LAPORAN AKHIR PENELITIAN TAHUN TUNGGAL

ID Proposal: 593c0677-0447-4283-8801-1eccdb94773a
Laporan Akhir Penelitian: tahun ke-1 dari 1 tahun

1. IDENTITAS PENELITIAN

A. JUDUL PENELITIAN

Uji Efek Farmakologi Ekstrak Etanol Daun Sukun (Artocarpus altilis) Terhadap Glukosa Darah, Kadar Insulin, Profil Lipid dan Histologi Pankreas Tikus Diabetes Mellitus Terinduksi Aloksan

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

Bidang Fokus RIRN / Bidang Unggulan Perguruan Tinggi	Tema	Topik (jika ada)	Rumpun Bidang Ilmu
Kesehatan	Pengembangan dan penguatan sistem kelembagaan, kebijakan kesehatan, dan pemberdayaan masyarakat dalam mendukung kemandirian obat	Pengetahuan lokal untuk penggunaan jamu dan herbal dalam kesehatan masyarakat, yang sensitif gender dan inklusif sosial	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	3	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
SUBEHAN S.Si, Apt, M.Pharm.Sc, Doctor of Philosophy	Universitas Hasanuddin	Ilmu Farmasi		5975282	10

Dosen Pembimbing Anggota 1					
Hesty Setiawaty 1	Mahasiswa S2 Universitas Hasanuddin	-	Farmasi Herbal	0	0
Hardi 2	Mahasiswa S2 Universitas Hasanuddin	-	Farmasi Herbal	0	0

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	Prosiding dalam pertemuan ilmiah Internasional	sudah terbit/sudah dilaksanakan	
1	Prosiding dalam pertemuan ilmiah Internasional	sudah terbit/sudah dilaksanakan	

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*)
1	Publikasi Ilmiah Jurnal Internasional	submitted	Jurnal Pharmacognosy Research

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. 53,710,000

Tahun 1 Total Rp. 53,710,000

Jenis Pembelanjaan	Item	Satuan	Vol.	Biaya Satuan	Total
Bahan	ATK	Paket	1	500,000	500,000
Bahan	Bahan Penelitian (Habis Pakai)	Unit	1	26,400,000	26,400,000
Bahan	Barang Persediaan	Unit	10	66,000	660,000
Pelaporan, Luaran Wajib, dan Luaran Tambahan	Biaya seminar internasional	Paket	2	3,700,000	7,400,000
Pelaporan, Luaran Wajib, dan Luaran Tambahan	Uang harian rapat di dalam kantor	OH	4	100,000	400,000

Jenis Pembelanjaan	Item	Satuan	Vol.	Biaya Satuan	Total
Pelaporan, Luaran Wajib, dan Luaran Tambahan	Biaya konsumsi rapat	OH	4	50,000	200,000
Pengumpulan Data	FGD persiapan penelitian	Paket	1	400,000	400,000
Pengumpulan Data	HR Pembantu Peneliti	OJ	1	600,000	600,000
Pengumpulan Data	HR Sekretariat/Administrasi Peneliti	OB	1	200,000	200,000
Pengumpulan Data	Tiket	OK (kali)	1	3,000,000	3,000,000
Pengumpulan Data	HR Pembantu Lapangan	OH	1	400,000	400,000
Pengumpulan Data	Transport	OK (kali)	4	100,000	400,000
Pengumpulan Data	Penginapan	OH	4	500,000	2,000,000
Pengumpulan Data	Biaya konsumsi	OH	4	50,000	200,000
Sewa Peralatan	Peralatan penelitian	Unit	1	9,950,000	9,950,000
Sewa Peralatan	Ruang penunjang penelitian	Unit	2	500,000	1,000,000

6. HASIL PENELITIAN

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

Diabetes melitus atau dikenal oleh masyarakat sebagai kencing manis adalah suatu kelainan metabolik yang ditandai dengan penurunan sekresi insulin dan atau resistensi insulin. Pada tahun 2015, Indonesia menempati peringkat ke tujuh dunia untuk prevalensi penderita diabetes teringgi bersama dengan China, India, Amerika Serikat, Brazil, Rusia dan Meksiko dengan jumlah estimasi orang dengan diabetes sebesar 10 juta orang. Prevalensi orang dengan diabetes di Indonesia menunjukkan kecenderungan meningkat yaitu dari 5,7 % (2007) menjadi 6,9 %.

Secara tradisional, tanaman yang digunakan secara turun temurun untuk pengobatan diabetes melitus salah satunya adalah sukun (Artocarpus altilis). Daun sukun banyak mengandung senyawa kimia yang berkhasiat, seperti saponin, polifenol, asam hidrosianat, asetilkolin, tanin, riboflavin, fenol, dan flavonoid. Kandungan senyawa aktif flavonoid dalam daun sukun diduga berperan dalam penyembuhan penyakit diabetes. Ekstrak etanol daun sukun memiliki efek dalam menurunkan kadar glukosa darah, kolesterol, dan perbaikan pulau langerhans tikus putih jantan Hiperkolesterolemia-Diabetes. Berdasarkan uraian di atas maka penting untuk melakukan penelitian lebih lanjut, untuk mengetahui efek pemberian ekstrak etanol daun sukun dan perbedaan dosis bertingkat ekstrak daun sukun terhadap kadar glukosa darah dan insulin, profil lipid dan histopatologi pankreas tikus putih jantan model diabetes yang diinduksi dengan aloksan. Adapun TKT dari penelitian ini mencapai TKT 3, dimana sebelumnya telah dilakukan penelitian eksperimental yang mengindikasikan efek ekstrak daun sukun terhadap tikus DM.

Penelitian ini bersifat eksperimen laboratorium dengan desain penelitian Pretest and Post-test Randomized Controlled Group Design dengan menggunakan tikus jantan. Dalam penelitian ini digunakan tikus jantan yang kemudian dikelompokkan ke dalam 5 kelompok, tiap kelompok terdiri dari 6 ekor, lalu diberi perlakukan sebagai berikut: kelompok I sebagai

kontrol negatif, diberikan larutan Na.CMC 1%, kelompok II sebagai kontrol positif, diberi suntikan insulin (4 U/200 g tikus per hari), kelompok III, IV dan V diberi suspensi Ekstrak daun Sukun, masing-masing dengan konsentrasi 100, 200 dan 400 mg/kg selama 2 minggu. Pemeriksaan kadar glukosa darah dilakukan setiap minggu dan pengukuran profil lipid, kadar insulin dan pengamatan histopatologi pankreas dilakukan pada akhir percobaan.

Hasil penelitian menunjukkan ekstrak daun sukun memiliki efek antihiperglikemik terutama pada dosis 400 mg/kg. Efek tersebut sejalan dengan perlindungan ekstrak daun sukun terhadap histologi pancreas terutama pada pulau Langerhans. Selain melindungi pancreas, ektrak daun sukun juga mampu memperbaiki kerusakan hati dan ginjal yang diinduksi aloksan. Walaupun demikian, peningkatan kadar insulin pada tikus yang diberi ekstrak daun sukun tidak berbeda signifikan dibanding placebo.

Hasil penelitian ini akan dipublikasikan di jurnal internasional bereputasi dan diseminasikan dalam seminar internasional. Selain itu, diharapkan hasil penelitian ini dapat menjadi sumber informasi baru untuk masyarakat tentang khasiat daun sukun sehingga dapat dimanfaatkan secara optimal utamanya dalam terapi diabetes, dan menjadi dasar untuk pengembangan produk ekstrak daun sukun sebagai fitofarmaka.

B. KATA KUNCI: Tuliskan maksimal 5 kata kunci.

Ekstrak daun sukun; Artocarpus altilis; aloksan; insulin; pankreas

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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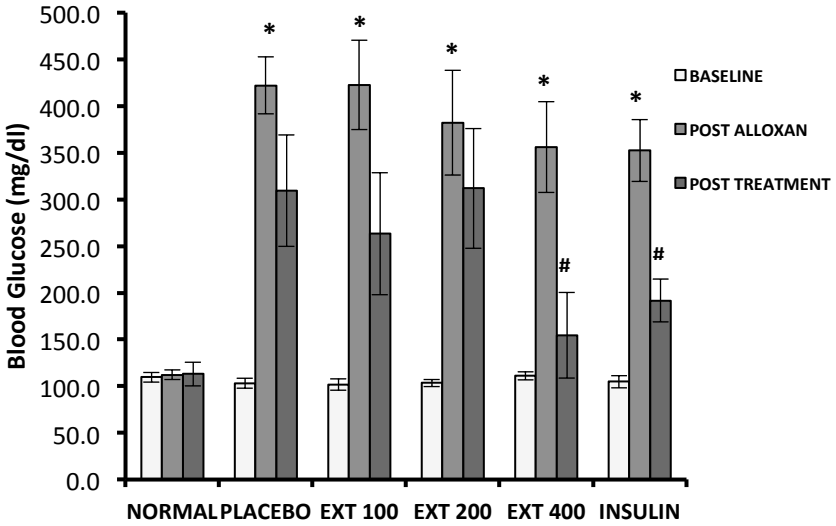
Pengaruh pemberian ekstrak daun sukun terhadap kadar gula darah tikus diabetik akibat induksi aloksan



Gambar 1. Profil gula darah sewaktu pada tikus normal dan tikus diabetik yang diinduksi aloksan sebelum dan setelah diberi perlakuan plasebo, extract 100 mg/kg, extract 200 mg/kg, extract 400 mg/kg dan insulin selama 14 hari.

Pengaruh pemberian daun sukun terhadap fungsi hati dan ginjal tikus diabetik akibat induksi aloksan

Pengukuran kadar SGPT dan kreatinin dilakukan untuk melihat fungsi sel hati dan ginjal setelah induksi aloksan dan pengaruh pemberian esktrak paliasa terhadap fungsi hati setelah tikus mengalami diabetes. SGPT dan kreatinin diukur sebanyak 3 kali, yaitu sebelum tikus diinduksi dengan aloksan (pra induksi), setelah tikus diinduksi (post induksi dan setelah diberikan terapi ekstrak etanol daun sukun (*Artocarpus altilis* (Parkinson) Fosberg) (post terapi). Hasil pengukuran kadar SGPT pra induksi, post induksi dan post terapi dapat dilihat pada tabel 2.

Tabel 1. Hasil pengukuran kadar rata-rata SGPT tikus yang diinduksi aloksan dengan dosis 155 mg/kg BB

Kelompok	Kadar Rata-rata SGPT (U/L)		
	Pra Induksi	Post Induksi	Post Terapi
I	57,81±3,33	62,63±5,19	62,56± 5,36
II	47,11±8,42	76,07±10,94	203,6±60,92
III	57,99±11,29	116,02±26,90	73,43±28,04
IV	59,86±9,93	80,25±12,04	62,41±9,94
V	50,88±3,15	108,34±19,44	47,99±4,90*

Keterangan:

I Kelompok Normal (tanpa perlakuan)
II Kelompok Negatif (Induksi aloksan dengan dosis 155 mg/kg BB)
III Kelompok Ekstrak 100(Induksi aloksan dengan dosis 155 mg/kg BB + ekstrak etanol daun sukun 100mg/kg BB)
IV Kelompok Ekstrak 200 (Induksi aloksan dengan dosis 155 mg/kg BB + ekstrak etanol daun sukun 200mg/kg BB)
V Kelompok Ekstrak 400 (Induksi aloksan dengan dosis 155 mg/kg BB + ekstrak etanol daun sukun 400 mg/kgBB)
Pra Induksi : Sebelum tikus diinduksi aloksan dengan dosis 155 mg/kg BB
Post Induksi: Setelah tikus diinduksi dengan dosis 155 mg/kg BB
Post Terapi : Setelah pemberian ekstrak etanol daun sukun selama 14 hari

Tabel 2. Hasil pengukuran kadar rata-rata kadar kreatinin tikus yang diinduksi aloksan

Kelompok	**Kadar Rata-rata Kreatinin (mg/dl)**		
	Pra Induksi	**Post Induksi**	**Post Terapi**
I	0,392± 0,126	0,402±0,085	0,400±0,087
II	0,339±0,075	1,049±0,175	0, 463±0,112
III	0,325±0,071	0,830±0,175	0,440±0,036
IV	0,414±0,111	0,916±0,225	0,423±0,104
V	0,246±0,036	1,070±0,165	0,461±0,005

Setelah tikus diinduksi terjadi peningkatan rata-rata kadar SGPT ($p < 0,05$) yang signifikan pada semua kelompok perlakuan kecuali kelompok I (normal tanpa perlakuan). Peningkatan kadar rata-rata SGPT pada kelompok II, III, IV dan V adalah 61,47%; 100,07%; 34,06%; 113,09%. Hal tersebut disebabkan karena kondisi hiperglikemik yang terjadi sehingga hati menjadi rusak. Hal ini menunjukkan bahwa pemberian ekstrak etanol daun sukun mampu memperbaiki fungsi hati yang ditandai dengan penurunan kadar rata-rata SGPT yang signifikan lebih baik dari kontrol negatif ($p<0,05$). Bahkan, pada pemberian ekstrak 200mg/kg BB dan 400 mg/kg BB menyebabkan kadar rata-rata SGPT tidak berbeda nyata dengan kelompok normal ($p> 0,05$).

Setelah tikus diinduksi terjadi peningkatan kadar rata-rata kreatinin signifikan lebih besar dari kelompok normal ($p < 0,05$) pada semua kelompok perlakuan (kelompok II, III, IV dan V) dengan persentase berturut-turut yaitu 209,44%; 155,38%; 121,26%; 334%. Peningkatan kadar rata-rata kreatinin oleh induksi aloksan terjadi karena adanya akumulasi glikogen dalam tubulus distal ginjal (lesi Ebstein-Armanni) dan mengalami hiperglikemia yang terus menerus.

