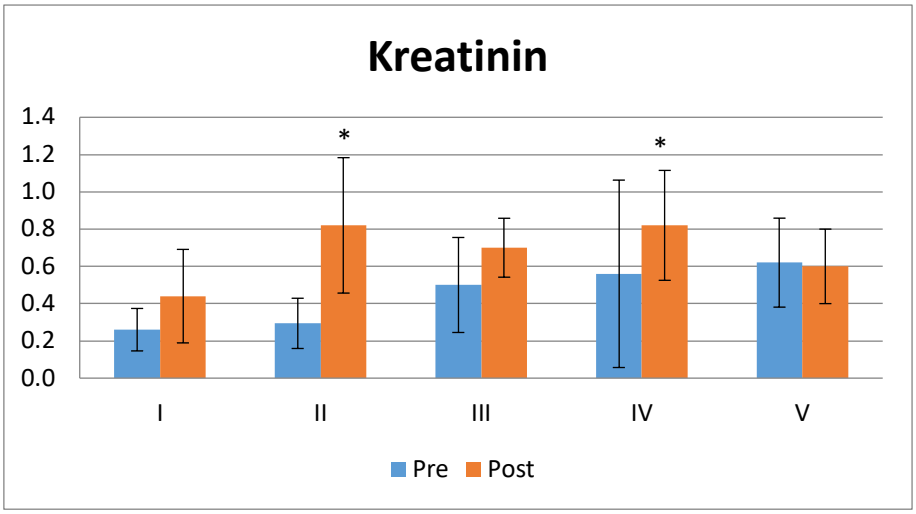
Pemberian dosis 93 mg/kg bb levofloksasin pada tikus terlihat dapat meningkatkan nilai pada biomarker fungsi hati (AST, ALT dan GGT) >100 %. Hal ini menandakan adanya gangguan pada fungsi hati. Pemberian minyak jintan hitam 1 ml, 2 ml dan 4 ml/kg bb terlihat dapat memberikan perubahan yang signifikan pada pemeriksaan biomarker dibandingkan dengan kelompok yang hanya diberikan levofloksasin saja. Pemberian minyak jintan hitam dosis 1 ml/kg bb memperlihatkan hasil paling baik pada semua parameter pemeriksaan biomarker fungsi hati yang diukur.

2. Pemeriksaan biomarker fungsi ginjal



Gambar 2. Grafik perbandingan kadar ureum setiap kelompok setelah perlakuan selama 28 hari. *p<0,05 dibandingkan dengan kelompok I setelah perlakuan. Kelompok I (NaCMC), Kelompok II (levofloksasin), kelompok III (minyak jintan hitam 1 ml/kg BB + levofloksasin), kelompok IV (minyak jintan hitam 2 ml/kgBB + levofloksasin), kelompok V (minyak jintan hitam 4 ml/kg BB + levofloksasin)

Pada gambar 2, terlihat bahwa pemberian perlakuan pada setiap kelompok dapat meningkatkan nilai pemeriksaan ureum. Pada kelompok kontrol, peningkatan nilai ureum menghasilkan peningkatan yang paling rendah dibandingkan dengan kelompok perlakuan lainnya. Pemberian dosis 93 mg/kg bb levofloksasin pada tikus meningkatkan nilai ureum yaitu dari 24,75 mg/dl menjadi 37,85 mg/dl. Peningkatan terjadi >50% dari nilai ureum sebelum perlakuan. Selain itu, peningkatan juga terlihat pada kelompok III (64%), IV (67%) dan V (58%) yang diberikan minyak jintan hitam 1 ,2 dan 4 ml/kg bb sebelum pemberian suspensi levofloksasin. Hal ini menandakan bahwa pemberian obat levofloksasin dapat meningkatkan nilai ureum pada tikus. Pemberian perlakuan juga memperlihatkan perubahan nilai ureum yang tidak berbeda signifikan, kecuali pada kelompok IV, dimana terjadi peningkatan ureum yang signifikan terhadap kelompok kontrol normal ($p<0.05$). Hal ini menunjukkan bahwa pemberian minyak jintan hitam dengan dosis 2 ml/ kg bb memiliki resiko peningkatan ureum dalam darah.



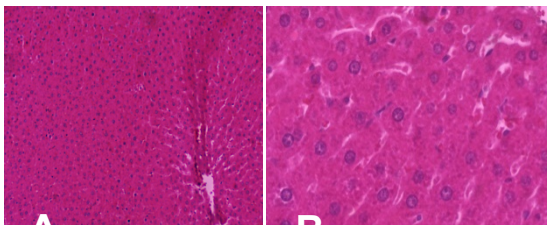
Gambar 3. Grafik perbandingan kadar kreatinin setiap kelompok setelah perlakuan selama 28 hari. *p<0,05 dibandingkan dengan kelompok I setelah perlakuan. Kelompok I (NaCMC), Kelompok II (levofloksasin), kelompok III (minyak jintan hitam 1 ml/kg BB + levofloksasin), kelompok IV (minyak jintan hitam 2 ml/kgBB + levofloksasin), kelompok V (minyak jintan hitam 4 ml/kg BB + levofloksasin)

Berdasarkan hasil pemeriksaan nilai kreatinin, pemberian dosis 93 mg/kg bb levofloksasin pada tikus dapat meningkatkan kadar kreatinin dari 0,3 mg/dl menjadi 0,8 mg/dl , peningkatan yang dihasilkan >100% dan melebihi nilai normalnya yaitu 0,3 – 0,5 mg/dl. Jika dibandingkan dengan perlakuan pada kelompok lainnya, pemberian suspensi levofloksasin menunjukkan presentase perubahan nilai kreatinin tertinggi (Gambar 3). Kelompok I yang merupakan kontrol sehat, tidak menunjukkan perubahan yang signifikan pada nilai kreatinin setelah 28 hari diberi NaCMC (lihat gambar 3). Selain pada kelompok II (pemberian suspensi levofloksasin), kelompok lainnya yang juga diberi levofloksasin (III, IV) juga mengalami peningkatan nilai kreatinin kecuali pada kelompok V terlihat adanya penurunan nilai kreatinin dibandingkan dengan sebelum perlakuan. Berdasarkan hasil uji statistik anova, kelompok II dan IV menunjukkan hasil yang berbeda secara signifikan p<0,05 jika dibandingkan dengan kelompok I (kontrol normal NaCMC).

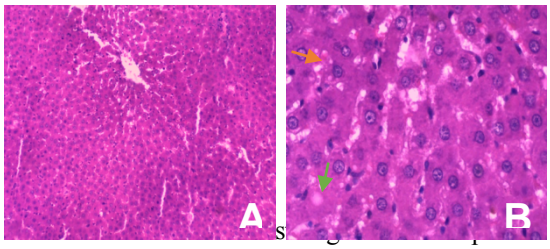
Perubahan kreatinin kelompok III dan V yang berbeda nyata dengan kelompok II dipengaruhi oleh pemberian minyak jintan hitam. Pemberian tambahan minyak jintan hitam ini terlihat dapat mengontrol naiknya kadar kreatinin terutama dengan dosis 1 ml/kg dan 4 ml/kg bb namun masih belum mencapai nilai normalnya yaitu 0,3 – 0,5 mg/dl.

