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THEME

Development of a natural dietary supplement utilizing the leaves of medicinal plants (Ficus carica and Olea europaea) for the regulation of blood glucose levels

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بسم الله الرحمن الرحيم

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فبينما كنتُ أتساءلُ كيف لي أن أجتازَ هذا الغِمارَ بمفردي واللهُ ونيسي؛ التفتُّ فألفيتُ أبويَّ هما الزميلينِ والشريكينِ لي في هذا المشروعِ.. لم يدخلا المخبرَ، ولم يخطّا في هذه المذكرةِ حرفاً، لكنهما كانا يجمعانِ لي العينات، ويتوليانِ عني كلَّ شؤونِ الخارجِ.. كانا يقولانِ لي دائماً: «كيف تظنينَ أنكِ بمفردكِ ونحنُ هنا؟ إلى الأمامِ يا صغيرتَنا، فنحنُ خلفكِ ندفعُ خطاكِ دفعاً». إلى جنتي وتفاحةِ قلبي: **بوعيطة نجاة**، وإلى ولدي وقرّة عيني **الفرحي**...أهدي هذا النجاحَ.

إلى مَن قالَ اللهُ في حقِّهم: ﴿**قَالَ سَنَشُدُّ عَضُدَكَ بِأَخِيكَ**﴾ **[القصص: 35].**

إلى سندي ومستندي، وعماد حياتي، فارسي البطلِ: **يونس**...

إلى مَن كنَّ يُنبّهنني، ويُرشدنَني، ويُعلّمنني كلَّ صغيرةٍ وكبيرة.. كيف أكونُ مسؤولةً، وكيف أكونُ أنثى، وكيف أكونُ مرنةً في مجابهةِ الحياةِ.. درر العائلةِ وزينتُها، أخواتي: **نور الهدى، قمر، وأشواق**..

إلى المشاغبينَ الصغار، أحفاد العائلةِ.. الذين مزّقوا دفاتري، وخربشوا أوراقي، وكنتُ حيزاً لتجاربِ أسنانِهم الأولى: **تقوى، شهد، عمر، غيث، عبد الفتاح، محمد أنس، ومحمد رياض** ..«خالتُكم الصغيرةُ تحبُّكم جداً».

إلى العظيمةِ التي تولّتِ المَهمّتينِ معاً، وصنعتْ من مِخبرِ العلمِ مِحراباً للأمومةِ.. إلى مَن واجهتْ ظروفَ المرضِ العاصفِ وعقباتِ الحياةِ، لتبقى طوداً شامخاً وعماداً نستندُ إليه.. إلى الروحِ المرحةِ التي لم تنلْ منها قساوةُ المحيطِ ولا أوجاعُ الجسدِ .إلى أمّي الثانيةِ، وملجئي في غُربتي، الدكتورة الغالية **:بوصبيع ابراهيم عايدة** خمسُ سنواتٍ خَلَتْ منذُ خطوتُ عتباتي الأولى في الجامعةِ، فما وجدتُكِ يوماً أستاذةً جافةً، بل أمّاً حانيةً بامتيازٍ؛ كنتِ الحضنَ الدافئَ الذي مَحا وحشةَ الغُربةِ وظلمةَ السكنِ الجامعيّ .أهدي إليكِ هذا الجهدَ، وأبتهلُ إلى اللهِ في كلّ سجدةٍ أن يَمُنَّ عليكِ بالشفاءِ العاجلِ، ويُلبسَكِ ثوبَ الصحةِ والعافيةِ، ويمدَّ في عُمركِ لتظلّي منارةً تُضيءُ دروبَنا.

إلى التي واجهتِ الإحباطَ والانكسارَ، فتحدّتْ وحاولتْ مراراً وتكراراً.. إلى التي لا ترضى بالقليلِ، ولا تَقْبلُ بشتاتِ الأمورِ على هامشِ الرحلةِ.. إلى نفسي، وحبيبتي:**رقية** التي آمنتْ بذاتِها وقدرتِها.. أقولُ لكِ: «هذهِ هي بدايةُ الطريقِ، فواصلي خُطاكِ وثبّتي مَسيركِ, وكوني كالرواسي الشامخاتِ كما عهدتُكِ؛ ثابتةً أمام العواصف، راسخةَ العزمِ والإرادة، لا تُثنيكِ الصعابُ عن بلوغِ ما تطمحين إليه. فما دام في القلبِ يقينٌ، وفي النفسِ إصرارٌ، فإنَّ القادمَ أجملُ بإذنِ الله» .

إلى كلِّ بيولوجيٍّ وبيولوجيةٍ، عملَ جاهداً ليكونَ هذا البحثُ بين أيديكم.. لكم مني ألفُ تحيةٍ وسلام.