Pengaruh pemberian daun sukun terhadap kadar profil lipid tikus diabetik akibat induksi aloksan

Tabel 3. Hasil rata-rata kadar profil lipid

Kelompok	Hasil rata-rata profil lipid			
	HDL (mg/dL)	Trigliserida (mg/dL)	LDL (mg/dL)	Kolesterol (mg/dL)
I	34.8 ± 8.779	72.7 ± 2.507	30.8± 0.626	103.7±2.126
II	28.6 ± 5.344	81.3 ± 10.770	28.4 ± 10.075	90.8±17.299
III	27.4 ± 2.768	70.7± 27.703	25.3 ± 2.518	106.9±11.574
IV	19 ± 0.577	44.5 ± 4.942	33.5 ± 8.648	98.1±23.504
V	24.8 ± 2.709	90.2 ± 21.208	41.7 ± 9.065	91.0±15.878

Keterangan:

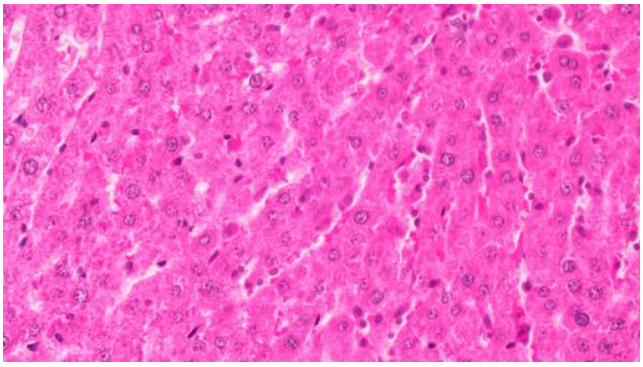
I Kelompok Normal (tanpa perlakuan)
II Kelompok Negatif (Induksi aloksan dengan dosis 155 mg/kg BB)
III Kelompok Ekstrak 100(Induksi aloksan dengan dosis 155 mg/kg BB + ekstrak etanol daun sukun 100mg/kg BB)
IV Kelompok Ekstrak 200 (Induksi aloksan dengan dosis 155 mg/kg BB + ekstrak etanol daun sukun 200mg/kg BB)
V Kelompok Ekstrak 400 (Induksi aloksan dengan dosis 155 mg/kg BB + ekstrak etanol daun sukun 400 mg/kgBB)

Table 9. Profil bobot badan tikus

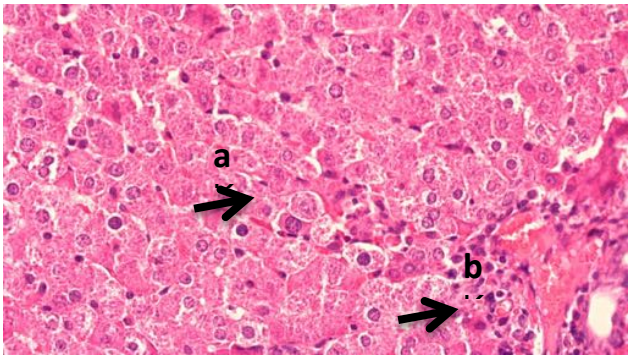
Kelompok	Bobot badan awal (a)	Bobot badan setelah perlakuan (b)	Erubahan bobot badan (b-a)	Jumlah hewan dengan penurunan bobot badan (%)
Normal (n=5)	243.4±20.0 g	259.2±18.3 g	+15.8±6.0 g	0
Placebo (n=5)	210.4±15.3 g	188.8±13.3 g	-21.6±13.1 g	80
Ext 100 (n=5)	239.0±17.8 g	224.6±35.5 g	-14.4±19.4 g	40
Ext 200 (n=5)	232.4±17.8 g	216.0±17.8 g	-16.4±9.3 g	80
Ext 400 (n=5)	206.0±3.3 g	205.0±16.2 g	-1.0±14.3 g	40
Insulin (n=5)	226.0±14.4 g	225.2±7.3 g	-0.8±11.3 g	40

+ weight change = weight gain; - weight change = weight loss

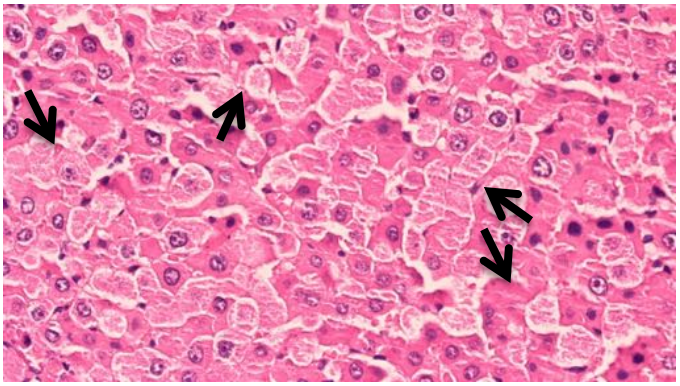
Pengaruh pemberian ekstrak daun sukun terhadap histologi hati tikus diabetik akibat induksi aloksan



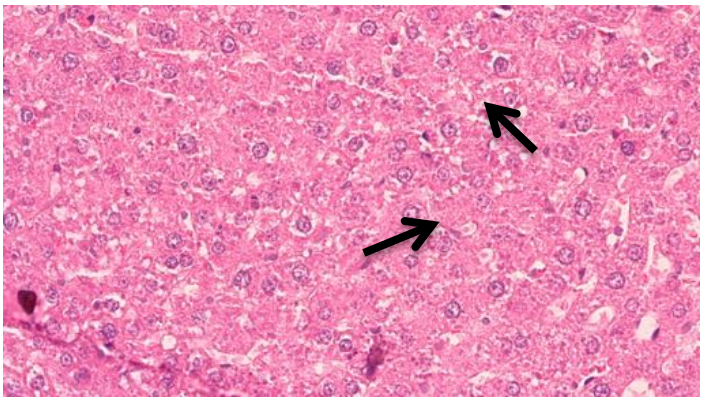
Gambar 2. Hepatosit Normal pada tikus kelompok I (normal tanpa perlakuan) dengan perbesaran 400x dan pewarnaan HE



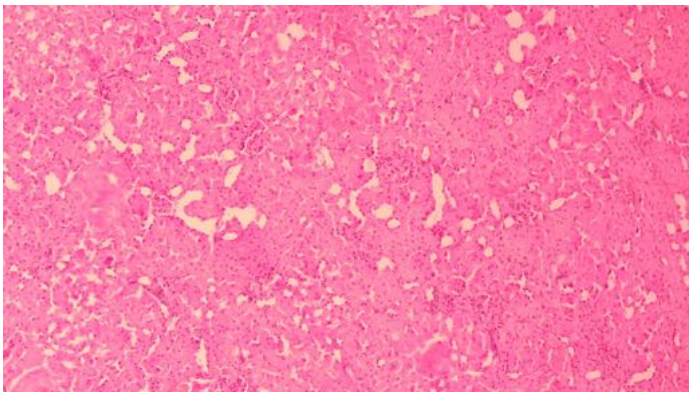
Gambar 3. Hepatosit dengan degenerasi lemak (a) dan degenerasi hidropik (b)skor 1 pada kelompok II (negatif, dengan induksi aloksan tanpa pemberian ekstrak)



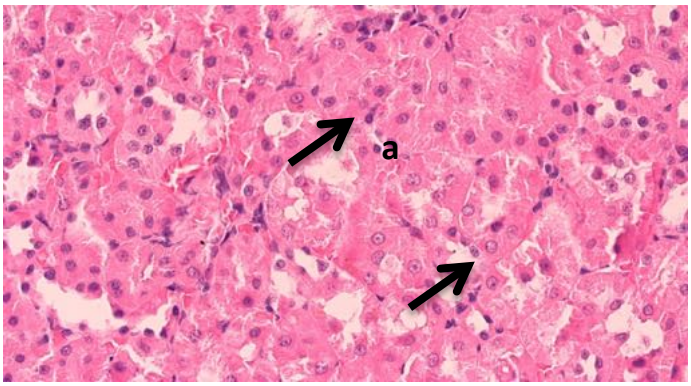
Gambar 4. Hepatosit dengan nekrosis pada kelompok II (negatif, dengan induksi aloksan tanpa pemberian ekstrak) dengan perbesaran 400x dan pewarnaan HE



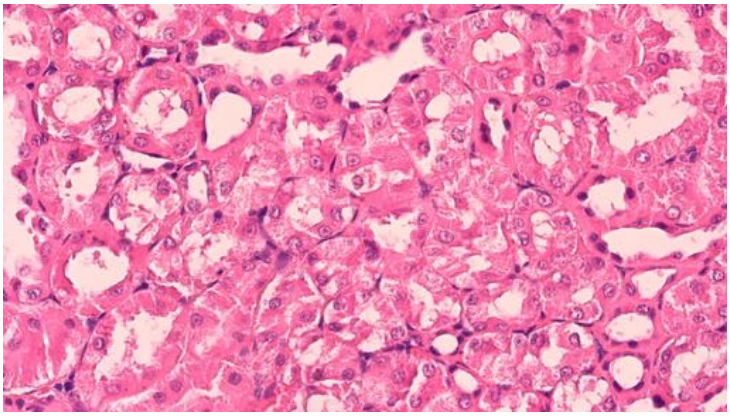
Gambar 5. Hepatosit dengan degenerasi hidropik skor 2 pada kelompok III (induksi aloksan dan pemberian ekstrak etanol dengan dosis 100 mg/kgBB) dengan perbesaran 400x dan pewarnaan HE



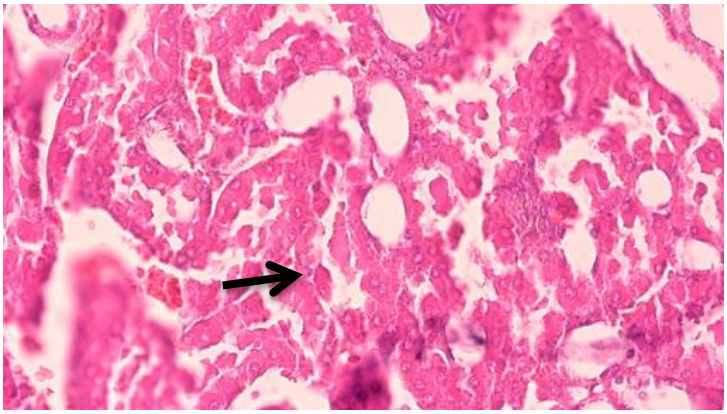
Gambar 6. Jaringan ginjal tikus pada kelompok normal (tanpa induksi aloksan 155 mg/kgBB tanpa pemberian ekstrak etanol daun sukun) dengan perbesaran 100x dan pewarnaan HE



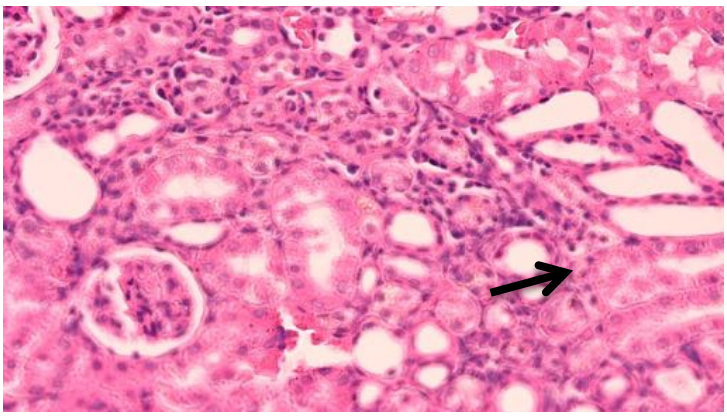
Gambar 7. Jaringan ginjal tikus dengan degenerasi lemak dan hidropik skor 1 pada kelompok II (Negatif, induksi aloksan 155 mg/kgBB tanpa pemberian ekstrak etanol daun sukun) dengan perbesaran 400x dan pewarnaan HE



Gambar 8. Jaringan ginjal tikus dengan degenerasi hidrofilik skor 3 pada kelompok III (induksi aloksan 155 mg/kg BB dengan pemberian ekstrak etanol daun sukun 100 mg/kg BB) dengan perbesaran 400x dan pewarnaan HE



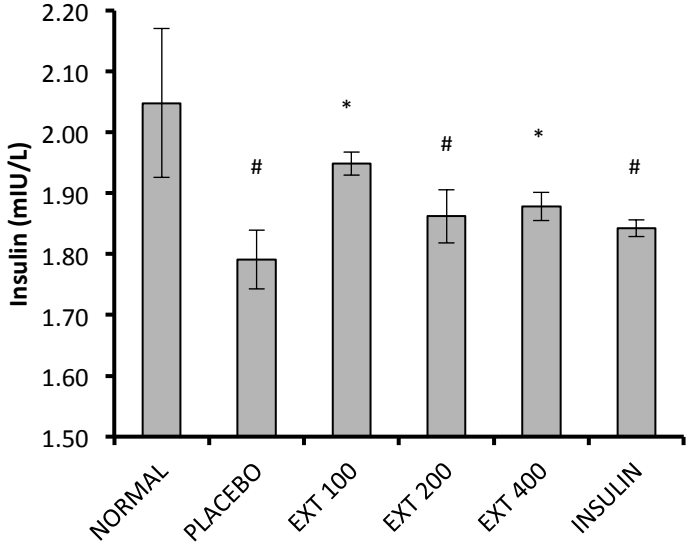
Gambar 9. Jaringan ginjal tikus dengan degenerasi tubulus skor 2 pada kelompok IV (induksi aloksan dan pemberian ekstrak etanol 200 mg/kg BB) dengan perbesaran 400x dan pewarnaan HE



Gambar 10. Jaringan ginjal tikus dengan peradangan skor 1 pada kelompok V (induksi aloksan dan pemberian ekstrak etanol 400 mg/kg BB) dengan perbesaran 400x dan pewarnaan HE

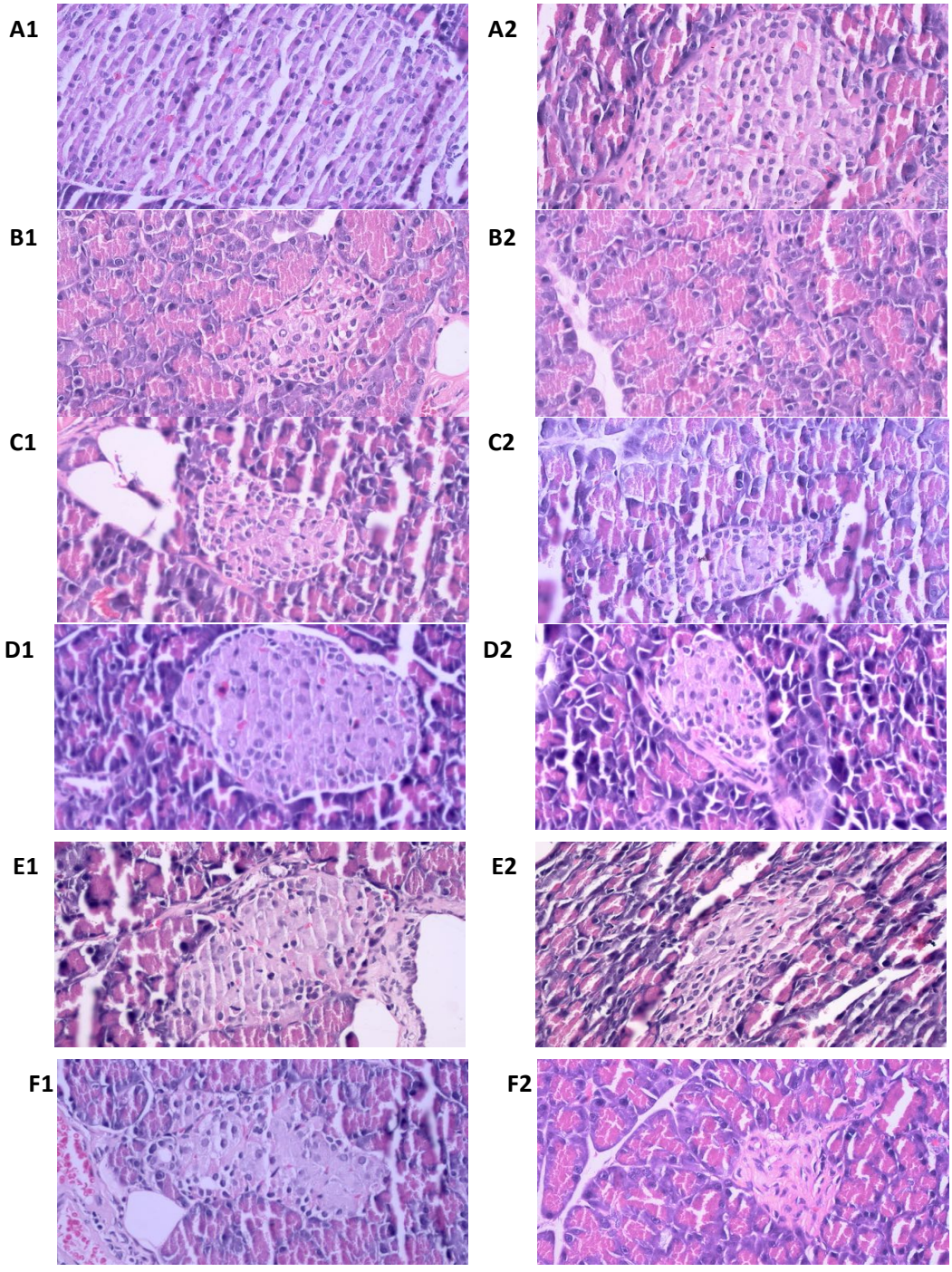
Pengaruh pemberian ekstrak daun sukun terhadap kadar insulin darah tikus diabetik akibat induksi aloksan

Induksi aloksan menyebabkan peningkatan gula darah yang kemungkinan disebabkan menurunnya produksi insulin pada pancreas.