3. Histopatologi hati

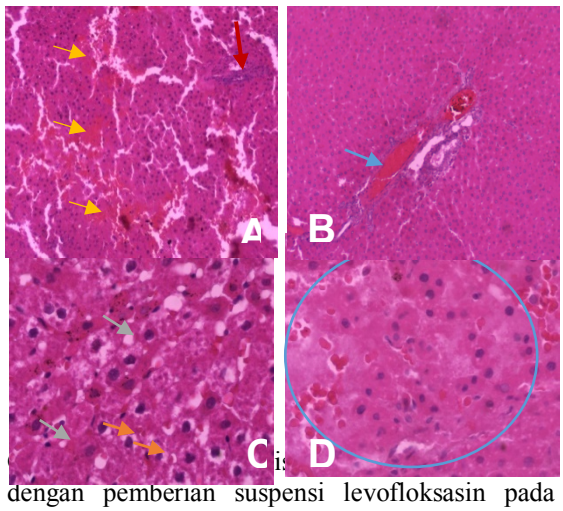
Hasil pengamatan terhadap histopatologi hati menunjukkan pada profil pengamatan yang mewakili kelompok I (kontrol sehat) memperlihatkan beberapa hasil yang normal dan kerusakan minimal (gambar 4). Sedangkan pada kelompok II (gambar 5) yang diberikan suspensi levofloksasin menunjukkan terjadinya kerusakan yang parah dengan tampaknya beberapa kerusakan seperti terjadi hemoragi, kongesti dan degenerasi. Kongesti merupakan keadaan dimana darah berakumulasi atau tertimbun di dalam pembuluh vena dan mengakibatkan pelebaran, sedangkan hemoragi merupakan kondisi terjadinya pendarahan pada pembuluh darah.



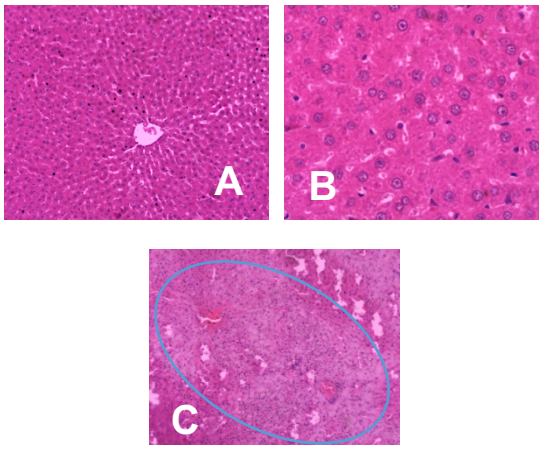
Gambar 4. Gambaran histologi hati dengan pemberian NaCMC 1% memperlihatkan sel hati dengan strultur yang normal pada perbesaran A (100x) dan B (400x).



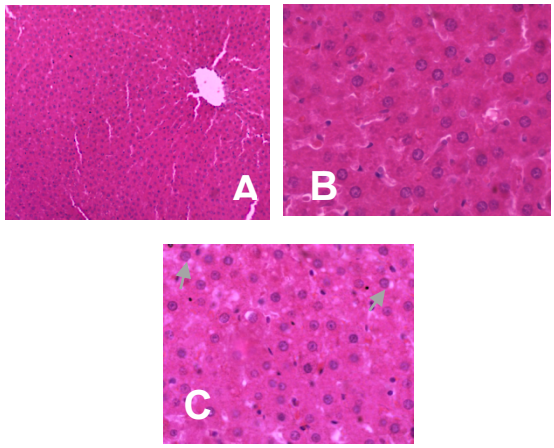
dengan pemberian minyak jintan hitam 1 ml/kg BB dan suspensi levofloksasin memperlihatkan pada perbesaran 100x (A) dan 400x (B) jaringan hati mengalami degenerasi hidropis (panah orange) dan degenerasi lipid (panah hijau).



dengan pemberian suspensi levofloksasin pada pengamatan memperlihatkan pada perbesaran 100x (A dan B) jaringan hati mengalami pendarahan (panah kuning), infiltrasi sel inflamasi (panah merah) dan kongesti (panah biru). Pada perbesaran 400x (C dan D) gambaran histologi hati memperlihatkan adanya degenerasi hidropis (panah orange), vakuolisasi (panah abu) dan area nekrosis (D).



Gambar 7. Gambaran histologi hati kelompok IV dengan pemberian minyak jintan hitam 2 ml/kg BB dan suspensi levofloksasin memperlihatkan pada perbesaran 100x (A) dan 400x (B) jaringan hati terlihat normal dan mengalami degenerasi (C).

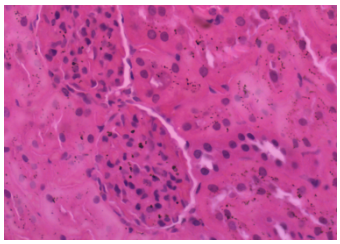


Gambar 8. Gambaran histologi hati kelompok V dengan pemberian minyak jintan hitam 4 ml/kg BB dan suspensi levofloksasin memperlihatkan pada perbesaran 100x (A) dan 400x (B) jaringan hati terlihat normal dan mengalami degenerasi lipid (C).

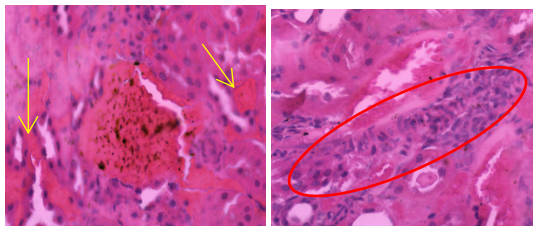
Pada kelompok III (gambar 6) yang diberi minyak jintan hitam 1 ml/kg BB dan suspensi levofloksasin, hasil pengamatan histologis menunjukkan tingkat kerusakan yang ringan di mana perubahan histologis hanya mempengaruhi 11 – 20% dari area yang diamati pada perbesaran 400x. Pada kelompok IV yang diberi minyak jintan hitam 2 ml/kg BB dan suspensi levofloksasin, secara umum hasil pengamatan histologis menunjukkan jaringan hati yang normal walaupun terdapat 1 tikus yang memiliki jaringan hati yang mengalami degenerasi (gambar 7). Kemudian pada kelompok V yang diberi minyak jintan hitam 4 ml/kg BB dan suspensi levofloksasin, secara umum hasil pengamatan histologis menunjukkan jaringan hati yang terlihat normal walaupun terdapat 1 tikus yang memiliki jaringan hati yang menunjukkan degenerasi lipid (gambar 8).

Kerusakan hepar secara histologi ditandai dengan adanya perubahan seluler, berupa perubahan reversibel dan ireversibel. Pola kerusakan sel reversibel dapat diamati melalui pemeriksaan mikroskopik berupa pembengkakan sel (degenerasi hidropik) dan perlemakan (steatosis) (3).

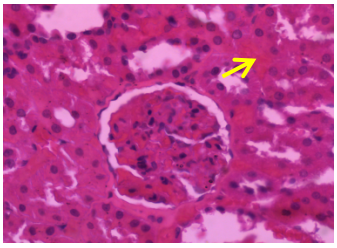
4. Histopatologi Ginjal



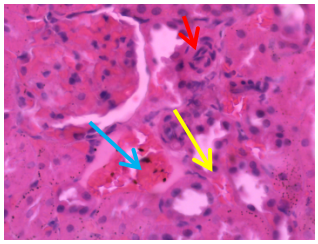
Gambar 9. Gambaran histologi ginjal dengan pemberian NaCMC 1% memperlihatkan strultur yang normal pada perbesaran 400x



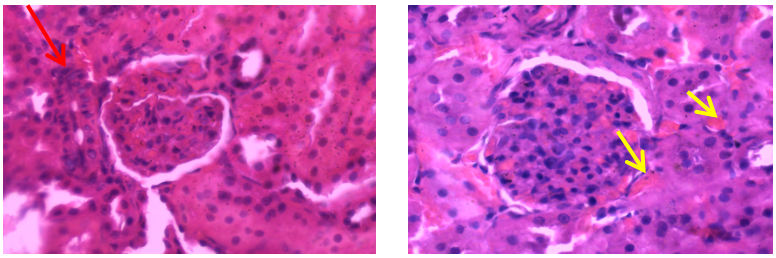
Gambar 10. Gambaran histologi ginjal kelompok II dengan pemberian suspensi levofloksasin pada pengamatan perbesaran 400x memperlihatkan jaringan hati mengalami pendarahan (panah kuning), area inflamasi (merah) dan kongesti (panah biru).



Gambar 11. Gambaran histologi ginjal kelompok III dengan pemberian minyak jintan hitam 1 ml/kg BB dan suspensi levofloksasin memperlihatkan pada perbesaran 400x jaringan ginjal mengalami hemoragi (panah kuning).



Gambar 12. Gambaran histologi ginjal kelompok IV dengan pemberian minyak jintan hitam 2 ml/kg BB dan suspensi levofloksasin memperlihatkan pada perbesaran 400x jaringan ginjal mengalami hemoragi (panah kuning), inflamasi (panah merah) dan kongesti (panah biru).



Gambar 13. Gambaran histologi ginjal kelompok V dengan pemberian minyak jintan hitam 4 ml/kg BB dan suspensi levofloksasin memperlihatkan pada perbesaran 400x jaringan ginjal mengalami hemoragi (panah kuning) dan inflamasi (panah merah).

Hasil pengamatan terhadap histopatologi ginjal menunjukkan pada profil pengamatan yang mewakili kelompok I (kontrol sehat) memperlihatkan beberapa hasil yang normal dan kerusakan minimal (gambar 9). Sedangkan pada kelompok II (gambar 10) yang diberikan suspensi levofloksasin menunjukkan terjadinya kerusakan yang parah yang mempengaruhi 41 – 100% area pengamatan pada perbesaran 400x dengan tampaknya kerusakan seperti terjadi inflamasi, hemoragi, kongesti. Profil pegamatan yang mewakili kelompok III (gambar 11) yang diberi minyak jintan hitam 1 ml/kg BB dan suspensi levofloksasin, menunjukkan tingkat kerusakan yang ringan di mana perubahan histologis nampak hanya mempengaruhi <11 - 20% area yang diamati pada perbesaran 400x. Pada kelompok IV (gambar 12) yang diberi minyak jintan hitam 2 ml/kg BB dan suspensi levofloksasin, secara umum hasil pengamatan histologis menunjukkan kerusakan yang sedang dengan mempengaruhi 21 – 40% area pengamatan pada perbesaran 400x. Kemudian pada kelompok V (gambar 13) yang diberi minyak jintan hitam 4 ml/kg BB dan suspensi levofloksasin, hasil pengamatan histologis yang mewakili kelompok V menunjukkan jaringan ginjal yang memperlihatkan kerusakan sedang yang mempengaruhi 21 – 40% area pengamatan pada perbesaran 400x. pada kelompok V nampak luasnya area dengan hemoragi maupun inflamasi.

Hepatotoksisitas dan nefrotoksitas dapat disebabkan oleh beberapa faktor dan penyebab utamanya adalah penggunaan obat. Hal ini berhubungan dengan fungsi hati dalam memetabolisme obat dan agen kimia lainnnya

(4). Mekanisme levofloksasin dalam menyebabkan hepatotoksisitas dan nefrotoksisitas belum diketahui secara jelas, namun efek toksisitas yang disebabkan oleh obat golongan fluorokuinolon secara umum disebabkan oleh pembentukan radikal bebas di hati.

Berdasarkan hasil yang diperoleh baik pada pemeriksaan biomarker fungsi hati dan ginjal serta pengamatan histologi dapat dijelaskan bahwa minyak jintan hitam cukup efektif dalam melindungi dan mengurangi hepatotoksisitas dan neftoksitas yang disebabkan oleh obat levofloksasin. Hal ini sesuai dengan beberapa hasil penelitian sebelumnya (5,6) yang menyatakan bahwa pemberian minyak jintan hitam menunjukkan aktivitas hepatoprotektif dan nefroprotektif pada beberapa induksi obat. Hal ini dikarenakan minyak jintan hitam memiliki sifat sebagai antioksidan dengan kandungan utama thymoquinon yang bertindak dalam melawan radikal bebas yang disebabkan oleh obat golongan fluorokuinolon salah satunya adalah levofloksasin.

KESIMPULAN

1. Minyak jintan hitam secara umum dapat mencegah peningkatan biomarker fungsi hati (AST, ALT dan GGT) serta biomarker fungsi ginjal (ureum dan kreatinin) secara drastis, terutama dengan dosis 1 ml/kg BB pada biomarker fungsi hati dan dosis 1 ml/kg dan 4 ml/kg bb untuk pemeriksaan biomarker fungsi ginjal pada tikus yang diinduksi levofloksasin.
2. Minyak jintan hitam dengan konsentrasi 1, 2 dan 4 ml/kg BB ditemukan dapat mengurangi kerusakan histologi hati dan ginjal yang disebabkan oleh induksi levofloksasin, terutama pada konsentrasi 2 dan 4 ml/kg BB pada hati dan konsentrasi 1 ml/kg BB pada ginjal.

D. **STATUS LUARAN**: Tuliskan jenis, identitas dan status ketercapaian setiap luaran wajib dan luaran tambahan (jika ada) yang dijanjikan. Jenis luaran dapat berupa publikasi, perolehan kekayaan intelektual, hasil pengujian atau luaran lainnya yang telah dijanjikan pada proposal. Uraian status luaran harus didukung dengan bukti kemajuan ketercapaian luaran sesuai dengan luaran yang dijanjikan. Lengkapi isian jenis luaran yang dijanjikan serta mengunggah bukti dokumen ketercapaian luaran wajib dan luaran tambahan melalui Simlitabmas.

Penelitian ini merupakan penelitian dasar TKT 2, hasil penelitian telah dipresentasikan di The 4th International Conference on Science (ICOS) tgl 22-23 Agustus 2020 dan manuskrip telah disubmit dan direview untuk dipublikasikan dalam *Journal of Physics: Conference Series (JPCS)*(SCOPUS Q4)

E**. PERAN MITRA:** Tuliskan realisasi kerjasama dan kontribusi Mitra baik *in-kind* maupun *in-cash* (untuk Penelitian Terapan, Penelitian Pengembangan, PTUPT, PPUPT serta KRUPT). Bukti pendukung realisasi kerjasama dan realisasi kontribusi mitra dilaporkan sesuai dengan kondisi yang sebenarnya. Bukti dokumen realisasi kerjasama dengan Mitra diunggah melalui Simlitabmas.

Tidak ada mitra

F. **KENDALA PELAKSANAAN PENELITIAN**: Tuliskan kesulitan atau hambatan yang dihadapi selama melakukan penelitian dan mencapai luaran yang dijanjikan, termasuk penjelasan jika pelaksanaan penelitian dan luaran penelitian tidak sesuai dengan yang direncanakan atau dijanjikan.

Tidak ada kendala yang berarti selama melaksanakan penelitian. Namun, dengan dana yang diperoleh (sekitar 25 juta) maka penelitian tidak dapat mengukur banyak variabel biomarker, sehingga mungkin saja akan berdampak pada *acceptance* jurnal internasional yang ditargetkan. Saat ini hasil penelitian telah disubmit dan direview untuk dipublikasikan dalam prosiding internasional IOP *Journal of Physics: Conference Series (JPCS)*

G**. RENCANA TAHAPAN SELANJUTNYA:** Tuliskan dan uraikan rencana penelitian di tahun berikutnya berdasarkan indikator luaran yang telah dicapai, rencana realisasi luaran wajib yang dijanjikan dan tambahan (jika ada) di tahun berikutnya serta *roadmap* penelitian keseluruhan. Pada bagian ini

diperbolehkan untuk melengkapi penjelasan dari setiap tahapan dalam metoda yang akan direncanakan termasuk jadwal berkaitan dengan strategi untuk mencapai luaran seperti yang telah dijanjikan dalam proposal. Jika diperlukan, penjelasan dapat juga dilengkapi dengan gambar, tabel, diagram, serta pustaka yang relevan. Jika laporan kemajuan merupakan laporan pelaksanaan tahun terakhir, pada bagian ini dapat dituliskan rencana penyelesaian target yang belum tercapai.