Gambar 11. Kadar insulin pada tikus normal dan diabetik yang diberi placebo, ekstrak 100, ekstrak 200 dan ekstrak 400. #$p<0.05$ using LSD test for placebo, EXT 200 and insulin compared to normal group; *$p<0.05$ using one-way anova analysis for EXT 100 and EXT 400 compared to placebo group.

Pengaruh pemberian ekstrak daun sukun terhadap kerusakan pancreas tikus diabetik akibat induksi aloksan



Gambar 12. Representatif hasil analisa histopatologi jaringan pancreas utamanya area pulau Langerhans. Perlakuan normal (A1 dan A2), plasebo (B1 dan B2), and tikus diabetik tyang diberi insulin insulin (C1 dan C2), tikus diabetik yang diberi ekstrak 100 (D1 dan D2), tikus diabetik yang diberi ekstrak 200 (E1 dan E2), tikus diabetik yang diberi ekstrak 400 (F1 dan F2)

D. **STATUS LUARAN**: Tuliskan jenis, identitas dan status ketercapaian setiap luaran wajib dan luaran tambahan (jika ada) yang dijanjikan pada tahun pelaksanaan penelitian. 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

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1.Data mengenai pengaruh ekstrak Daun Sukun terhadap fungsi hati dan ginjal tikus serta perbaikan histopatologinya telah dibuat dalam bentuk artikel yang disubmit pada Journal of Applied Pharmaceutical Science (Scopus, Q2).

2. Data mengenai efek antihiperglikemik dan kadar insulin plasma serta kerusakan pancreas tikus telah dipresentasikan dan akan diterbitkan dalam prosiding internasional terindeks scopus (Proceeding of Pakistan Academic of Science)

3. Data mengenai bobot badan dan profil lipid telah dipresentasikan dan akan diterbitkan dalam prosiding internasional terindeks scopus (Proceeding of Pakistan Academic of Science)

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Menunggu hasil revisi dari Jurnal dan prosiding

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2. Surat keterangan accepted dari editor

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Contribution of pancreatic islets protection of Breadfruit (*Artocarpus altilis)* leaf extract on blood glucose and insulin level in alloxan-induced diabetic rats

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Abstract. The antioxidant capacity of *Artocarpus altilis* compounds may offer protection against stress oxidative-induced damage to pancreatic cells. This study aimed to examine the effect of *Artocarpus altilis* leaf extract on pancreatic islets protection, blood glucose and serum insulin levels in alloxan-induced diabetic rats. Diabetes mellitus was induced in rats with an i.p injection of alloxan (155 mg/kg). Rats blood glucose were measured daily and only rats with blood glucose >200 mg/dl proceeded to treatment protocols. The diabetic rats (n=25) were divided into 5 treatment groups: placebo (NaCMC 1%); Artocarpus leaf extract 100 mg/kg; Artocarpus leaf extract 200 mg/kg; Artocarpus leaf extract 400 mg/kg; and insulin 4 IU/200 g. Meanwhile, healthy rats (n=5) that did not inject with alloxan were also involved as healthy controls. All treatments were received daily for 14 days before blood sampling and pancreatic tissue removal. Result shows only rats treated with *Artocarpus altilis* extract at 400 mg/kg had significantly reduced blood glucose level, which was similar to that in insulin-treated rats. Although did not reach significance, the insulin level of *Artocarpus altilis* extract-treated groups were higher than placebo and similar to insulin-treated group. Alloxan injection induced atrophy of pancreatic islets. The administration of *Artocarpus altilis* leaf extract at all given doses reduced the severity of pancreatic islet's atrophy. In conclusion, *Artocarpus altilis* leaf extract protects pancreatic islets of rats against alloxan-induced damage. This protection tends to reduce blood glucose levels and improve insulin plasma levels in Artocarpus altilis treated rats.

Keywords: Alloxan, *Artocarpus altilis*, blood glucose, insulin plasma, pancreatic islets

1. Introduction

Diabetes mellitus (DM) is a complex metabolic disorder characterized by chronic hyperglycemia, frequent urination, excessive hunger and increased thirst. The prevalence of diabetes mellitus has been increasing in the past decades in Asian countries, including Indonesia [1]. In fact, there is an increase in the number of DM incidents in adolescents, which is almost doubled in 2013 compared to those in 2007 [2]. The increasing number of people who suffer from DM has led to a massive increase in the cost of diabetes treatments since it requires a lifelong medication. The high cost of DM treatment has driven people to turn to traditional medicine as an alternative treatment [3].

Several plants have been acclaimed for their anti-diabetic properties. Among them is Breadfruit or *Artocarpus altilis,* which belong to the family of Moraceae. The fruits of *Artocarpus altilis* have been reported to contain triterpenes, flavonoids, stillbenes, and sterols that provide antioxidant, anti-hyperglicemic and antimicrobial properties [4]. Meanwhile, the leaves of *Artocarpus altilis* have been recognized to endow with rich phenolic content, providing potent antioxidant activities [5]. The anti-hyperglycemic effect of *Artocarpus altilis* leaf ethanolic extract has been demonstrated in streptozotocin-induced diabetic rats [6], which is This is an important finding as beta pancreatic cells are the insulin-producing cells that mainly impaired during diabetic condition.

The current anti-diabetic medications mainly act to enhance insulin secretion, improve insulin sensitivity, prevent liver gluconeogenesis, or to reduce glucose absorption. However, there is a lack of anti-diabetic drugs that enhance beta pancreatic cell regeneration and prevent further loss of beta pancreatic cells. The antioxidant capacity of *Artocarpus altilis* compounds may offer protection against stress oxidative induced damage to pancreatic cells. Therefore, this present study aimed to examine the effect of *Artocarpus altilis* leaf extract on pancreatic islets protection, blood glucose and serum insulin levels in alloxan-induced diabetic rats.

2. Methods

2.1. Chemicals and drugs

Alloxan monohydrate (Sigma Aldrich®), Formaldehyde in 10% PBS and diethyl ether was purchased from official chemical distributor in Makassar, Indonesia. Insulin glargine (Lantus®) used in this study was obtained from regional hospital in Makassar, Indonesia. The level of plasma insulin was measured using rat insulin ELISA kit 96T (BT-Lab®, Gaithersburg).

2.2. Artocarpus altilis leaf collection, extraction and preparation

Artocarpus altilis [(Parkinson) Fosberg] leaves were handpicked from the trees in the area of Gowa, South Sulawesi. Leaves with green or yellow appearance were collected and extracted separately. Extraction was carried out with maceration method with 70% ethanol. However, since the mature leaves of *Artocarpus altilis* were traditionally claimed to have a better anti-diabetic effect than the young leaves [7], only the yellow leaf extract that was involved in the animal study. The thick extract of *Artocarpus altilis* leaves was prepared as a suspension with 1% Sodium CMC (NaCMC) immediately prior to treatment in animals.

2.3. Total flavonoid analysis

The total flavonoid content of breadfruit leaf (green and yellow) extract was determined as rutin content using an Ultra Fast Liquid Chromatography (…). The mobile phase used comprises of methanol, acetonitrile, and 5% formic acid with a ratio of 25:65:10 and a flow rate of 1 mL/minute. The stationary phase used was the octadesyl carbon chain (C18) bound to silica. The flavonoid compound was measured at a wavelength of 370 nm and calculated as the area under curve (AUC).

2.4. Animal procedures

Male Wistar rats age ranging from 2 to 3 months and minimum weight of 180 g were purchased from rat laboratory breeding facility in Yogyakarta, Indonesia. The rats were housed in Pharmacology and

Toxicology Laboratory of Hasanuddin University and cared according to the institutional standard procedure and care of animal laboratory (animal ethical clearance no. 544/UN4.6.4.5.31/PP36/2019). Rats were accustomed to their environment for 14 days prior to initiating treatment protocols. Access to standard pellets and water were provided at all times.

Diabetes mellitus was induced in rats with an intraperitonial injection of alloxan (155 mg/kg). An oral administration of 5% glucose (2 ml) was given after 10 minutes of alloxan injection to prevent hypoglycaemia in rats [8]. Rats blood glucose were measured every day using glucometer (Easy Touch®) and rats that exhibited significant elevation of blood glucose (>200 mg/dl) were defined as diabetic and preceded to treatment protocols. The diabetic rats (n=25) were divided into 5 treatment groups: placebo (NaCMC 1%); Artocarpus leaf extract 100 mg/kg; Artocarpus leaf extract 200 mg/kg; Artocarpus leaf extract 400 mg/kg; and insulin 4 IU/200 g. Meanwhile, healthy rats (n=5) that did not subjected with alloxan injection were also involved in this study to serve as healthy controls. All treatments were received daily for 14 days before blood sampling and pancreatic tissue removal were performed.

2.5. Blood glucose test

Following injection of alloxan and during 14 days of treatment, rat blood glucose levels were checked daily using Glucometer (Easy Touch®) to record the day-to-day fluctuation of blood glucose level.

2.6. Insulin level assay

At the end of experiment, the rats were anesthetized with diethyl eter. Blood samples were collected from tail lateral vein using BD vacutainers. Blood samples were centrifuged at the rate of 3000 rpm at 25^{o}C for 20 minutes to obtain serum. The level of serum insulin was measured using ELISA reader (Thermo scientific®) according to kit's instruction (rat insulin ELISA kit, BT-Lab) at 450 nm.

2.7. Histopathology analysis of pancreatic islets

Following blood collection, rats were euthanized with cervical dislocation. Organ pancreas were removed and fixed in 10% formaldehyde for 2 days before undergone an automatic tissue processing (Thermo Scientific®). Pancreatic tissues were embedded in paraffin cassettes and cut into 4-µm thickness with a microtome (Thermo Scientific®) before put in to a histological slide. The tissues were stained using hematoxylin and eosin and observed under a light microscope. An observer who is blinded to the treatment performed a histopathological analysis on the pancreatic islets based on the average area of the islets and the density of cells in islet tissues at the 400x magnification [9].

2.8. Statistical analysis

Numerical data is presented as mean ± SEM. All data was analysed using SPSS 24 software. The distribution of data was tested for normality using Kolmogorov-Smirnov test and homogeneity of the data was confirmed with Lavene's test. If the data is normally distributed, a one way anova analysis was performed, followed by LSD post hoc test. Paired t- test was used to analyse changes in blood glucose levels in rats following alloxan injection and after 14-day treatments. Significant difference is defined if the p value is <0.05.

3. Results and Discussion

3.1. Total flavonoid content of Artocarpus altilis leaf extract

Artocarpus altilis has been claimed to possess antioxidant activities, which is strongly associated with the flavonoid content of the plants. It is interesting that the mature (yellow) leaves of *Artocarpus altilis* is believed to have a more potent antidiabetic effect compared to the green leaves. A comparison of the flavonoid content of the green and yellow leaf extracts of *Artocarpus altilis* is shown figure 1. From the graph, it is clear that the yellow leaf extract had four times higher concentration of flavonoid compared to the green leaf extract (0.476 vs 0.144 µg/mg).

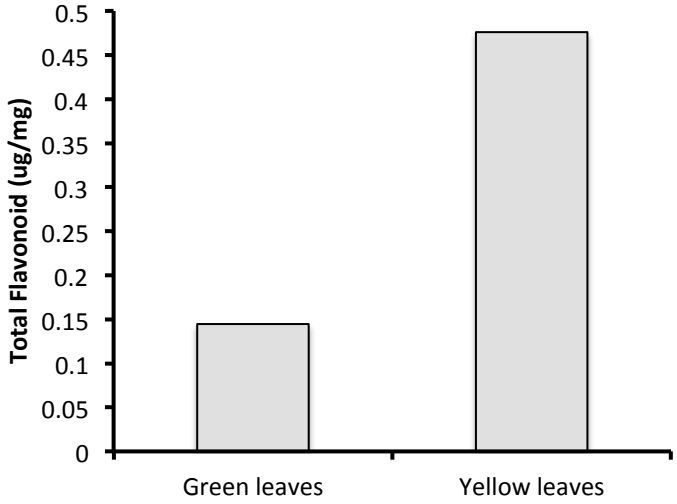

Figure 1. The total flavonoid content calculated as rutin content of the green and yellow leaf extracts of *Artocarpus altilis*

3.2. The effect of Artocarpus altilis leaf extract administration on blood glucose level

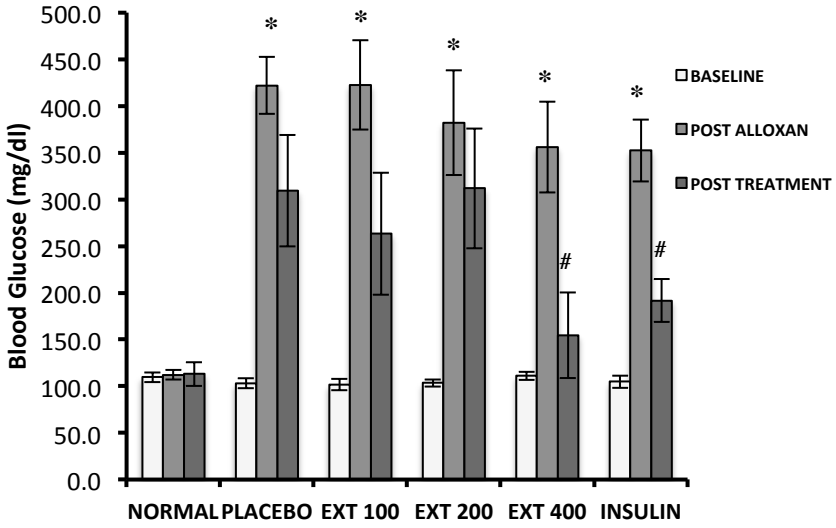

Figure 2. The profiles of blood glucose levels of normal and diabetic rats treated with placebo, extract 100 mg/kg, extract 200 mg/kg, extract 400 mg/kg and insulin. Baseline: BG prior to alloxan injection; Post alloxan: BG post 3 –day from alloxan injection; Post treatment: 14-day post treatments. *$p<0.05$ using paired t-test between post alloxan and baseline values; #$p<0.05$ using paired t-test between post treatment and post alloxan values.