Penelitian telah selesai dilakukan dan telah dibuat manuskrip yang telah dipresentasikan pada conference international the 4th ICOS 2020.

H. DAFTAR PUSTAKA: Penyusunan Daftar Pustaka berdasarkan sistem nomor sesuai dengan urutan pengutipan. Hanya pustaka yang disitasi pada laporan kemajuan yang dicantumkan dalam Daftar Pustaka.

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Dokumen pendukung luaran Wajib #1

Luaran dijanjikan: Artikel di Jurnal Internasional Terindeks di Pengindeks Bereputasi

Target: Accepted
Dicapai: Sedang direview

Dokumen wajib diunggah:
1. Naskah artikel
2. Bukti sedang direview

Dokumen sudah diunggah:
1. Naskah artikel
2. Bukti sedang direview

Dokumen belum diunggah:
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Judul artikel: The Protective Effect of Black Seed Oil (Nigella sativa L.) Against Liver Dysfunction Due to Levofloxacin Use in Rats

The Protective Effect of Black Seed Oil (*Nigella sativa L.*) Against Liver Dysfunction Due to Levofloxacin Use in Rats

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Abstract. Levofloxacin is a fluoroquinolone antibiotic which is the first choice of long-term MDR-TB therapy. The use of long periods can cause interference and damage to the liver. The existence of liver disorders is characterized by an increase in the level of AST and ALT. This study aimed to determine the effect of black seed oil on AST and ALT levels after subacute administration of levofloxacin in rats. Fifteen rats were divided into 5 groups. Group I served as healthy control was only given 1% NaCMC suspension, group II was given levofloxacin suspension, while groups III, IV, and V were given black seed oil 1 ml, 2 ml, 4 ml/kg body weight, respectively, and after 2 hours, rats were given levofloxacin suspension. Treatment was given for 21 days orally. Blood sampling was carried out before treatment and 24 hours following the last treatment using a photometric method. The results showed that the administration of levofloxacin for 21 days increased the ALT and AST level for a minimum of 16.8%. The treatment with black seed oil, especially at 2 ml/kg was able to decrease the level of ALT for 43% compared to the placebo group ($p<0.05$). The AST levels with black seed oil in all doses were also reduced by 16% compared to the placebo group, but it was not statistically significant. It can be concluded that the administration of black seed oil, especially at a concentration of 2 ml/kg, can reduce liver dysfunction due to levofloxacin use in rats.

1. Introduction

Tuberculosis (TB) is an infectious disease that has become a major problem in the world, including Indonesia. Tuberculosis infection in 34 Indonesian provinces has reached 360,770 cases in 2017 and increased to 511,873 cases in 2018 [1]. Furthermore, World Health Organization annual report (2017) has ranked Indonesia as a second country with the most multidrug-resistant tuberculosis (MDR-TB) cases in Southeast Asia after India [2]. It appears that the incidence of MDR-TB in Indonesia increases every year. In 2012, there were 696 MDR-TB cases found in Indonesia, and after 3 years, this number increased exponentially to 1,860 confirmed and 15,380 suspected MDR-TB cases [3].

Treatment of MDR-TB is more difficult when compared to the treatment of TB sensitive. The success of the MDR-TB treatment is dependent on how quickly the cases are identified and the availability of the MDR-TB treatment. Drugs used for the MDR-TB are the second-line anti tuberculosis (AT) drugs, which have more potential toxicity than the first-line drugs [4]. These include levofloxacin antibiotic from the fluoroquinolone group. Fluoroquinolone induces hepatotoxicity characterized by elevated

levels of alanine transferase (ALT) and aspartate aminotransferase (AST) [5]. A retrospective data with 746 subjects showed an increase in the incidence of hepatotoxicity during fluoroquinolone use, especially levofloxacin and moxifloxacin [6]. Cases of hepatotoxicity have also been reported in a number of patients who received levofloxacin in the management of MDR-TB [7]. In animal model, levofloxacin toxicity can be found using different doses, from 5 to 20 mg/kg of rat body weight [8].

The black seed oil contains thymoquinone, an active substance that protect the liver function against hepatotoxicity [9]. It has been shown that the black seed oil extract is nontoxic and safe for consumption [10]. The chronic toxicity test with an oral dose of 2 ml/kg of black seed oil for 12 days did not cause alterations in hepatic enzyme levels (ALT, AST) [11]. The oral use of the black seed oil 2 ml/kg even shown to reduce the AST and ALT levels against Thioacetamide-induced hepato-renal toxicity in rats [12]. Black seed oil has also been found to have a hepatoprotective effect in rats induced by isoniazid [13]. Therefore, the aim of this study was to determine the effect of black seed oil on AST and ALT levels after a subacute administration of levofloxacin in rats.

2. Material and Methods

2.1. Preparation of Experimental Animal

Fifteen male rats weighed 200 – 300 g were used in the study. The animals were provided standard pellets and drinking water 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.

2.2. Preparation of levofloxacin and black seed oil

The dose of levofloxacin used in this study was based on the human dose and converted to rat body weight (93 mg/kg). The levofloxacin powder was suspended in 1% sodium carboxymethyl cellulose (Na CMC) suspension. Black seed oil was prepared in 3 different concentrations, i.e. 10%, 20%, and 40% in corn oil and the volume of administration was 1 ml/200 g rats. This equivalent to black seed dose of 1 ml/kg, 2 ml/kg and 4 ml/kg, respectively.

2.3. Experimental Protocol

The study was carried out using 15 male rats, which were divided into 5 groups consisted of 3 rats: Group I was given a 1% NaCMC suspension, which is a neutral suspending agent that acts as vehicle in this study. Group II was given levofloxacin suspension at a dose of 93 mg/kg with a volume of 1 mL/100 g. Group III, IV and V was given black seed oil with the dose of 0.1 ml/kg, 0.2 ml/kg, and 0.4 ml/kg, respectively before treated with levofloxacin suspension (2 hours interval from black seed oil administration).

2.4. ALT and AST Measurement

As much as 2 ml blood samples were taken before (day 0) and after treatment (day 21). The blood samples were centrifuged at 3000 rpm for 20 minutes to separate serum from the whole blood cells. Measurements were made using ALT and AST (Human®) reagent kits. The blood plasma (100 µl) was mixed with 1000 µl buffer then homogenized and incubated for 5 minutes at 37°C. After incubation, 250 μl of substrate kit was added, homogenized and re-incubated for 1 minute at 37°C. The ALT or AST level was measured using a humalyzer 3500 (Human®) at 340 nm.

2.5. Statistical Analysis

Data is presented in mean ± SD. Statistical analysis was performed using SPSS 26. The normal distribution of data was determined using Shapiro-Wilk analysis. Analysis was continued with one-way ANOVA followed by Tukey's HSD test to see significant differences between groups. Statistical significance is achieved if $p<0.05$.

3. Results and Discussion

The concentration of AST and ALT plasma before treatments are presented in table 1. There was no significant difference found between groups prior to treatments, indicating a similar baseline levels between groups.

Table. 1 The baseline AST and ALT levels before treatments in the experimental animals

Group	Treatment	Baseline	
		AST (U/L)	ALT (U/L)
I	1% Na CMC	85 ± 23	59 ± 33
II	Levo suspension	129 ± 41	77 ± 28
III	BS oil 1 ml/kg + Levo	106 ± 38	79 ± 6
IV	BS oil 2 ml/kg + Levo	134 ± 6	78 ± 11
V	BS oil 4 ml/kg + Levo	107 ± 35	68 ± 10

BS: black seed; Levo: levofloxacin

The results shown that the use of levofloxacin (93 mg/kg) increased the AST level from 129 U/L to 162 U/L after treatment in rats. An increase in AST level may indicate an alteration in the liver function or even cell damage. Levofloxacin treatment induced an increase in the level of AST around 25% from its baseline and significantly higher than the group I (normal control) after treatment (see Figure 1). The administration of black seed oil led to a maintenance of the AST level in some animals, but others still experienced an increase in AST levels. However, when compared to group II, the post treatment AST levels in group III, IV and V were somewhat lower (around 13-16%) and did not reach significant different from the normal controls.