The anti-diabetic effect of *Artocarpus altilis* leaf extract was examined against alloxan-induced diabetes in rats. The changes seen in blood glucose levels in response to alloxan injection was multiphasic, starting with a transient hypoglycemic phase that could lasts for a maximum of 30 minutes followed by a rapid elevation of blood glucose levels within hours post injection. This is caused by a transient hyperinsulinemia, which is more likely caused by a temporary increase in ATP

levels as a consequence of alloxan inhibition on glucokinase [10]. To prevent the hypoglycemic phase in rats, 5% glucose was administered in this study 10 minutes after administering i.p injection of alloxan. The result showed three days following alloxan injection (155 mg/kg bw), around 50 % rats experienced a significant elevation of blood glucose levels to more than 200 mg/dl (figure 2). It seems that the condition of the animal itself, including nutritional status and weight, may contribute to their response to alloxan injection [11]. The mechanisms of alloxan-induced diabetes is associated with its two different pathogenesis: 1) a selective inhibition of insulin secretion through the inhibition of glucokinase, the glucose sensor in beta pancreatic cells, and 2) a stimulation of ROS formation in leading to necrosis of pancreatic beta cells [12]. Following treatments, as shown in figure 2, only rats treated with Artocarpus altilis extract at the dose of 400 mg/kg (EXT 400) was able to significantly reduced the blood glucose level to less than 200 mg (154 ± 45 mg/dl). Reduction of blood glucose in the EXT 400 group was similar to that in rats treated with insulin (191 ± 23 mg/dl).

3.3. The effect of Artocarpus altilis leaf extract administration on serum insulin level

The induction of diabetes in alloxan-injected rats is associated with reduced production of insulin due to alloxan inhibition on glucokinase and alloxan-induced damage in insulin-producing cells of pancreatic tissue.

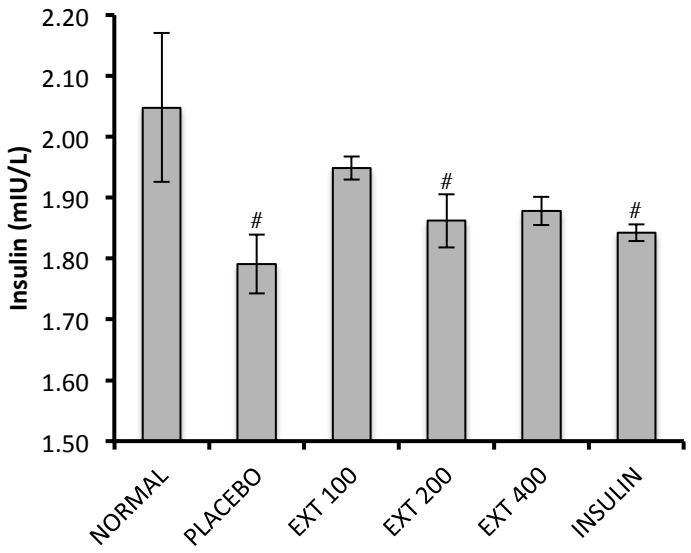

Figure 3. The insulin levels of normal and diabetic rats treated with placebo, extract 100 mg/kg, extract 200 mg/kg, extract 400 mg/kg and insulin. #p<0.05 using LSD test for placebo, EXT 200 and insulin compared to normal group; *p<0.05 using one-way anova analysis for EXT 100 and EXT 400 compared to placebo group.

In this study, it is shown that serum insulin level in placebo-treated rats was significantly reduced from 2.05 ± 0.12 mIU/L in normal rats to 1.79 ± 0.05 mIU/L compared to normal rats (figure 3). The reduction of insulin level was also experienced in diabetic rats treated with *Artocarpus altilis* extract at all doses or insulin, but those treated with *Artocarpus altilis* extract at 100 and 400 mg/kg doses was not considered significant compared to normal rats. Although did not reach significant difference, the insulin level of Artocarpus altilis extract treated groups were higher than those in placebo group and similar to insulin-treated group.

3.4. The effect of Artocarpus altilis leaf extract administration on pancreatic islets

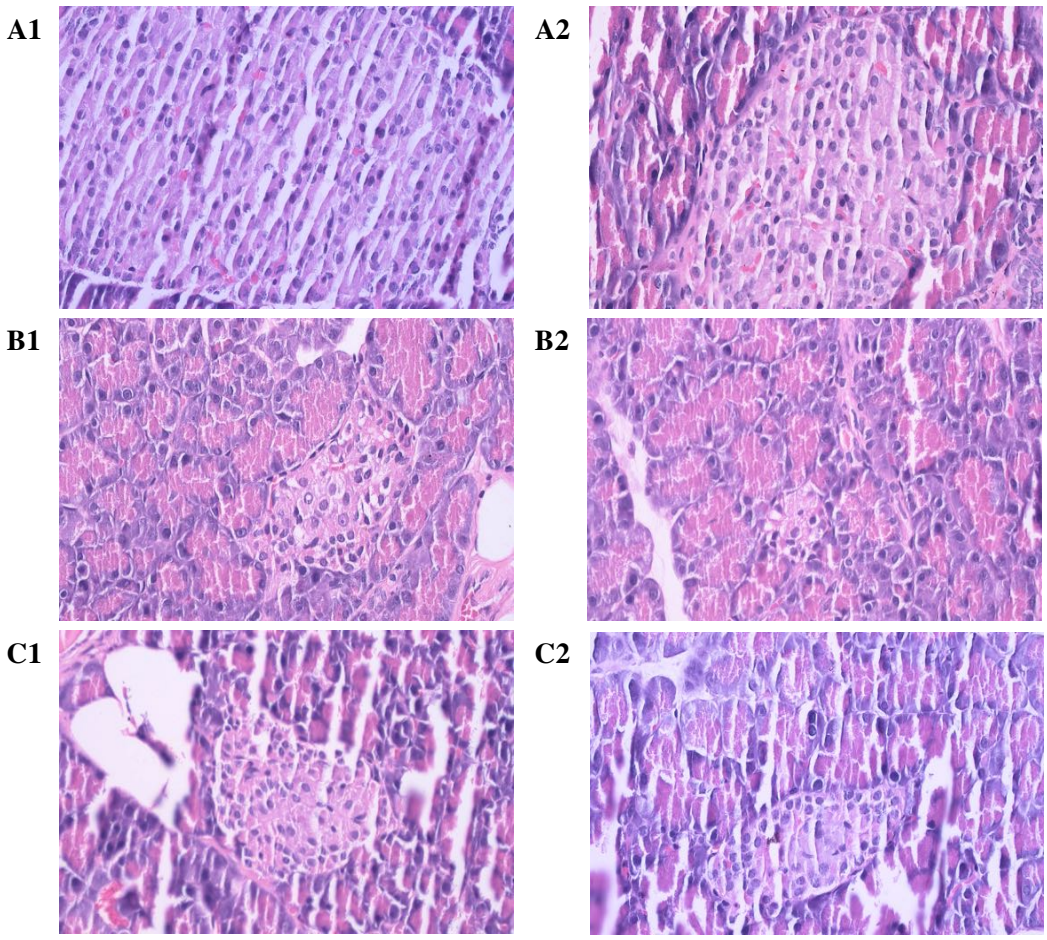

Figure 4. The representative photomicrograph of pancreatic islets of normal rats (A1 and A2), diabetic rats treated with placebo (B1 and B2), and diabetic rats treated with insulin (C1 and C2). The left-hand side picture represent the islets with largest diameter found in the group and the right-hand side pictures represent the smallest islets in the group

Figure 4 and 5 presents the photomicrographs of pancreatic islets of normal rats and diabetic rats treated with different treatments. Normal rats showed normal size of pancreatic islets. Meanwhile, the injection of alloxan induces the shrinkage (atrophy) of pancreatic islets in all treatment groups. The shrinkage of pancreatic islets was severely found in the placebo-treated rats. While in insulin-treated rats, the shrinkage of pancreatic islets was still dominantly found in the pancreatic tissue. However the treatment of Artocarpus altilis extract significantly improved the shrinkage of pancreatic islets, as seen in figure 5. Previously, *Artocarpus altilis* leaf extract has been demonstrated to improve liver tissue and also reduce SGPT levels of alloxan-treated rats [13]. This is mostly contributed by the antioxidant compounds contained in the leaf of *Artocarpus altilis*.

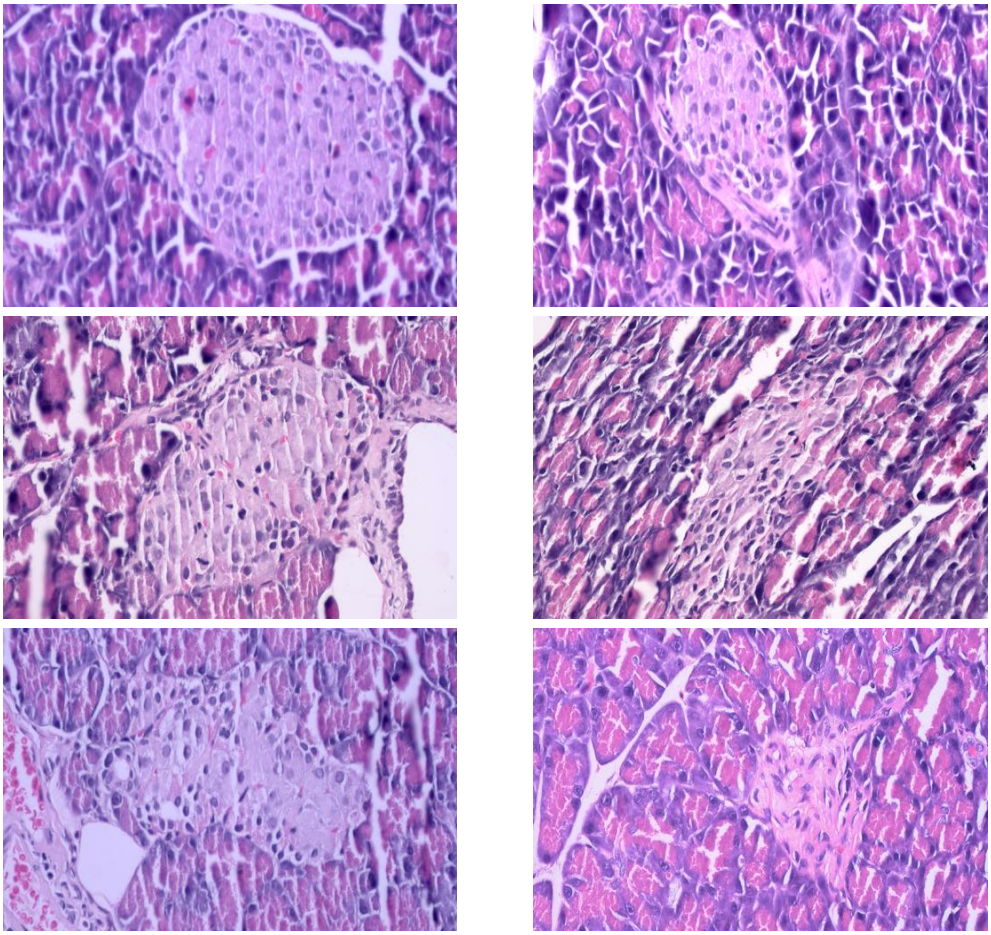

Figure 5. The representative photomicrograph of pancreatic islets of diabetic rats treated with Artocarpus leaf extract 100 mg/kg (D1 and D2), diabetic rats treated with Artocarpus leaf extract 200 mg/kg (E1 and E2), and diabetic rats treated with Artocarpus leaf extract 400 mg/kg (F1 and F2). The left-hand side picture represent the islets with largest diameter found in the group and the right-hand side pictures represent the smallest islets in the group

4. Conclusion

Alloxan injection induced damage in pancreatic tissue shown by the atrophy of pancreatic islets. However, the administration of *Artocarpus altilis* leaf extract at all given doses appeared to reduce the severity of pancreatic islet atrophy, shown by the greater size of pancreatic islets in extract groups. This protection to pancreatic islets by the extract contributes to a reduction in blood glucose levels and slightly higher plasma insulin level in extract groups compared to placebo group. This result could be translated to clinical study using diabetic patients.

5. Acknowledgement

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6. Conflict of interest

The authors declared no conflict of interests.

References

1. Pulungan AB, Afifa IT, and Annisa D. Type 2 diabetes mellitus in children and adolescent: An indonesian perspective. *Annals of pediatric endocrinology & metabolism* 2018; **23**(3): 119-125.
2. Moelyo AG, Himawan IW, Pateda V, and Fadil R. Indonesia national registry of children with type 2 diabetes mellitus. *International journal of pediatric endocrinology* 2013; **2013**(1): P8.
3. Egede LE, Ye X, Zheng D, and Silverstein MD. The prevalence and pattern of complementary and alternative medicine use in individuals with diabetes. *Diabetes Care* 2002; **25**(2): 324-329.
4. Baba S, Chan HT, Kezuka M, Inoue T, and Chan EWC. Artocarpus altilis and pandanus tectorius: Two important fruits of oceania with medicinal values. *Emirates Journal of Food and Agriculture* 2016: 531-539.
5. Leng LY, binti Nadzri N, Yee KC, and Shaari AR. *Antioxidant and total phenolic content of breadfruit (artocarpus altilis) leaves*. in *MATEC Web of Conferences*. 2018. EDP Sciences.
6. Indrowati M, Pratiwi R, Rumiyati, and Astuti P. Levels of blood glucose and insulin expression of beta-cells in streptozotocin-induced diabetic rats treated with ethanolic extract of artocarpus altilis leaves and gaba. *Pakistan Journal of Biological Science* 2017; **20**: 28-35.
7. Riasari H, Zainuddin A, and Handayani DY. Karakterisasi senyawa fenol dari fraksi terpilih daun sukun (artocarpus altilis (parkinson) fosberg) kuning nempel sebagai antioksidan. *Indonesian Journal of Pharmaceutical Science and Technology* 2015; **4**(2): 18-35.
8. Chauhan P, Pandey I, and Dhatwalia VK. Evaluation of the anti-diabetic effect of ethanolic and methanolic extracts of centella asiatica leaves extract on alloxan induced diabetic rats. *Adv Biol Res* 2010; **4**: 27-30.
9. Jelodar Gholamali A, Maleki M, Motadayen M, and Sirus S. Effect of fenugreek, onion and garlic on blood glucose and histopathology of pancreas of alloxan-induced diabetic rats. *Indian J Med Sci* 2005; **59**: 64-69.
10. Ighodaro OM, Adeosun AM, and Akinloye OA. Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. *Medicina* 2017; **53**(6): 365-374.
11. Federiuk IF, Casey HM, Quinn MJ, Wood MD, and Ward KW. Induction of type-1 diabetes mellitus in laboratory rats by use of alloxan: Route of administration, pitfalls, and insulin treatment. *Comparative medicine* 2004; **54**(3): 252-257.
12. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia* 2008; **51**(2): 216-226.
13. Setiawati H, Djabir YY, Hardi H, Lallo S, and Cangara MH. Breadfruit leaf (*artocarpus altilis*) extract reduces hepatic and renal damages in alloxan-induced diabetic rats. *Journal of Applied Pharmaceutical Sciences* xxxx; **x**: xxx.

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ACCEPTANCE LETTER

Dear, Mr./ Mrs./ Miss., **Yulia Yusrini Djabir, Hardi Hardi, Hesty Setiawati, Subehan Lallo and M Husni Cangara.**

Thank you for your interest in the International Seminar on Bioscience and Drug Discovery (ISBDD) 2019 and the submission of your abstract.

We are pleased to state that your paper based on abstract entitled:

Contribution of pancreatic islets protection of Breadfruit (Artocarpus altilis) leaf extract on blood glucose and insulin level in alloxan-induced diabetic rats

has been **ACCEPTED** by the committee.

We are looking forward to seeing you at ISBDD 2019, Makassar, South Sulawesi.

Thank you.

Sincerely yours,

Muhammad Aswad, Ph.D., Apt.
Chairman of the Organizing Committee

Dokumen pendukung luaran Wajib #2

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1.

Dokumen sudah diunggah:
1. Naskah artikel
2. Surat keterangan accepted dari editor

Dokumen belum diunggah:
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The effect of *Artocarpus altilis* leaf extract administration on lipid profiles and weight loss in alloxan-induced diabetic rats

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Abstract. Diabetes mellitus is associated with abnormalities in lipid metabolism, which increase the risk of atherogenesis. Artocarpus artilis leaves have been used empirically to treat diabetes mellitus. This study aimed to examine the effect of Artocarpus artilis leaf extract administration on lipid profiles and weight loss in diabetic rats induced by alloxan injection. Forty-five male wistar rats were injected with alloxan (155 mg/kg). After 72 hours, the blood glucose (BG) levels were checked with glucometer. Only rats that had BG > 200 mg/dl were included in experiment (n=20). Rats were divided into groups and treated daily with either placebo, Artocarpus leaf extract (100 mg/kg, 200 mg/kg, or 400 mg/kg) or insulin (4U/200 g) for 14 days. Five additional rats were included as normal controls. It is found that following 3 days of alloxan injection, only 20 of 45 rats experienced hyperglycemia. Following 14 days of treatments, placebo-treated rats had significantly increased total cholesterol compared to normal controls (175 mg/dl vs 71 mg/dl). However, the triglyceride, LDL and HDL levels were similar to controls. Diabetic rats receiving either Artocarpus leaf extract (100, 200 and 400 mg/kg) or insulin did not show a significant increase in their cholesterol levels as well as triglyceride, LDL, and HDL levels. Reduced body weight was experienced in 80% rats in placebo group, while only 40% rats in Artocarpus extract 100 and 400 groups losing weight. In conclusion, treatment of Artocarpus extract may improve lipid metabolism and weight loss in alloxan-induced diabetic rats.

Keywords: Alloxan, Artocarpus altilis, diabetes mellitus, lipid profiles, weight loss

1. Introduction

Diabetes mellitus is a condition characterized by hyperglycemia, insulin resistance, and relative insulin deficiency. It is estimated that people with diabetes mellitus have reached 451 million worldwide [1]. Indonesia was claimed as the top seventh for the highest prevalence of diabetes in the world with more than 10 millions of incidence found in 2015 [2].

In addition to hyperglycemia, diabetes mellitus is associated with dyslipidemia as a consequence of insulin modulation on lipid metabolism, which alters the activities or transport of lipid

metabolism enzymes. The impact of diabetic-induced dyslipidemia include vascular complications such as atherosclerosis, which may lead to increased comorbidity in DM patients [3].

The use of herbal products and supplements has increased rapidly over the past three decades with no less than 80% of people relying on herbal products [4]. It has been reported that more than 1200 traditional plants may have been used to benefit diabetes treatment [5]. Breadfruit (*Artocarpus altilis*) is one of the plants that has been empirically used for diabetes mellitus and lipid disorders such as hypercholesterolemia. In animal study, Artocarpus leaf extract was found to increase pancreatic beta cell number in streptozotocin-induced diabetic rats [6]. Artocarpus leaf extract has been also shown to improve Langerhans islands of beta pancreatic cells in alloxan-induced diabetic rats [7]. The protective effects of Artocarpus altilis extract is not limited to pancreatic cells, but also expand to liver cells. As shown in the previous study, Artocarpus leaf extract was demonstrated to protect liver damage due to alloxan injection, shown by SGPT level and histopathological analysis [8]. Furthermore, it is shown that Artocarpus leaf extract was beneficial to reduced free fatty acid levels in obese rats [9]. Therefore, this study aimed to examine the effect of Artocarpus artilis leaf extract administration on lipid profiles (cholesterol, triglycerides, LDL and HDL) and weight loss in diabetic rats induced by alloxan injection.

2. Methods

2.1. Sample preparation and extraction

Artocarpus altilis leaves were harvested in Gowa, South Sulawesi. Only leaves with yellowish color were handpicked from the trees based on empirical use of breadfruit leaves as diabetes treatment. The leaves were cleaned with tap water, sorted, dried and finely chopped. The *Artocarpus altilis* leaves (150 g) were macerated with 70% ethanol (2.5 L) for 3 days with occasional stirring. Re-maceration was done until the solvent was clear. The result of maceration was evaporated using a rotary vacuum evaporator at 40^0C until a thick extract was obtained.

2.2. Chemical preparation

Alloxan monohydrate was purchased from Sigma Aldrich (PT. Gilang Medica Jaya, Makassar). Diagnostic kits for cholesterol, triglycerides, LDL and HDL were purchased from Human Diagnostics Worldwide (PT. Rayhan Alkesindo, Makassar).

2.3. Animal preparation

Fifty male wistar rats were purchased from animal laboratory breeding facility (UD. Wistar, Yogyakarta, Indonesia). Animals were acclimatized for 14 days prior to experiment in Pharmacology and Toxicology Laboratory of Hasanuddin University. Animals received standard food for rodents and water ad libitum. All animal procedures has been approved by institutional ethic committee at Hasanuddin University, with the ethical clearance number of 544/UN4.6.4.5.31/PP36/2019

2.4. Experimental procedures

Forty-five rats were intraperitonially injected with alloxan at the dose of 155 mg/kg. The dose was chosen based on previous study [10] and adjusted in our preliminary study. Ten minutes after injection, rats were given 5% glucose (2 ml) through oral gavage to prevent hypoglycaemia in rats. The blood glucose levels were checked daily and only rats that had blood glucose level > 200 mg/dl were defined diabetic and received treatments according to their groups.

Group I was given placebo (NaCMC 1%, n=5); group II was given Artocarpus leaf extract at the dose of 100 mg/kg; group III was given Artocarpus leaf extract at the dose of 200 mg/kg; group IV was given Artocarpus leaf extract at the dose of 400 mg/kg; group V received insulin injection at the dose 4 IU/200 g. Additional group of rats (n=5) that did not receive alloxan injection were also involved to serve as normal controls.

Rats were weigh every day before receiving treatments to adjust the dose accordingly. The extract treatments as well as insulin injection were done once daily for 14 consecutive days. Blood samples were withdrawn at the end of experiments to measure cholesterol, triglyceride, LDL and HDL levels. All lipid fractions were measured using Humalyzer 3500 according to the kit instruction.

2.5. Statistical analysis

All data is presented as mean ± SEM. Data analysis was performed using SPSS 24 software. Kolmogorov-Smirnov analysis is used to test data distribution, while Lavene's test is used to determine homogeneity of the data. Data that is normally distributed was analysed using One-way ANOVA followed by Tukey's HSD post hoc test. Significant difference was considered achieved when $P<0.05$.

3. Results and Discussion

Dyslipidaemia in diabetic patients is common since insulin dependent pathways of lipid metabolisms were considerably altered [11]. Effective treatment of diabetic dyslipidemia can significantly reduce the risk of cardiovascular disorders. In an effort to find effective treatment for diabetic dyslipidemia, this study try to examine the effectiveness of Artocarpus leaf extract in improving lipid metabolism in diabetic rats.

This study measured four lipid biomarkers in alloxan-induced diabetic rats: cholesterol, triglycerides, LDL and HDL. Figure 1A shows the levels of total cholesterol in alloxan-induced diabetic rats following 14 days of treatment. It is revealed that the normal groups had total cholesterol levels of 41 to 116 mg/dl, with the average of 71 mg/dl. Meanwhile, the diabetic rats that only received placebo had increased cholesterol level to 175 mg/dl, which is more than doubled the normal value. The lowest mean of total cholesterol level was achieved by the insulin group (62.27 mg/dL), while Artocarpus extract 100, 200 and 400 mg/ kg had cholesterol levels of 71, 107 and 91 mg/dl, respectively. Treatments insulin or Artocarpus leaf extract (at all given doses) were able to significantly reduce total cholesterol levels in alloxan-induced diabetic rats compared to placebo. This may indicate the potential roles of *Artocarpus altilis* leaf extract as an alternative treatment for diabetic hypercholesterolemia.

The common characteristics of diabetic dyslipidaemia include hypertriglyceridemia, low HDL cholesterol and elevated LDL, with hypertriglyceridemia being more dominant [12]. Interestingly, alloxan-induced hyperglicemia in this study did not seem to induce hypertriglyceridemia. As we can see in Figure 1B, none of the diabetic groups had significantly increased triglyceride levels. The discrepancy may originate from the fact that the experimental diabetes mellitus induced by alloxan had different mechanisms than that develops in human. Dyslipidaemia is mainly found in diabetes mellitus type 2 patients, which is chronically developed leading to insulin resistance. In contrast, alloxan injection acutely damage the Langerhans island of pancreatic tissue, led to degeneration of beta pancreatic cells resulting in massive reduction in insulin production and release [13]. Therefore, we found that instead of increasing the level of triglycerides, i.p injection of alloxan in this study was more likely to induce hypercholesterolemia.

It is found in this study normal rats had the range of LDL level of 22-54 mg/dl and HDL level of .21-30 mg/dl. When looking at the HDL level, there was no significant change found between groups (Figure 1C). Similarly, the mean level of LDL was not significantly elevated in placebo treated diabetic rats compared to the non-diabetic rats (Figure 1D). Nonetheless, there was a trend towards increased LDL in diabetic groups. When looking at the number of animal experienced elevated LDL level (>60 mg/dl), we found 1 out of 5 rats in the placebo group that had elevated LDL, 2 in Ext 100, 1 each in Ext 200 and Ext 400, but none in insulin group. This suggest that treatment with insulin but not Artocarpus extract somewhat has reduced LDL level of alloxan-induced diabetic rats.

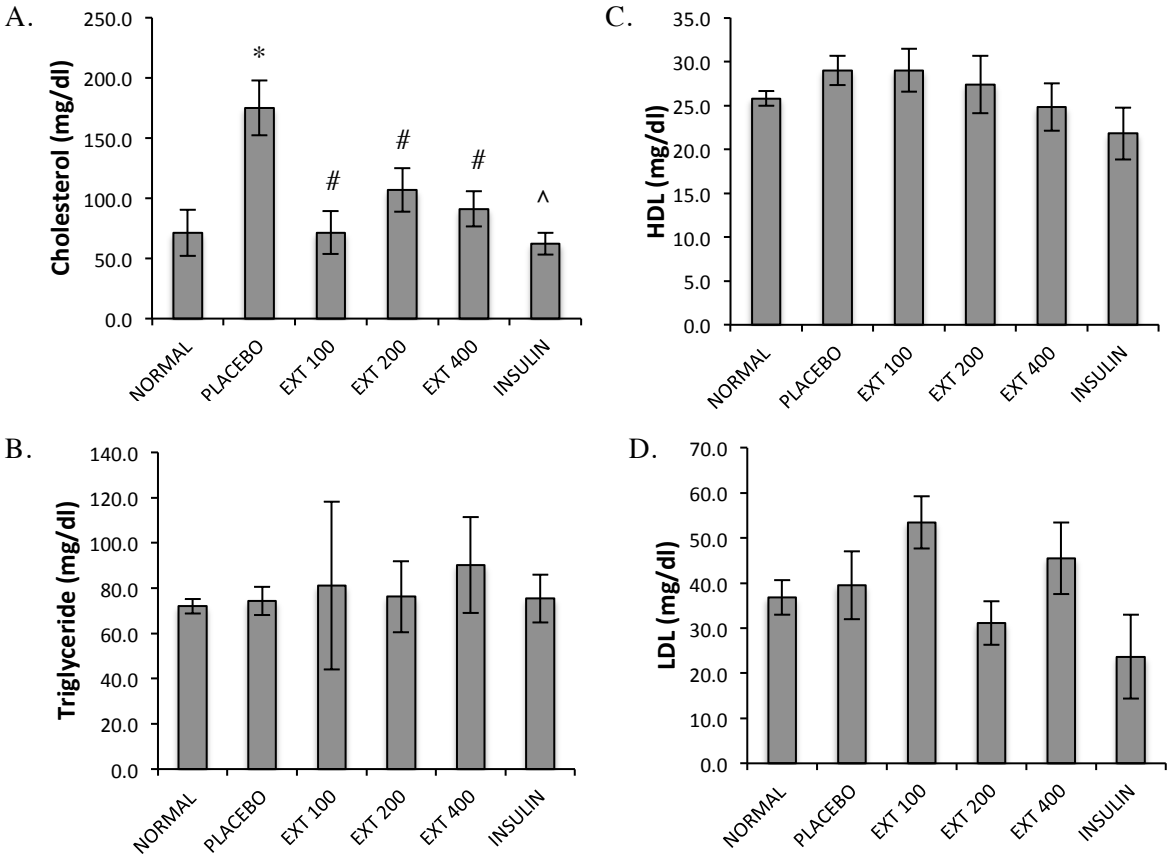

Figure 1. Lipid profiles in non-diabetic (normal) and diabetic rats receiving placebo, Artocarpus leaf extract 100, 200 and 400 mg/kg, or insulin injection. A) Total Cholesterol; B) Triglyceride; C) High Density Lipoprotein; D) Low Density Lipoprotein
*P<0.05 between placebo group and normal controls
#P<0.05 between extract-treated groups and placebo
^P<0.05 between insulin group and placebo

Weight loss is one of the symptoms of hyperglycemia in diabetes. Diabetic weight loss is caused by a decrease in insulin's anabolic effect to increase body weight by stimulating the synthesis of carbohydrates, proteins and fats (lipogenesis). In the absence of insulin, glucose absorption decreases in body tissues and lipid mobilization increases from adipocytes [14]. The results of body weight measurement of alloxan-induced diabetic rats are depicted in table 1.