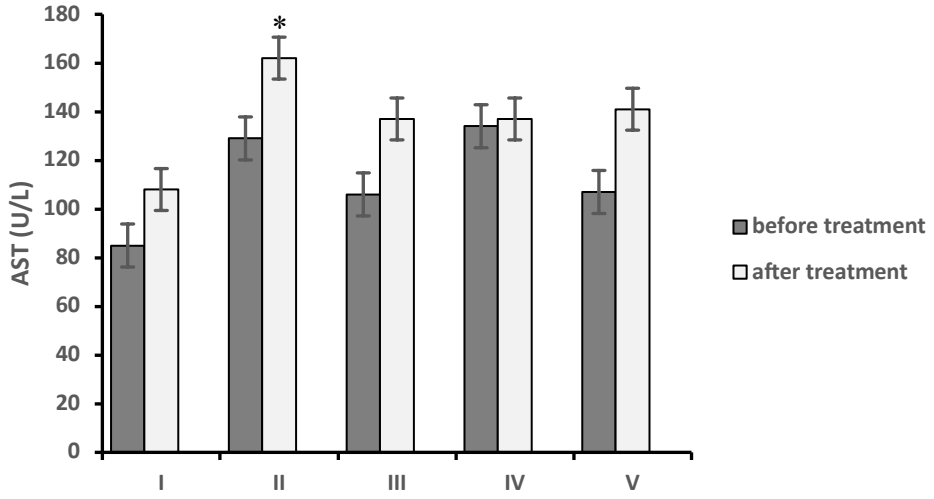

Figure 1. The comparison of AST level for each group before and after treatment. *p<0.05 compared to group I after treatment. I: 1% Na CMC; II: Levofloxacin; III: Black seed oil 1 ml/kg + Levofloxacin; IV: Black seed oil 2 ml/kg + Levofloxacin; V: Black seed oil 4 ml/kg + Levofloxacin.

The administration of levofloxacin in the dose of 93 mg/kg in rats also showed an elevation in the ALT level from 77 to 90 U/L. As seen in Figure 2, the increase in ALT level in group II was significantly different from the normal control ($p<0.05$). The administration of 1 and 2 ml/kg body weight of black seed oil (group III and IV) showed significantly lower ALT values compared to the levofloxacin group (group II) and was similar to the normal value (Group I). Group V with 4 ml/kg black seed oil was also

able to maintain the ALT level of levofloxacin treatment, although when compared with one-way anova analysis, it was not significantly different from the levofloxacin group (Figure 2).

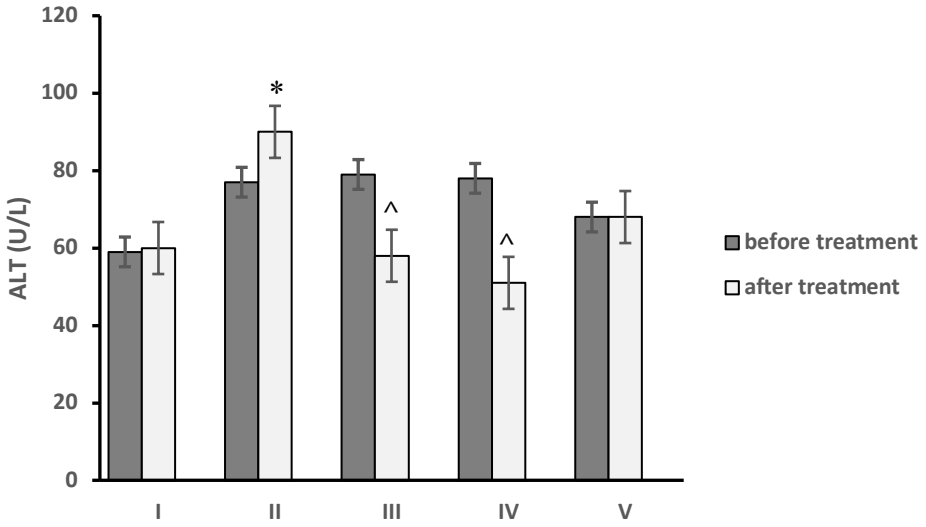

Figure 2. The comparison of ALT level for each group before and after treatment. *p<0.05 compared to group I after treatment, ^p<0.05 compared to group II after treatment; I: 1% Na CMC; II: Levofloxacin; III: Black seed oil 1 ml/kg + Levofloxacin; IV: Black seed oil 2 ml/kg + Levofloxacin; V: Black seed oil 4 ml/kg + Levofloxacin.

Liver damage can be caused by many factors and drugs has become one of the primary risk factors since liver is the main organ that metabolizes drugs and other chemical agents [14]. Levofloxacin induces damage on liver tissue may manifest from asymptomatic elevation of liver enzymes to liver failure [15]. Specific mechanisms of how levofloxacin can induce hepatotoxicity have not been reported yet, but it generally involves oxidative radical production in the liver. Black seed oil through its antioxidant properties can enhance the antioxidant defense system and the elimination of oxidative radicals, which is important to prevent cellular damage. In this study, the use of black cumin oil with a dose of 1 ml and 2 ml/kg bw can significantly reduce the ALT and AST levels. A previous study using 1 ml and 2 ml/kg bw black cumin oil has been also shown to provide hepatoprotective effects in rats induced by cisplatin [16]. Presumably, the result of this study may support the use of black cumin oil as an alternative hepatoprotector in patients who are required to use levofloxacin in a long-term.

4. Conclusion

Black seed oil at the dose of 2 ml/kg may improve liver dysfunction due to levofloxacin administration in experimental animals shown by a decrease in the AST and ALT levels following 21 days of treatment.

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Research Paper

Black Seed Oil Protects Against Levofloxacin Hepatotoxicity: Analyses of the Biochemical and Histopathological Effects

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ABSTRACT

Background: Long-term use of levofloxacin can cause alterations in the liver function. This study aimed to determine the protective effect of black seed oil (BSO) against liver injury due to levofloxacin administration in rats.

Methods: The chemical composition of BSO was analyzed with gas chromatography and mass spectrophotometry (GC-MS). Rats (n=30) were treated daily with levofloxacin and BSO at three doses (1, 2 or 4 mL/kg) orally for 28 days. The presence of liver injury was determined based on serum biomarkers and liver malondialdehyde (MDA) levels, and histopathological examinations.

Results: The GC-MS analyses showed that BSO contained 25 chemical compounds, including thymoquinone (10.14%). The levofloxacin administration significantly increased the liver enzymes and MDA levels, and induced a marked alteration in the liver histological structures. Treatments of rats with one or two mL/kg BSO significantly decreased the liver enzymes, and MDA levels compared to those that received levofloxacin alone ($P<0.05$). However, the highest dose (4 mL/kg) BSO failed to improve liver MDA levels. The recovery of liver histological damages was also observed in rats treated with BSO.

Conclusion: It was concluded that the BSO administration reduced the liver dysfunction due to levofloxacin at doses of 1 or 2 mL/kg, but not at 4 mL/kg. Further research is warranted to explore if the protective effect of BSO is associated with its antioxidant properties.

Keywords: Black seed oil, Histopathology, Levofloxacin, Liver injury, *Nigella Sativa*

Introduction

The eradication of tuberculosis is challenged by a rise in the number of multidrug-resistant tuberculosis (MDR-TB) cases worldwide [1]. Levofloxacin, an antibiotic from the fluoroquinolone group, is one of the most common drugs used for MDR-TB cases [2]. This drug inhibits DNA supercoiling in *Mycobacterium tuberculosis*, thus damaging the DNA replication by interfering with its gyrase activity [3]. However, levofloxacin may induce hepatotoxicity characterized by elevated liver enzymes, i.e. alanine and aspartate aminotransferases (ALT & AST) levels [4]. The hepatotoxicity secondary to levofloxacin administration may occur after 5-14 days of the

therapy initiation [5, 6]. Cases of hepatotoxicity have been reported in a number of patients who received levofloxacin for the management of their MDR-TB [7]. In this context, retrospective data from 746 patients have demonstrated an increase in the incidence of hepatotoxicity during treatment with fluoroquinolone drugs, especially levofloxacin and moxifloxacin [8].

The hepatotoxicity management remains limited due to lack of approved drugs with adequate hepatoprotective properties. Studies have reported the beneficial effects of black seed oil (BSO) extracted from the plant, *Nigella sativa* , on the prevention of drug-induced liver injury [9]. This oil has been shown to contain several bioactive compounds, including thymoquinone, thymohydroquinone, dithymoquinone, p-cymene, carvacrol, 4-terpineol, and t-anethol [10]. Thymoquinone has been demonstrated to inhibit oxidative stress by increasing the activity of the antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase, and reducing the lipid peroxidase activity [11]. Since the toxicity by levofloxacin occurs mainly due to diminished glutathione supplies [12], strengthening the body's defense system with antioxidants would be essential to overcome the oxidative stress-related cellular damages. This study was planned to investigate the antitoxic effect of black seed oil on the liver function in rats after levofloxacin administration at subacute doses.

Materials and Methods

Chemicals and drugs: The black seed oil (BSO) was obtained from black cumin seed (*Nigellasativa*) purchased from Al-waqia'ah supplier (Makassar, Indonesia). Levofloxacin tablets (Hexpharm Jaya®) were obtained from a licensed pharmaceutical store in Makassar, Indonesia. Thiobarbituric acid (TBA, Sigma-Aldrich), 1,1,3,3-tetra methoxy propane (TMP, Sigma-Aldrich), and Trichloroacetic Acid (TCA, Merck) were ordered via the official suppliers in Indonesia. The diagnostic kits for AST, ALT, and GGT were obtained from Human Diagnostics Worldwide (Wiesbaden, Germany).

Chemical constituents analyses: Prior to the in vivo experiments, a laboratory analysis was conducted to identify the chemical constituents of the BSO samples used in this study. The analysis was performed using a Trace 1310 gas chromatography with TSQ 8000 Evo mass spectrometry (Thermo Scientific; Mundelein, IL, USA). The column size was 20 mm x 0.18 mm (TG-5MS) with helium gas used as the carrier. The oven had an initial and final temperature of 50°C and 330°C, respectively, at an increasing rate of 10 to 25°C/minutes.

Preparation of animals: The experimental animals used in this study were 30 male albino rats (*Rattus norvegicus*) at an average weight of 200-300 g each. They were acclimatized to the laboratory environment for 14 days prior to the treatments. The rats had free access to standard food pellets and drinking water throughout the study. The animal care protocol used was based on the institution's animal care standards, which had already been granted an ethical clearance (318/UN4.6.4.5.31/PP36/2020).

Preparation of Levofloxacin and Black Seed Oil: The levofloxacin dosage used in this study was based on that for humans (15 mg/kg/body weight/day), which was converted to animal dosage as described by Nair and Jacob's guideline [13]. Accordingly, the levofloxacin dose was 93 mg/kg per rat. At this dose, levofloxacin has previously been shown to sufficiently induce renal toxicity in rats [14]. The levofloxacin powder was prepared as suspension, using 1% sodium carboxymethyl cellulose (Na-CMC) immediately before administration. The BSO was diluted in corn oil at three different concentrations, i.e.10%, 20%, and 40%, and the volume of administration was one mL/200g per rat's body weight. These concentrations were equivalent to the BSO doses of one, two or four mL/kg, respectively.

Experimental protocol: The animals were divided into five groups of six each as follows: 1) healthy controls, 2) levofloxacin group (rats received levofloxacin suspension and corn oil as a placebo), and, 3, 4, 5) treatment groups that received BSO at either of three doses of one, two or four mL/kg body weight. The BSO treatment was given two hours before the daily levofloxacin administration for 28 days. The blood samples were collected one day before starting the study (day 0) and a day after the last treatment (day 29). Following the final blood sampling, a necropsy was performed to harvest the rats' livers for further analyses. The right lobe of the liver was fixed in 10% formaldehyde diluted in phosphate buffered saline (PBS) for histopathological examination. The left lobe was immersed in liquid nitrogen and stored in a freezer at minus 20°C for malondialdehyde (MDA) analysis.

Serum biomarkers analyses: The collected blood samples were centrifuged at 3000 rpm for 20 minutes. Next, we analyzed the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT) according to the kits' instructions provided by the supplier. A 100 µl aliquot of each blood plasma was mixed with 1000 µl PBS, homogenized, and incubated for five minutes at

37°C. After the incubation, 250 µl of the substrate kit was added, homogenized, and re-incubated for one minute at 37°C. All of the serum biomarker levels were measured at 340 nm, using Humalyzer 3500 (Hamburg, Germany).

Liver Malondialdehyde Analyses: The MDA analysis was carried out according to the method described by a previous study [15]. A rat liver sample, weighing 400mg, was ground in a mortar and pestle, and homogenized in PBS at pH 7.4. The homogenate was centrifuged at 3000 rpm for 10 minutes. A 0.5 mL supernatant from each homogenate was mixed with 1 mL of 1% TBA and 1 mL of 10% TCA before being placed in a water bath at 90°C for 20 minutes. The absorbance was then measured at 531 nm, using a UV-visible spectrophotometer.

Histopathological examinations: After 48-hour storage in formaldehyde, the liver specimens were cut into 0.5-1cm thickness, stored in embedding cassettes, and homogenized in a tissue processor (Thermo Scientific; Bedford, MA, USA). The specimens were embedded in paraffin blocks and sliced into 4-5 µm thickness, using a microtome, then floated on a warm water bath at 40°C. The specimens were placed on glass slides and dried, using an electric hotplate for at least 2 hours before being stained with hematoxylin and eosin (H&E) and then covered with glass slips. The histopathological examination was conducted under a light microscope (Olympus; Tokyo, Japan) equipped with a Nikon camera. The photomicrographs were taken at 100X and 400X magnifications. The liver histological damages were examined independently by two expert murine pathologists who were blinded to the animal grouping.

The antioxidative activity of the BSO samples was tested against radicals, using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH). The half maximal inhibitory concentration (IC_{50}) values were determined by measuring the absorbance of a series of BSO concentrations, using a UV-visible spectrophotometer at 515nm. The IC_{50} values were then plotted in the form of a concentration-response curve.

Statistical analyses: The normal distribution of data was determined using Shapiro-Wilk's and one-way ANOVA analyses, and finally by Tukey's HSD test to determine significant differences among the groups. The data were presented as the means±standard deviations. The level of statistical significance was set at $P<0.05$.