Table 1. The profile of rat body weight before alloxan injection (baseline), after treatment and overall changes in body weight of rats

Groups	Baseline weight (a)	Post treatment (b)	Weight change (b-a)	Percentage of animal with weight loss (%)
Normal (n=5)	243.4±20.0 g	259.2±18.3 g	+15.8±6.0 g	0
Placebo (n=5)	210.4±15.3 g	188.8±13.3 g	-21.6±13.1 g	80
Ext 100 (n=5)	239.0±17.8 g	224.6±35.5 g	-14.4±19.4 g	40
Ext 200 (n=5)	232.4±17.8 g	216.0±17.8 g	-16.4±9.3 g	80
Ext 400 (n=5)	206.0±3.3 g	205.0±16.2 g	-1.0±14.3 g	40
Insulin (n=5)	226.0±14.4 g	225.2±7.3 g	-0.8±11.3 g	40

+ weight change = weight gain; **-**weight change = weight loss

Changes in body weight varied in each treatment group. The normal group experienced an average of body-weight gain of 15.8 grams. Significant weight loss was shown in the placebo, extract 100 and extract 200 groups, with an average of weight loss of 21.6 grams, 14.4 grams, and 16.4 grams respectively. Meanwhile, the insulin group and the 400 mg/kg extract group experienced only a slight decrease in body weight (<1.0 gram). Additionally, the number of rats experienced weight loss in placebo group was 80%, while only 40% of rats treated with extract 400 mg/kg or insulin experienced weight loss. This could suggest the potential role of Artocarpus leaf extract to improve the lipid metabolism in diabetic rats. The effect of Artocarpus leaf extract may be provided by its flavonoid compound that has been shown to be cytoprotective against LDL oxidation .

4. Conclusion

The administration of *Artocarpus altilis* extract at all given doses reduced the total cholesterol in diabetic rats, but only at the dose of 400 mg/kg Artocarpus extract could prevent weight loss in alloxan-induced diabetic rats.

5. Acknowledgement

The authors would like to thank The Indonesian Ministry of Research, Technology and Higher Education for the research grant support.

6. Conflict of interest

The authors declared no conflict of interests.

References

[1] Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, et al. IDF diabetes atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Research and Clinical Practice* 2018; **138** pp 271-281.

[2] Rudijanto A, Saraswati MR, Yunir E, Kumala P, Puteri HH, and Mandang VV. Indonesia cohort of io hat study to evaluate diabetes management, control, and complications in retrospective and prospective periods among insulin-treated patients with type 1 and type 2 diabetes. *Acta Medica Indonesiana* 2018; **50**(1) pp 26-37.

[3] Parhofer KG. Interaction between glucose and lipid metabolism: More than diabetic dyslipidemia. *Diabetes & metabolism journal* 2015; **39**(5) pp 353-362.

[4] Bodeker G and Ong C-K, *Who global atlas of traditional, complementary and alternative medicine*. Vol. 1. 2005: World Health Organization.

[5] Jung M, Park M, Lee HC, Kang Y-H, Kang ES, and Kim SK. Antidiabetic agents from medicinal plants. *Current medicinal chemistry* 2006; **13**(10) pp1203-1218.

[6] Hidayatulloh AS and Hikmayani NH. Pengaruh pemberian ekstrak daun sukun (artocarpus altilis f.) terhadap perbaikan jumlah sel-β Pankreas pada tikus (rattus norvegicus) model diabetes melitus. *Nexus Biomedika* 2014; **3**(2) pp 14-15
[7] Wahyudin, Massi MN, Natzir R, Alam G, and Stevani H. Analysis of leaf extract breadfruit (artocarpus altilis (park.) fosberg) effect against insulin resistance in rat (rattus norvegicus) obese study on cell morphology of langerhans island. *International Journal of Sciences: Basic and Applied Research* 2017; **31** pp 316-317.
[8] Setiawati H, Djabir YY, Hardi H, Lallo S, and Cangara MH. Breadfruit leaf (*Artocarpus altilis*) extract reduces hepatic and renal damages in alloxan-induced diabetic rats. *Journal of Applied Pharmaceutical Sciences* xxxx; **x**: xxx.
[9] Wahyudin MNM, Natzir R, Alam G, and Bukhari AS. Effect of sukun leaf extract [artocarpus altilis (park.) fosberg] on insulin resistance in obese rats (rattus norvegicus): A study of free fatty acid (ffa) levels. *Pakistan Journal of Nutrition* 2017; **16**(7) pp 521-524.
[10] Ismail IS, Gezawa ID, Yahya MF, Chedi B, Nafisatu K, and Imam A. Anti-hyperglycemic effect of gasca d herbal formulation on alloxan induced diabetic rats. *Diabetes Management* 2018; **8**(4) pp 85-93.
[11] Wu L and Parhofer KG. Diabetic dyslipidemia. *Metabolism* 2014; **63**(12) pp 1469-1479.
[12] Mooradian AD. Dyslipidemia in type 2 diabetes mellitus. *Nature Reviews Endocrinology* 2009; **5**(3): 150-159.
[13] Rohilla A and Ali S. Alloxan induced diabetes: Mechanisms and effects. *International journal of research in pharmaceutical and biomedical sciences* 2012; **3**(2): 819-823.
[14] Basciano H, Federico L, and Adeli K. Fructose, insulin resistance, and metabolic dyslipidemia. *Nutrition & metabolism* 2005; **2**(1): 5.

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ACCEPTANCE LETTER

Dear, Mr./ Mrs./ Miss., **Hardi Hardi, Yulia Yusrini Djabir, Hesty Setiawati and Subehan Lallo**

Thank you for your interest in the International Seminar on Bioscience and Drug Discovery (ISBDD) 2019 and the submission of your abstract.

We are pleased to state that your paper based on abstract entitled:

he effect of Artocarpus altilis leaf extract administration on lipid profiles and weight loss in alloxan-induced diabetic rats

has been **ACCEPTED** by the committee.

We are looking forward to seeing you at ISBDD 2019, Makassar, South Sulawesi.

Thank you.

Sincerely yours,

Muhammad Aswad, Ph.D., Apt.
Chairman of the Organizing Committee

Dokumen pendukung luaran Tambahan #1

Luaran dijanjikan: Publikasi Ilmiah Jurnal Internasional

Target: submitted
Dicapai: Submited

Dokumen wajib diunggah:
1. Bukti submit
2. Naskah artikel

Dokumen sudah diunggah:
1. Naskah artikel
2. Bukti submit

Dokumen belum diunggah:
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BREADFRUIT LEAF (*Artocarpus altilis*) EXTRACT REDUCES HEPATIC AND RENAL DAMAGES IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

Alloxan is widely used to induce diabetes mellitus; however, it may also cause liver and renal injuries. This study aimed to examine breadfruit leaf (*Artocarpus altilis*) extract effects on hepatic and renal injuries in alloxan-induced diabetic rats. Rats were divided into 5 groups: healthy controls, alloxan group that was injected with alloxan without extract treatments, and treatment groups that was injected with alloxan, followed by breadfruit leaf (BL) extract treatment at the dose of 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight, respectively. SGPT and creatinine levels were measured at baseline, post alloxan injection and following 14-day treatments. The results showed a significant increase in rat SGPT and creatinine levels 3-day post alloxan injection ($P<0.05$). SGPT level was reduced significantly with BL extracts 200 mg/kg and 400 mg/kg ($P<0.05$), however, the decrease in creatinine levels was similar to alloxan group. The damages in the liver tissues due to alloxan was alleviated by BL extract administration, especially at 400 mg/kg dose. However, renal tubule degeneration was still found following BL extract treatment. In conclusion BL extract at 400 mg/kg dose may improve liver dysfunction and damage due to alloxan, but less effective to recover renal injury.

Keywords: Breadfruit leaf extract, Artocarpus altilis, alloxan, hepatic damage

INTRODUCTION

Alloxan (2,4,5,6-tetraoxypyrimidine) is a chemical compound that is widely used to induce diabetes mellitus in experimental animals. The molecule structure of alloxan that resembles glucose molecules enables its uptake through glucose transport GLUT-2 in beta cells of

pancreas (Lenzen, 2008). This facilitates the selective entry of alloxan into beta cells of pancreas and ultimately damages these insulin-producing cells. As a result, animals that are injected with alloxan will experience a decrease in insulin production leading to hyperglycemia.

Although alloxan was initially thought to merely damage the pancreatic beta cells (Gorus*, et al.*, 1982), increasing number of studies have shown that alloxan injection is not only toxic to pancreatic beta cells, but also toxic to other organs expressing GLUT-2, including liver hepatocytes and kidney tubular cells (Gargouri*, et al.*, 2016; Terayama*, et al.*, 2016). Thus, it is often found that alloxan-induced diabetic animals also experience liver and kidney dysfunction.

Since alloxan toxicity is mediated by reactive oxygen species, from superoxides, hydrogen peroxydes and finally radical hydroxyl (Lenzen, 2008), it is believed that plant extract that rich in antioxidant compounds may reduce the oxidative damage induced by alloxan. Breadfruit leaf (*Artocarpus altilis* (Parkinson) Fosberg) extract has been known to contain tannins, phenols, glycosides, saponins, steroids, terpenoids and anthraquinones with moderate antioxidant activities (Sikarwar*, et al.*, 2014a; Sikarwar*, et al.*, 2014b). It has been shown that breadfruit leaf extract was able to reduce oxidative damage in rat testicular induced by cadmium (Adaramoye & Akanni, 2016). Based on that, this study aimed to explore the potential use of breadfruit leaf extract to reduce hepatic and renal damages in diabetic rats induced by alloxan.

MATERIALS AND METHODS

Chemical preparation

Alloxan monohydrate (Sigma Aldrich) and other chemicals including 70% ethanol were purchased from official chemical distributors in Indonesia. Reagents for Glutamate-Pyruvate Transaminase (GPT (ASAT) IFCC mod.liquiUV and Creatinine (creatinine liquicolor) measurements were purchased from HUMAN Diagnostic Worldwide (Indonesia).

Breadfruit leaf extract preparation

Leaves of breadfruit plant (*Artocarpus altilis* (Parkinson) Fosberg) that has turned into a yellowish color were picked from the trees and used as samples. This particular type of leaves has been used empirically in Indonesia region to treat hyperglycemia in diabetic patients. The leaves were then washed, dried and cut into 0.5 cm simplicia. Simplicia was macerated with 1:10 ratio in 70% ethanol for 5 days. The maceration process was protected from sunlight. Remaceration is performed twice using the same volume of solvent. The ethanolic extract obtained was then thickened using a rotary evaporator (Heidolp®) and preserved in a desiccator at a room temperature (25^{o}C) to reduce extra solvent. Prior to administration, the extract was prepared in a 1% NaCMC suspension to facilitate extract administration to animals.

Animal preparation

Male Wistar rats with 12 weeks of age (180-300 g) were placed in plastic cages with wood shaving as bedding where food and water were prepared ad libitum. All animal protocols were performed in accordance with international principle of laboratory animal care. The experiment has granted ethical clearance from Institutional Animal Ethics Committee under Hasanuddin University with the number of UH19050277. The number of rats used in this study was calculated based on sample calculations for a complete random design using the Federer formula with equation $(t-1)(n-1) \geq 15$. Based on the formula, each treatment group in

this study consisted of 5 animals. All animals were adapted at least 14 days prior to initiation of experiment.

Experimental protocols

Thirty rats were intraperitonially injected with alloxan (155 mg/kg bw). After alloxan injection, rats were given a 5% glucose solution orally (2 ml/200 g bw) to avoid acute hypoglycemia. Blood glucose level was measured everyday post alloxan injection and after 72 hours blood glucose level was also confirmed with Humalyzer (Human®). Only rats that had blood glucose level of >200 mg/dl 3 days post alloxan injection was included in the study, while those that did not experience hyperglycemia was excluded (n=10).

Another group of rats (n=5) that were not injected with alloxan was added to serve as healthy controls (Group I). Meanwhile, the hyperglycemic rats (n=20) were randomly assigned into 4 groups and were treated accordingly: 1% Na CMC suspension without extract (Group II), Breadfruit leaf (BL) extract 100 mg/kg bw (Group III), Breadfruit leaf (BL) extract 200 mg/kg bw (Group IV), and Breadfruit leaf (BL) extract 400 mg/kg bw (Group V). Treatments were administered for 14 days. Blood samples were taken prior to alloxan injection, 3 days after alloxan injection and after 14 days of treatments.

Biomarker analysis

Blood samples (3 ml) were withdrawn from lateral vein and placed in vacutainers containing EDTA. Blood was immediately centrifuged at 3000 rpm for 20 minutes to obtain the serum, which was then placed in a refrigerator (-20^{o}C) until further analysis. The SGPT and creatinine levels were analyzed using reagent kits for Humalyzer 3000 (Human®) based on the kit instruction.

Histopathological analysis

Following the 14 days of treatment, the animals were euthanized by cervical dislocation. Rat livers and kidneys were removed then washed with PBS before fixated with 10% formaldehyde. The tissue specimens were processed in a tissue processor and made in to tissue blocks with paraffin. The tissue blocks were sliced using microtome about 4-5 μm thickness then stained using Hematoxylin and Eosin (HE). The tissue specimens were observed under light microscope connected to a camera (Nikon®). The presence of histopathological changes, such as necrotic cells, tissue degeneration, and inflammation were analyzed by an anatomic pathologist blinded to the treatment groups. The histopathological scores were determined by the pathologist using methods that described in other study (Gibson-Corley*, et al.*, 2013).

Statistical Analysis

The data obtained were analyzed with SPSS program. The SGPT and creatinine levels were tested for normal distribution with Kolmogorov Smirnov test. One Way ANOVA statistical test was performed at a 95% confidence level to determine significant difference for the group treatments followed by post hoc Tukey's HSD test. Paired T test was used to compare baseline and post alloxan injection data, as well as post-injection and post-treatment data within each group.

RESULTS AND DISCUSSION

Blood glucose level and rat body weight following alloxan injection

Prior to experiment, rats included in this study had blood glucose level ranged from 82-110 mg/dl and body weight of 180-300 g. Following alloxan intraperitonial injection (155 mg/kg

bw), most rats experienced a marked increase in blood glucose levels and randomly assigned to either receive no treatment (Alloxan group) or BL extract treatments. The increase of blood glucose following 3-day post alloxan injection is presented in table 1. The blood glucose of rats post alloxan injection ranged from 200 to 449 mg/dl, which is 2 to 4 times higher than control rats that did not receive alloxan injection. Meanwhile, in the control group, blood glucose levels were within the normal range of <140 mg / dl. There are three mechanism by which alloxan induces hyperglycemia: 1) it selectively inhibits insulin secretion through glucokinase inhibition, 2) detection of sugar by beta cells, and 3) inducing ROS formation, causing selective necrosis of beta cells, which ultimately leads to an insulin-dependent diabetes condition (Lenzen, 2008).

Table 1. Rat blood glucose and body weight prior to and following 3 days from alloxan injection before treatment initiation

Treatment group	**Blood glucose (mg/dl)**		**Body weight (g)**	
	Before injection	**After injection**	**Before injection**	**After injection**
Control (no alloxan)	104 ± 4.0	136 ± 22.5	196 ± 11.5	201 ± 11.7
Aloxan	107 ± 5.2	276 ± 26.6*	224 ± 26.2	212 ± 26.7
Alloxan + BL 100 mg/kg	103 ± 6.4	292 ± 43.7*	236 ± 15.1	220 ± 21.3
Alloxan + BL 200 mg/kg	107 ± 4.8	261 ± 25.3*	234 ± 22.9	221 ± 25.5
Alloxan + BL 400 mg/kg	111 ± 4.5	372 ± 63.2*	206 ± 3.3	197 ± 7.3

The body weight of rats following alloxan injection slightly decreased, even though it was not statistically significant. Each group that injected with alloxan lost weight 9 g to 16 g only after 3 days from injection, while the control group gain weight as much as 5 g in average (table 1). The features of diabetic rats include weight loss (>10% of body weight), lethargy and soft stool (Wang-Fischer & Garyantes, 2018), and we did observe these features in groups that received alloxan injection.

Liver function and tissue structure following alloxan injection and post treatment

Measurement of SGPT levels was carried out 3 times: prior to alloxan injection, 3-day post alloxan injection and post 14-day treatment. It is shown in figure 1 that alloxan injection significantly increased the level of SGPT following 3 days of injection. The increase in the mean level of SGPT in alloxan group, alloxan + BL 100, alloxan + BL 200 and alloxan BL 400 were 62%; 100%; 34%; 113%, respectively. Indeed, the SGPT level of rats in the alloxan group continued to increase to >200 mg/dl, which was five times higher than their baseline level ($P<0.05$).

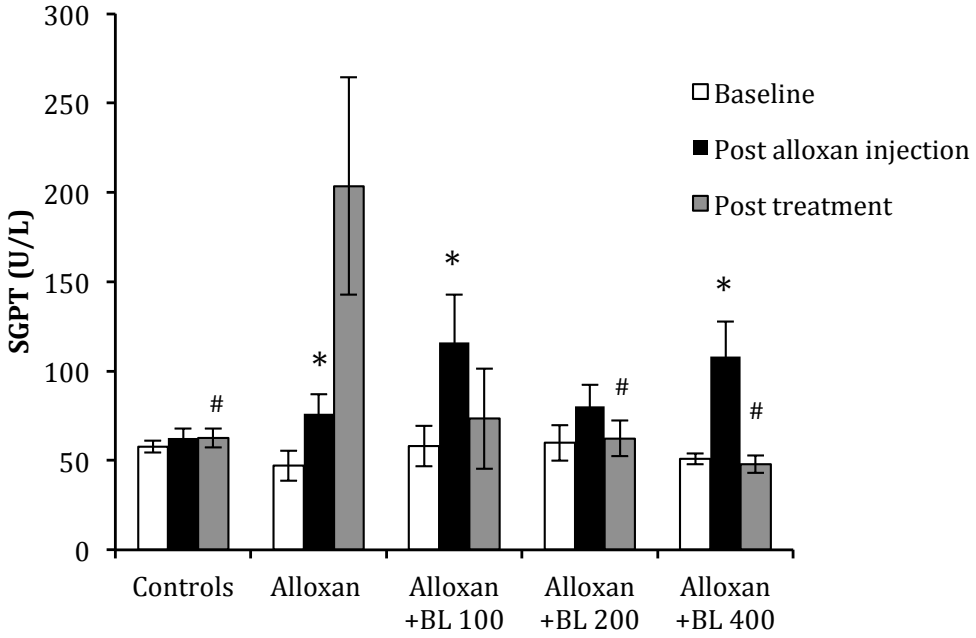

Figure 1. Serum glutamic pyruvic transaminase (SGPT) level of treated rats at baseline, post alloxan injection and post 14 days of treatments. *shows $P<0.05$ compared to baseline level. #shows $P<0.05$ compared to alloxan group post treatment.

Increased SGPT is a specific indicator for liver dysfunction as well as liver damage. The presence of liver damage can be triggered during uncontrolled hyperglycemia (Gargouri*, et al.*, 2016). If this condition continues, it increases ROS production that will bind to hepatocytes, causing hepatocyte degeneration to cellular necrosis (Lucchesi*, et al.*, 2015). Supporting this, it is shown that induction of alloxan at a dose of 155 mg/kg BW in this study

led to fatty and hydrophic degeneration and most importantly alloxan injection causes widespread necrosis in the liver of alloxan group (Figure 2B and 2C).

From figure 1, it is shown that Breadfruit leaf (BL) extract treatment can protect the increase of SGPT. The most effective dose was found to be 400 mg/kg bw. With that given dose, the SGPT level returned to its normal level despite of initial increase post alloxan injection ($P < 0.05$). The levels of SGPT after treatment in group alloxan + BL 200 were also significantly lower than alloxan group, but when compared to alloxan + BL 400 the reduction of SGPT was less significant.

Oral administration of breadfruit leaf ethanol extract appears to improve hepatocyte cell structure. In lower dose (100 mg/kg body weight), the rat's liver experiences hydropic degeneration (figure 2D), reaching 50% of damage. As for the dose 200 mg/kg (figure 2E) and 400 mg/kg (figure 2F) hepatocytes still shown a sign of hydropic changes but significantly less inecrotic cells was found compared to alloxan group without extract treatment. Liver damage that occurs due to alloxan may varied ranging from inflammation to necrosis (Bilal*, et al.*, 2016). The improvement of liver structure seen with BL extract is enabled by the presence of potent antioxidants and high phenolic content in BL (Leng*, et al.*, 2018), which can stabilize reactive oxygen species produced by alloxan .

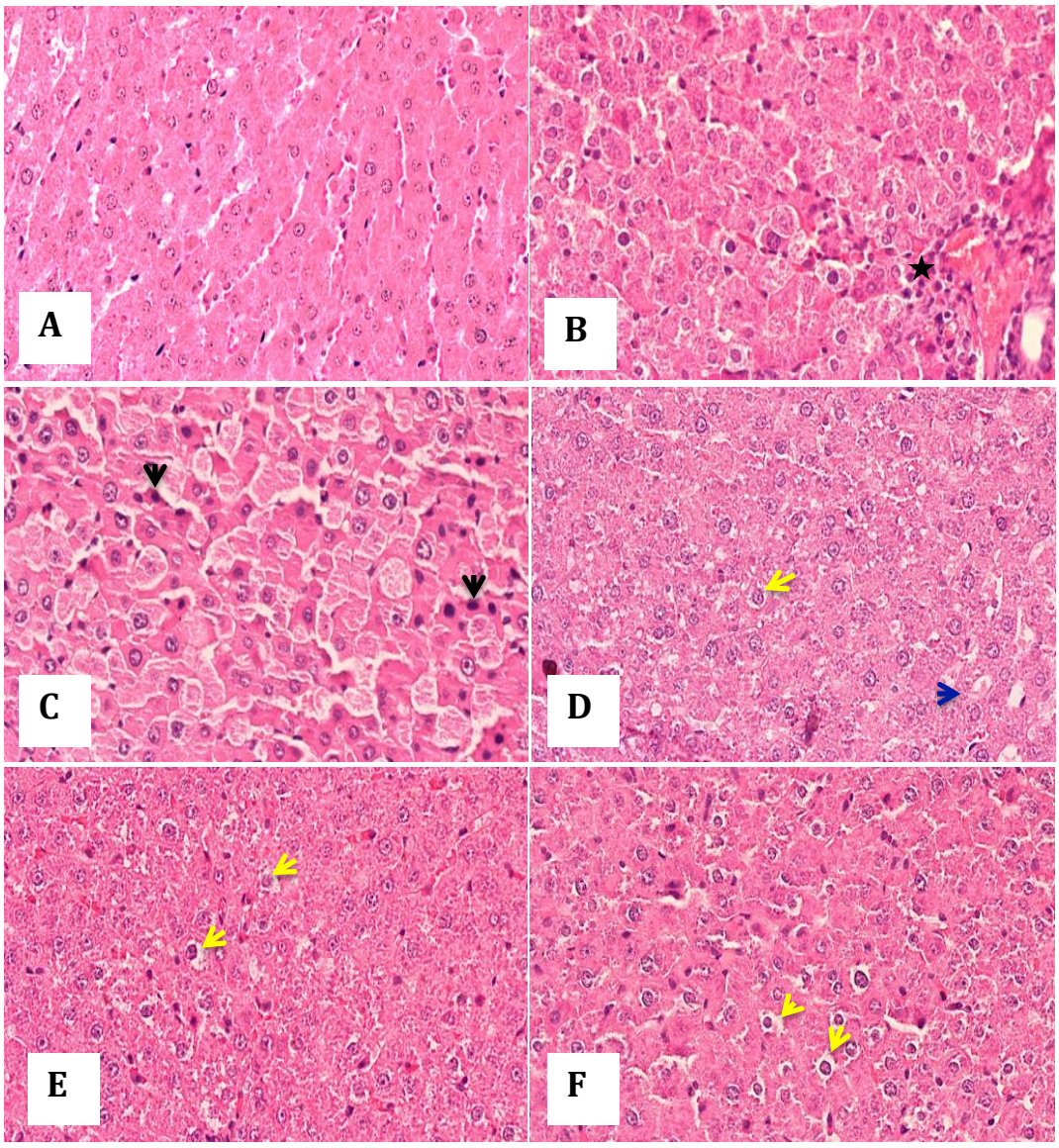

Figure 2. The photomicrograph of liver tissues of rats following 14 days of treatments (H&E stained, 400X magnification). **A**) Control group showed normal architecture of hepatocytes and portal triad. **B**) Alloxan group showed infiltration of inflammatory cell in liver tissue (★). **C**) Diffuse necrotic area and necrotic cells were found scattered in Alloxan-treated rat (black arrow). **D**) Liver from rat treated with Alloxan + BL extract 100 mg/kg showed hydropic degeneration (yellow arrow) and dilated sinusoid (blue arrow). **E**) Rat's liver treated with Alloxan + BL extract 200 mg/kg mostly showed hydropic changes (yellow arrow). **F**) Rat's liver treated with Alloxan + BL extract 400 mg/kg showed hydropic degeneration (yellow arrow).

Renal function and tissue structure following alloxan injection and post treatment

Figure 3 depicted the serum creatinine level of normal rats and rats treated with alloxan at baseline, 3-day post alloxan injection and 14 day post treatment. After alloxan injection, the creatinine level significantly elevated in alloxan groups and all alloxan + BL groups compared to the normal control group ($P < 0.05$). Rats treated with alloxan experiences at least 120 % elevation in creatinine level in 3-day post alloxan injection. It is believed that elevated creatinine level occur due to the accumulation of glycogen in the distal tubules of the kidney when rats experiencing continuous hyperglycemia (Terayama*, et al.*, 2016). However, the creatinine level of alloxan treated rats simultaneously decreased by 50% following 14-day from alloxan injection, regardless whether the rats were treated with BL extract or not (Figure 3). This renders none of the treatment groups reaches significant differences since serum creatinine of all groups returned to normal level after 14 days of alloxan injection.

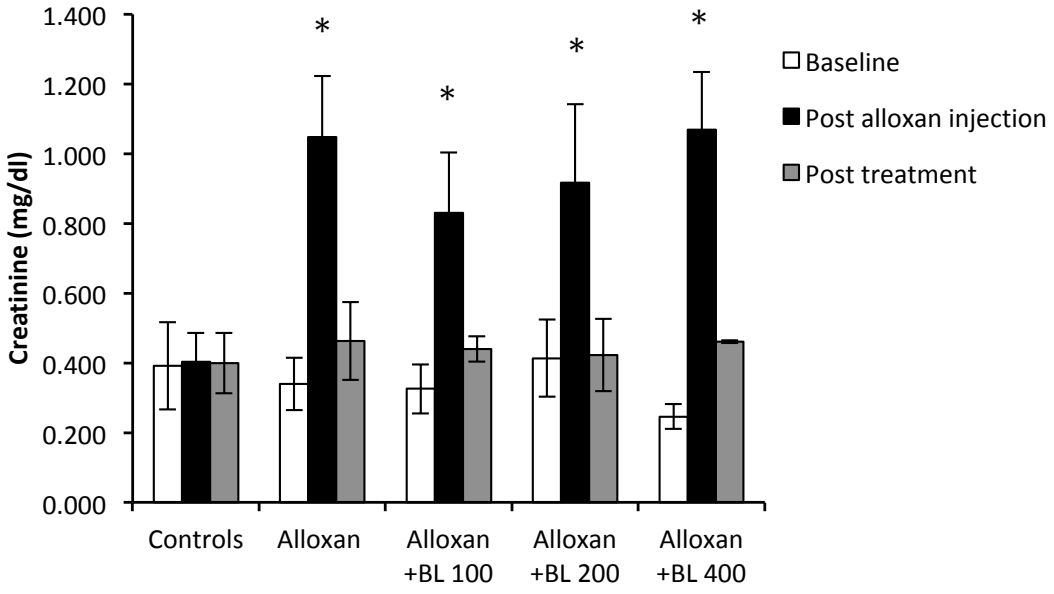

Figure 3. Serum creatinine level of rats at baseline, post alloxan injection and post 14 days of treatments. *shows $P<0.05$ compared to baseline level