Results

Chemical Constituents: The gas chromatography and mass spectrophotometry (GC-MS) analyses of BSO revealed 25 peaks on the chromatogram, suggestive of 25 volatile chemical compounds being pres-

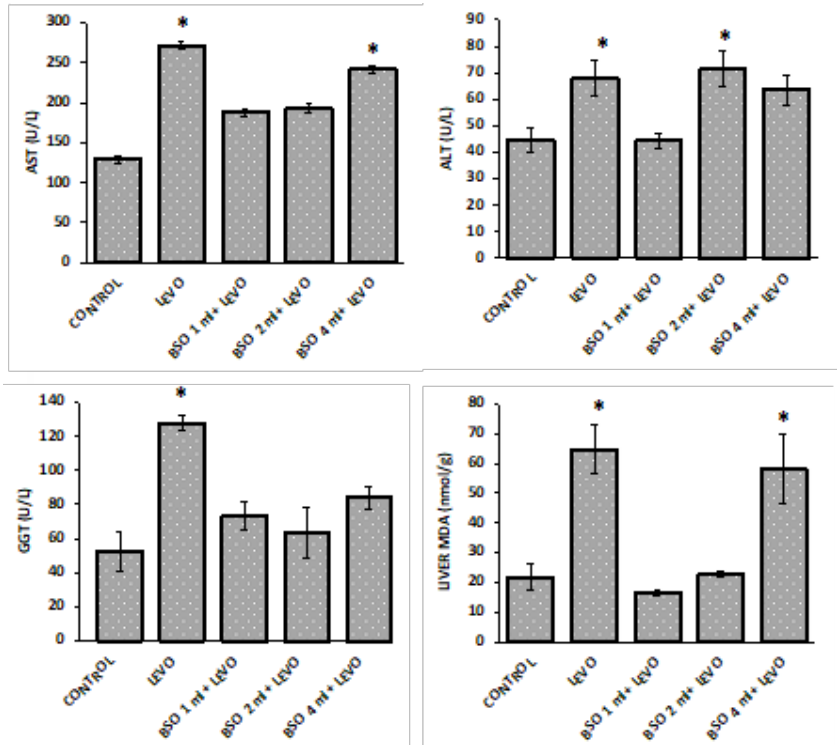

Figure 1. Comparison of liver biomarker levels among the rat groups after treatments

*P<0.05 compared to the control group.

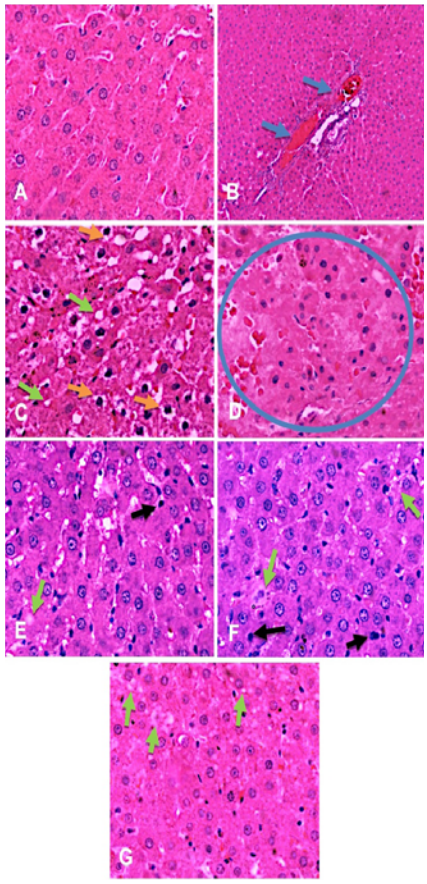

Figure 2. Comparison of rats' liver microphotographs after 28 days of exposure to different treatments

A: Control; B-D: Levofloxacin; E: Black seed oil 1 mL/kg; F: Black seed oil 2 mL/kg; G: Black seed oil 4 mL/kg. Congestion (blue arrow), fatty degeneration and vacuolization (green arrow), hydropic and ballooning degeneration (yellow arrow), necrotic cells (black arrow), and areas of necrosis (blue circle). H&E staining at 100x and 400x magnification.

ent in BSO (Table 1). These tests revealed six major constituents as follows: a) 9,12-octadecadienoic acid (Z, Z) (44.94%); b) bicyclo (3.1.0) hex-2-ene, 4-methyl-1-(1-methylethyl) (10.23%); c) thymoquinone (10.14%); d) o-Cymene (9.05%); e) n-hexadecanoic acid (8.25%); and f) 9,12-Octadecadienoic acid (Z, Z)-2,3-dihydroxypropyl ester (6.95%).

Liver Biomarkers: The liver enzymes, such as AST, ALT, and GGT, were significantly elevated after the rats received levofloxacin (93 mg/kg) for 28 days ($P<0.05$; Figure 1). The elevations of the liver enzymes suggest the occurrence of damages to the hepatocytes. Simultaneously, the MDA levels in the liver tissue samples also increased by approximately 3-fold compared to those of the controls ($P<0.05$). Compared to the levofloxacin group, pre-treatment with black seed oil at 1 mL/kg induced a substantial decrease in the liver biomarkers' levels ($P<0.05$; Figure 1). The reductions in the MDA levels were not dose-dependent. Compared to the 1 mL/kg dose, the administration of BSO at 2 mL/kg reduced the AST, GGT, and MDA levels but did not change the ALT levels. Interestingly, the liver biomarkers were not reduced, in the groups treated with BSO at 4 mL/kg.

Histopathological Analyses: Figure 2 represents the results of the histopathological observations under light microscopy after H&E staining. In the normal controls, the rat liver samples showed normal cellular architecture with no or minimal damages. In contrast, the liver tissue samples from the rats treated with levofloxacin but without BSO were characterized by large areas of hydropic degeneration, ballooning hepatocytes, lipid degeneration, vacuolization, congestion and signs of hemorrhage (Figures 2B & 2C). The histological damages were diffuse and mostly evident in over 50% of the observed areas at 400x magnification (Figure 2D).

In general, the group that was pre-treated with BSO at 1 mL/kg showed minimal damages, with the cells demonstrating lipid degeneration and necrosis (Figure 2E). These histopathological changes only affected a

Table 1. Chemical compounds found in the black seed oil based on GC-MS analysis

No	RT.	Molecular Formula	Compound Title**	% Area
1	4.00	$C_{10}H_{16}$	Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methyl ethyl)-	10.23
2	4.89	$C_{10}H_{14}$	o-Cymene	9.05
3	5.58	$C_{11}H_{20}O$	cis-4-methoxy thujane	3.19
4	6.00	$C_{10}H_{18}O$	3-Cyclohexen-1-ol,4-methyl-1-(1-methylethyl)-, (R)-	0.80
5	6.16	$C_{10}H_{16}O$	1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-	0.53
6	6.50	$C_{10}H_{12}O_2$	Thymoquinone	10.14
7	7.29	$C_{15}H_{24}$	Tricyclo [5.4.0.0 (2,8)] undec-9-ene	0.55
8	7.74	$C_{15}H_{24}$	Longifolene	1.81
9	8.60	$C_{15}H_{24}O$	Phenol, 4-methoxy-2,3,6-trimethyl-	1.24
10	8.86	$C_{14}H_{26}O$	7-Tetradecenal, (Z)-	0.08
11	9.68	$C_{15}H_{26}O$	Humulene-1,6-dien-3-ol	0.09
12	9.92	$C_{12}H_{20}O$	4,8-Decadienal,5,9-dimethyl-	0.69
13	10.17	$C_{16}H_{32}O_2$	Butyric acid, dodecyl ester	0.07
14	11.42	$C_{16}H_{32}O_2$	n-Hexadecanoic acid	8.25
15	12.76	$C_{18}H_{32}O_2$	9,12-Octadecadienoic acid (Z,Z)-	44.94
16	16.36	$C_{27}H_{56}O_4Si_2$	9-Octadecatrienoic acid, 2-[trimethylsilyl)oxy]-1-[(trimethylsilyl) oxymethyl] ethyl ester	0.32
17	16.67	$C_{12}H_{38}O_4$	9,12-Octadecadienoic acid (Z,Z)-2,3-dihydroxy propyl ester	6.95
18	17.58	$C_{37}H_{76}O$	1-Heptatriacotanol	0.22
19	18.00	$C_{27}H_{54}O_4Si_2$	1-Monolinoleoylglycerol trimethylsilyl ether	0.20
20	18.47	$C_{20}H_{26}N_2O_2$	Dasycarpidan-1-methanol-acetate (ester)	0.13
21	18.91	$C_{27}H_{52}O_4Si_2$	9,12,15-Octadecatrienoic acid	0.06
22	19.12	$C_{27}H_{54}O_4Si_2$	1-Monolinoleoylglycerol trimethylsilyl ether	0.10
23	19.68	$C_{27}H_{54}O_4Si_2$	1-Monolinoleoylglycerol trimethylsilyl ether	0.07
24	20.62	$C_{27}H_{54}O_4Si_2$	1-Monolinoleoylglycerol trimethylsilyl ether	0.06
25	22.10	$C_{27}H_{54}O_4Si_2$	1-Monolinoleoylglycerol trimethylsilyl ether	0.23
		Total		100

small number of cells as observed under light microscopy at 400x magnification (Figure 2E). Similarly, most rats treated with BSO at 2 mL/kg also showed normal liver tissue features apart from a low number of cells with lipid degeneration and necrosis (Figure 2F). Meanwhile, the group treated with BSO at 4 mL/kg appeared to have minimal to mild liver damages (Figure 2G). The histopathological changes observed were mostly characterized as lipid degeneration, swollen cytoplasms, and vacuolization of hepatocytes due to lipid accumulations.

DPPH Scavenging Activity: The DPPH assay estimated the antioxidant activity of BSO by the mechanism associated with free radical scavenging. It was found

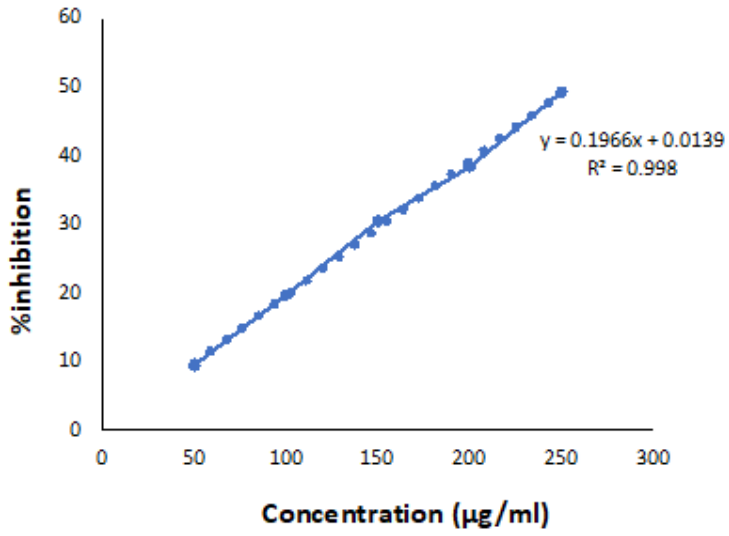

Figure 3. Dose-response plot of black seed oil free radical scavenging activity, using DPPH test

Based on the equation sown above, the IC_{50} of clove oil was less than 250 µg/mL.

that the IC_{50} of the BSO samples used was greater than 250 µg/mL based on the concentration-response curve as shown in Figure 3.

Discussion

Since the hepatotoxic side effects of levofloxacin might be serious or even life-threatening, it is imperative to explore therapeutic strategies aimed at preventing or minimizing the induced toxicity. Several studies have reported the beneficial effects of black seed oil (BSO) and some parts of *Nigellasativa* plant, suggestive of significant antioxidant and anti-inflammatory properties [16]. These therapeutic effects are likely to be very useful in protecting against levofloxacin-induced toxicity in the liver.

It has been reported that BSO may contain 22-38% fixed oil, including linoleic acid, oleic acid, tocopherols, retinol, carotenoids, thymoquinone, and about 0.40-1.50% volatile oil [17]. Among the essential oils, the main constituent is mostly thymoquinone (14-28%), a potent antioxidant and anti-inflammatory agent [17-19]. However, based on GC-MS analyses, the BSO sample used in this study had a lower thymoquinone content (10.14%) compared to those reported by earlier studies [17-19]. The thymoquinone content of BSO may vary based on the origin of the plants and the extraction method used. This may emphasize the need for standardizing the BSO products that are commercially available as food supplements.

In this study, BSO's protective effect on the liver was evaluated versus the levofloxacin's toxicity. Fluoroquinolone antibiotics, including levofloxacin, can have serious side effects since they cause permanent damage to the liver that may be fatal [20]. The incidences of levofloxacin induced-hepatotoxicity have been reported by many studies, which is characterized by increased levels of liver enzymes, such as AST, ALT, ALP, and bilirubin, diffuse hepatocellular necrosis, and intense cellular degeneration [4-6]. The results of this study demonstrated that daily administration of levofloxacin for 28 days caused marked elevations of AST, ALT, GGT and MDA levels. However, treatment with BSO at 1 mL/kg before levofloxacin administration halted the elevation of all liver injury biomarker levels, including the MDA.

MDA is a product of lipid peroxidation, hence, the surge in the liver MDA level is suggestive of increased lipid peroxidation in the rats' liver after levofloxacin administration. The hepatotoxic effect of drugs is often associated with oxidative stress, resulting from an imbalance between the antioxidant cellular system and the generation of reactive oxygen species [21]. Nevertheless, the BSO treatment only reduced the liver MDA levels at 1 or 2 mL/kg, but not at 4 mL/kg. This finding suggests that the hepatoprotective effect of BSO was dose-dependent.

To confirm the antioxidative effect of BSO, a DPPH assay was conducted. This compound is highly stable and reacts with antioxidants by accepting hydrogen atoms [22]. The IC_{50} value of the BSO in this study was higher than 250 µg/mL. Another study has shown

that the capability of BSO to trap radical DPPH may vary depending on its variety. For instance, BSO from Australia has an IC_{50} value of about 460 µg/mL, while the Turkish variety presents an IC_{50} of 515 µg/mL [18]. It is important to note that the antiradical activities of BSO can be influenced by a range of factors, including the plant's growth environment, seed and oil storage, and the extraction process. In this study, we did not directly compare the IC_{50} value of the BSO samples with other standard antioxidants. Obviously, it may be necessary to provide a quantitative basis for the BSO radical scavenging activity [23]. Further studies are warranted to clarify this matter.

The levofloxacin administration not only increased the liver enzyme levels, but also induced marked degenerations in the hepatocytes. Unlike other drug-induced hepatotoxicities where the inflammatory reaction is often evident in the liver tissues [24, 25], there was no inflammatory cell infiltration in the rats treated with levofloxacin. Instead, lipid and hydropic degenerations were the hallmarks of the histopathological changes observed in our study. A similar result has also been observed in mice treated with levofloxacin, where swollen hepatocytes, necrosis, vacuolization, and pyknosis have been the most evident changes in the liver [26].

With BSO treatment, the presence of histological damages in the levofloxacin-treated rats were markedly reduced at all given doses, especially at one or two mL/kg groups. This is consistent with the results of other studies that tested the hepatoprotective effects of BSO against paracetamol [27], carbon tetrachloride [28], and vitaminosis-induced toxicities [29]. The protective effects of *Nigella sativa* have been not only demonstrated in the liver, but also in other organs, including heart [30], gastrointestinal tract [31], and the kidneys [14]. Its antioxidant compound, i.e. thymoquinone, has been shown in an earlier study [32] to prevent a decline in the activity of liver antioxidants, such as superoxide dismutase, catalase, and glutathione peroxidase, and non-enzymatic antioxidants, such as Vitamins A, E, and C. Given the findings of this and other studies, the protective effect of black seed oil is likely to stem from its radical scavenging activity and inhibiting the lipid peroxidation in the liver, which protect the integrity of the liver hepatocytes.

Conclusions

Black seed oil from the Nigella sativa plant has protective effects against liver toxicity induced following 28 days of levofloxacin administration. The hepatoprotective effect was confirmed by significant declines in the liver enzymes found in the serum, improved liver tissue injury, and reduced liver malondialdehyde level. The most protection was found when the black seed oil was administered at doses of 1 or 2 mL/kg per rat. This protective effect is likely to find useful applications in clinical settings in humans.

Ethical Considerations

Compliance with ethical guidelines

The animal care protocol was carried out based on the institution's animal care guidelines. The study protocol was reviewed and approved prior to conducting the experiments (Institutional Registration #: 318/UN4.6.4.5.31/PP36/2020).

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declare no conflict of interests with any internal or external entities in conducting this study.

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