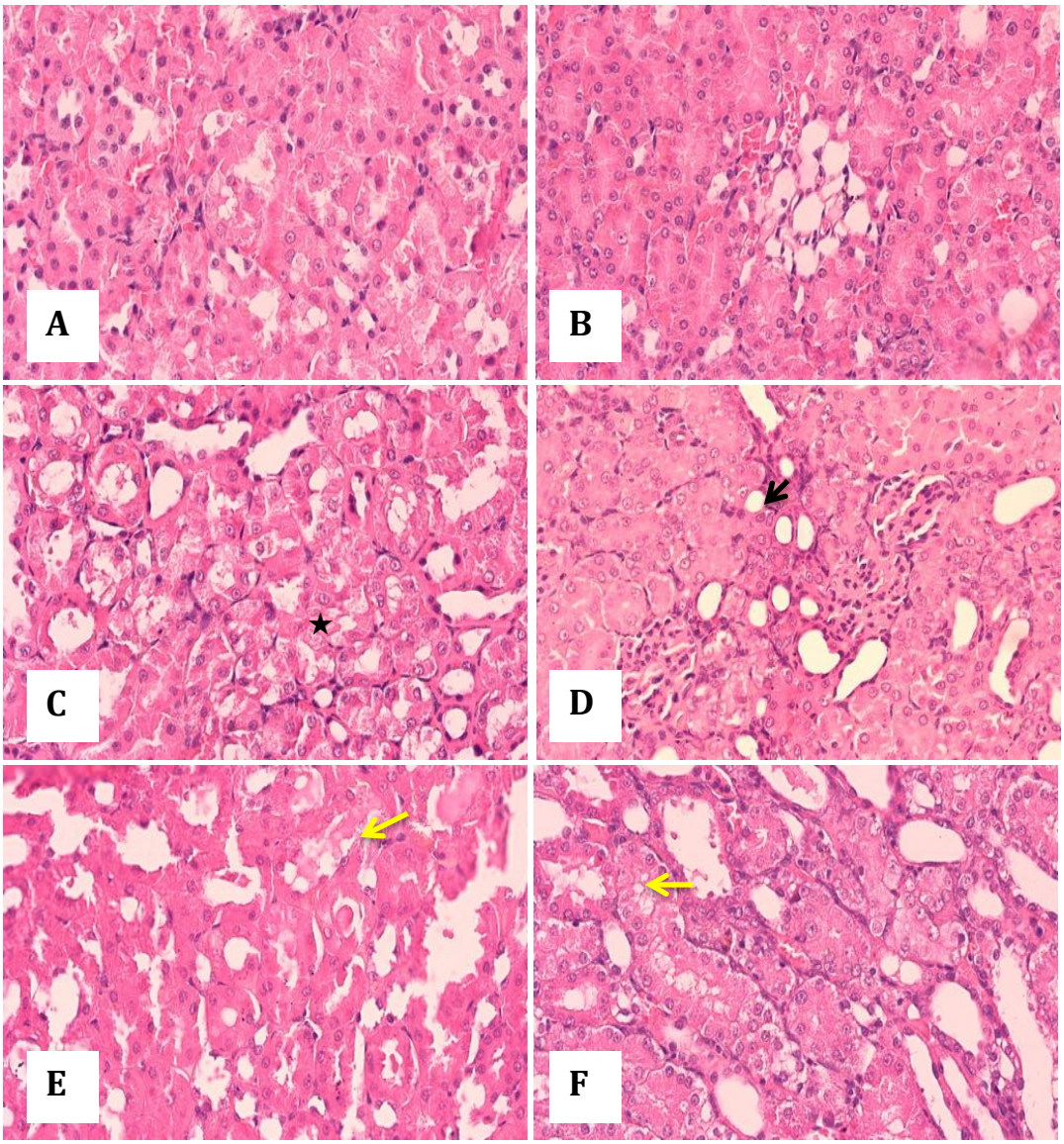

Figure 4. The photomicrograph of renal tissues of rats following 14 days of treatments (H&E stained, 400X magnification). **A**) Control group showed normal architecture of renal tubules. **B**) Alloxan group showed substantial lipid degeration in the tubules. **C**), Alloxan group experienced degeneration in the renal tubule (★). **D**) Rat treated with Alloxan + BL extract 100 mg/kg showed lipid degeneration (black arrow). **E**) Rat treated with Alloxan + BL extract 200 mg/kg showed hydropic degeneration (yellow arrow). **F**) Rat treated with Alloxan + BL extract 400 mg/kg showed hydropic degeneration (yellow arrow).

The fact that serum creatinine level back to baseline level could become an indication that alloxan did not induce permanent damage to the kidneys. Similar result was also found by other study showing no increased in creatinine level after 30 days of alloxan injection in diabetic rabbits (Ahmad*, et al.*, 2008).

It is believed that the increase of creatinine is more likely to reflect an acute renal dysfunction rather than permanent renal damage as it return to baseline level after 14 days of injection. Interestingly, at the same time, renal histological changes were still prominent (25 to 50% of the field of view at 400X magnification) in rats that treated with alloxan only (figure 4B and 4C), where fatty degeneration and hydropic changes were observed in their renal tubules. The administration of BL extract seemed to slightly reduce the score of the damage, but most rats showed alteration in renal structures, mostly presenting hydropic degeneration. In alloxan + BL 100 group, lipid degeneration was observed (figure 4D), while in alloxan + BL 200 and 400 showed some hydropic degeneration in renal tubules (figure 4E and 4F). The discrepancy between serum creatinine level and histopathological changes found in the tubules suggests that serum creatinine may not be the best biomarker for tubular injury (Chu*, et al.*, 2016). Further study needs to include other biomarkers to measure the degree of kidney injuries due to alloxan injection.

CONCLUSION

The injection of alloxan 155 mg/kg in rats led to hyperglycemia 3-day post injection. In addition, the rats experienced liver and renal dysfunction as well as histopathological changes in liver and kidneys. Administration of breadfruit leaf extract is shown to reduce SGPT level, indicating its potential role in improving alloxan-induced liver dysfunction. Breadfruit extract can also alleviate the liver injuries as well as renal damage to a lesser extent. Due to spontaneous return of serum creatinine level, it is difficult to assess breadfruit leave extract significance in alloxan-induced renal dysfunction.

REFERENCES

Adaramoye, O.A., and Akanni, O.O. Protective effects of Artocarpus altilis (Moraceae) on cadmium-induced changes in sperm characteristics and testicular oxidative damage in rats. *Andrologia,* 2016*;* 48(2): 152-163.

Ahmad, M., Zaman, F., Sharif, T., and Ch, M.Z. Antidiabetic and Hypolipidemic Effects of Aqueous Methanolic Extract of Acacia Nilotica Pods in Alloxan-Induced Diabetic Rabbits. *Scand. J. Lab. Anim. Sci,* 2008; 35(1): 49-53.

Bilal, H.M., Riaz, F., Munir, K., Saqib, A., and Sarwar, M.R. Histological changes in the liver of diabetic rats: A review of pathogenesis of nonalcoholic fatty liver disease in type 1 diabetes mellitus. *Cogent Medicine,* 2016*;* 3(1): 1275415.

Chu, X., Bleasby, K., Chan, G.H., Nunes, I., and Evers, R. The Complexities of Interpreting Reversible Elevated Serum Creatinine Levels in Drug Development: Does a Correlation with Inhibition of Renal Transporters Exist? *Drug Metabolism and Disposition,* 2016*;* 44(9): 1498-1509.

Gargouri, M., Magné, C., and El Feki, A. Hyperglycemia, oxidative stress, liver damage and dysfunction in alloxan-induced diabetic rat are prevented by Spirulina supplementation. *Nutrition Research,* 2016; 36(11): 1255-1268.

Gibson-Corley, K.N., Olivier, A.K., and Meyerholz, D.K. Principles for valid histopathologic scoring in research. *Veterinary pathology*, 2013; 50(6): 1007-1015.

Gorus, F.K., Malaisse, W.J., and Pipeleers, D.G. Selective uptake of alloxan by pancreatic B-cells. *Biochemical Journal,* 1982; 208(2): 513-515.

Leng, L., binti Nadzri, N., Khor, C.Y., Abdul razak, N., and Razak Shaari, A. Antioxidant and Total Phenolic Content of Breadfruit (Artocarpus altilis) Leaves. *MATEC Web of Conferences*, 2018*;* 150: 06007.

Lenzen, S. The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia,* 2008*;* 51(2): 216-226.

Lucchesi, A.N., Cassettari, L.L., and Spadella, C.T. Alloxan-induced diabetes causes morphological and ultrastructural changes in rat liver that resemble the natural history of chronic fatty liver disease in humans. *Journal of diabetes research,* 2015*;* 2015: 494578-494578.

Sikarwar, M.S., Hui, B.J., Subramaniam, K., Bavani, Valeisamy, D., Yean, L.K., et al. A Review on Artocarpus altilis (Parkinson) Fosberg (breadfruit). *J App Pharm Sci,* 2014a; 4(08): 091-097.

Sikarwar, M.S., Hui, B.J., Subramaniam, K., Valeisamy, B.D., Yean, L.K., and Balaji, K. Antioxidant activity of Artocarpus altilis (Parkinson) Fosberg leaves. *Free Radicals & Antioxidants,* 2014b; 4(2).

Terayama, Y., Kodama, Y., Matsuura, T., and Ozaki, K. Acute alloxan renal toxicity in the rat initially causes degeneration of thick ascending limbs of Henle. *J Toxicol Pathol,* 2016*;* 30: 7–13.

Wang-Fischer, Y., and Garyantes, T. Improving the Reliability and Utility of Streptozotocin-Induced Rat Diabetic Model. *Journal of diabetes research,* 2018*;* 2018: 8054073-8054073.

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RESEARCH ARTICLE

Potential Use of Breadfruit (*Artocarpus altilis*) Leaf Extract to Recover Hepatic and Renal Damage in Alloxan-Induced Diabetic Rats

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Potential Use of Breadfruit (Artocarpus altilis) Leaf Extract to Recover Hepatic and Renal Damage in Alloxan-Induced Diabetic Rats

SUMMARY

The antihyperglycemic effect of breadfruit leaf (Artocarpus altilis) extract has been demonstrated in a preclinical study using an alloxan-induced diabetic model. This study aimed to examine whether breadfruit leaf extract also ameliorated liver and kidney injury in alloxan-induced diabetic rats. Male Wistar rats (n=35) were used in the study. All other animals except control group (group I, n=5) were injected with alloxan (155 mg/kg body weight). After 3 days, the hyperglycemic rats with blood glucose >200 mg/dl were divided into 4 treatment groups: placebo (alloxan group), Breadfruit Leaf (BL) extract 100 mg/kg, BL extract 200 mg/kg, and BL extract 400 mg/kg. Treatments were administered daily for 14 days, and blood samples were drawn at baseline, after alloxan injection, and following treatments to obtain serum glutamic pyruvic transaminase (SGPT) and creatinine levels. Alloxan was found to cause a significant increase in rat blood glucose, SGPT, and creatinine levels three days post alloxan injection (P<0.01). After treatment, rats that received 200 mg/kg and 400 mg/kg BL extracts had significantly lower SGPT levels compared to those treated with placebo alone (P<0.05). Liver histological damage was also significantly alleviated, especially with the 400 mg/kg dose of BL extract. Although serum creatinine level was restored, alloxan-induced tubular degeneration in renal tissue was still evident. In conclusion, BL extract at a dose of 400 mg/kg improved alloxan-induced liver dysfunction and tissue damage but was less effective at alleviating kidney damage. This result may support the use of breadfruit leaf extract as herbal drug with a hepatoprotective effect.

Key Words: *Breadfruit leaf, Artocarpus altilis, diabetic rats, alloxan, liver damage, kidney damage*

Alloxan Nedenli Diyabetik Sıçanlarda Hepatik ve Renal Hasarı İyileştirmek İçin Ekmek Meyvesi (Artocarpus altilis) Yaprak Ekstresinin Potansiyel Kullanımı

ÖZ

Ekmek meyvesi yaprağı (Artocarpus altilis) ekstresinin antihiperglisemik etkisi, alloksan nedenli diyabet modeli kullanılarak yapılan in vivo bir çalışmada gösterilmiştir. Bu çalışma, ekmek meyvesi yaprak ekstresinin, alloksan nedenli diyabetik sıçanlarda karaciğer ve böbrek hasarını iyileştirip iyileştirmediğini incelemeyi amaçlamıştır. Çalışmada erkek Wistar sıçanları (n=35) kullanılmıştır. Kontrol grubu (grup I, n=5) dışındaki tüm diğer hayvanlara alloksan (155 mg/kg vücut ağırlığı) enjekte edilmiştir. Üç gün sonra, kan şekeri >200 mg/dl olan hiperglisemik sıçanlar 4 tedavi grubuna ayrılmıştır: plasebo (alloksan grubu), ekmek meyvesi yaprak (BL) ekstresi 100 mg/kg, BL ekstresi 200 mg/kg; ve BL ekstresi 400 mg/kg. Tedaviler 14 gün boyunca günlük olarak uygulanmış ve başlangıçta, alloksan enjeksiyonundan sonra ve tedavileri takiben serum glutamik piruvik transaminaz (SGPT) ve kreatinin düzeylerini belirlemek için kan örnekleri alınmıştır. Alloksanın, enjeksiyondan 3 gün sonra sıçan kan şekeri, SGPT ve kreatinin seviyelerinde önemli bir artışa neden olduğu bulunmuştur (P<0.01). Tedaviden sonra, 200 mg/kg ve 400 mg/kg BL ekstreleri uygulanan sıçanların, tek başına plasebo ile tedavi edilenlere kıyasla önemli ölçüde daha düşük SGPT seviyelerine sahip olduğu görülmüştür. (P<0.05). Karaciğer histolojik hasarı da, özellikle 400 mg/kg dozda BL ekstresi ile önemli ölçüde azalmıştır. Serum kreatinin düzeyi eski haline gelmesine rağmen, böbrek dokusunda alloksan nedenli tübüler dejenerasyonun hala belirgin olduğu gözlenmiştir. Sonuç olarak, 400 mg/kg vücut ağırlığı dozunda uygulanan BL ekstresi, alloksan nedenli karaciğer fonksiyon bozukluğunu ve doku hasarını iyileştirmiş, ancak böbrek hasarını iyileştirmede daha az etkili bulunmuştur. Bu sonuç, hepatoprotektif etkili bir bitkisel ilaç olarak ekmek meyvesi yaprağı ekstresinin kullanımını destekleyebilir.

Anahtar Kelimeler: *Ekmek meyvesi yaprağı, Artocarpus altilis, diyabetik sıçanlar, alloksan, karaciğer hasarı, böbrek hasarı.*

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The effect of breadfruit (*Artocarpus altilis* (Parkinson) Fosberg) leaf extract on blood glucose, lipid profiles, and weight loss in alloxan-induced diabetic rats

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ABSTRACT

Diabetes mellitus is associated with abnormalities in lipid metabolism and weight loss. This study aimed to examine breadfruit (*Artocarpus altilis* (Parkinson) Fosberg) leaf extract's effects on lipid profiles and weight loss in alloxan-induced diabetic rats. Forty-five male Wistar rats were injected with alloxan and divided into treatment groups: placebo, *Artocarpus altilis* leaf extract (100, 200, or 400 mg/kg) or insulin (6U/200 g). Five additional rats were included as normal controls. Following 14 days of treatments, *Artocarpus altilis* extract lowered the blood glucose (BG) level, but only significant at 400 mg/kg dose. Eighty percent of rats in the placebo group had a significant weight loss compared to 40% of rats in the 400 mg/kg group. The placebo group had significantly higher total cholesterol (TC) compared to controls ($p<0.05$) and the *Artocarpus altilis* extract treatment significantly reduced the TC level ($p<0.05$). In conclusion, *Artocarpus altilis* extract treatment improves BG, lipid metabolism, and weight loss in alloxan-induced diabetic rats.

Keywords: *Artocarpus altilis*, breadfruit, alloxan, dyslipidemia, weight loss

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