Abstract

Diabetes mellitus is a chronic metabolic disorder marked by persistent hyperglycemia, which leads to severe microvascular and macrovascular complications that impair hepatic and pancreatic functions. While synthetic drFcms like metformin are effective in regulating glucose levels, their use is often limited by side effects such as gastrointestinal issues, the development of secondary drFcm resistance, and their lack of direct tissue-protective capabilities. Consequently, research into biocompatible, multi-targeted natural alternatives has gained considerable traction in the field of antidiabetic therapies.

This study explores the therapeutic potential, phytochemical composition, and antioxidant properties of *Ficus carica* L. (fig) leaves from two distinct Algerian ecosystems: the hyper-arid desert region of El FCmhair (FCm) and the relatively humid northern region of Souk Ahras (FCs). Additionally, the study assesses the synergistic effects of their polyherbal formulation (Mgol) when combined with *Olea europaea* L. (olive) leaves sourced from El Oued. The primary aim is to evaluate the individual and blended extracts' ability to mitigate oxidative stress, regulate carbohydrate digestion, and restore cellular architecture in vivo.

Methods: Plant materials underwent polar solvent extraction, with yield, organic matter content, and total ash quantified. Phytochemical profiling was conducted to analyze total phenolics, flavonoids, and condensed tannins. Antioxidant capacity was determined using a range of assays, including DPPH radical scavenging activity, Ferric Reducing Antioxidant Power (FRAP), and Catalase (CAT) activity. In vitro antidiabetic potential was assessed throFcmh pancreatic α-amylase inhibition and yeast cell glucose uptake/diffusion assays.

In vivo experiments were performed on Wistar rats to assess the effects of the dry plant and its extract. Initially, acute toxicity tests were conducted on healthy rats using three dose levels: 100, 2000, and 5000 mg/kg. Following this, diabetes was experimentally induced in the animals to investigate the antidiabetic potential of the extract at two specific doses, 100 and 400 mg/kg. The study design included a healthy control group, a negative control group comprising untreated diabetic rats, and a positive control group treated with metformin. Furthermore, hematological assessments and histopathological evaluations were carried out to examine the impact of the extracts and the herbal formulation on the liver and pancreas.

Results: The FCm extract displayed the highest extraction yield (57.49%), attributed to its high-recovery efficiency during polar maceration. Mineral analysis revealed greater total ash content in FCm (15.48%) compared to FCs (9.71%), sFcmgesting distinct ecological adaptations to arid conditions. Phytochemically, FCs exhibited higher concentrations of total phenolics (15.57 ± 0.29 FCm GAE/g) and flavonoids (34.59 ± 1.47 FCm QE/g), while both ecotypes showed comparable levels of condensed tannins. The superior polyphenol content in FCs corresponded to enhanced antioxidant activity in DPPH and FRAP assays. Conversely, FCm demonstrated higher enzymatic antioxidant activity via catalase (25.32 ± 1.35 U/min/FCm), highlighting an adaptive reliance on non-phenolic protective metabolites. In enzymatic activity assays, significant variations emerged among the extracts. Concernant les essais d'activité enzymatique, l'efficacité variait selon les différents extraits. The FCm extract exhibited a moderate inhibitory effect on the

enzyme α-amylase, with an IC_{50} value of 320.9 ± 9.9 µg/mL. In contrast, the FCs and Ol extracts, as well as the polyherbal formulation Mgol, showed a limited inhibitory effect, with inhibition rates not exceeding 5.30% at the highest concentration tested (40 mg/mL). This mild activity could potentially help mitigate gastrointestinal issues associated with excessive use of commercial starch inhibitors. However, the Mgol formulation demonstrated a positive impact on regulating postprandial glucose absorption, reducing glucose availability by 13.80% between 30 and 60 minutes. This effect is attributed to molecular entrapment mechanisms and a slowdown in diffusion.

Safety evaluations revealed no adverse effects at low doses (100 FCm/kg), with preserved cellular architecture observed in all test subjects. However, acute high doses (2000 FCm/kg and 5000 FCm/kg) highlighted dose-dependent riFCss, including leukopenia, microcytic erythroid shifts, hepatic congestion, and pancreatic islet dissociation. In terms of pharmacological efficacy, a low-dose Mgol mixture (100 FCm/kg) did not significantly control diabetes symptoms; however, a higher optimized dose (400 FCm/kg) achieved impressive glycemic stabilization by reducing fasting glucose levels to 1.09 g/L and normalizing glycated hemoglobin (HbA1c) levels to 3.55%, comparable to or exceeding the effectiveness of metformin treatment. Furthermore, this dose successfully corrected diabetes-associated leukocytosis (12.70 × 10^3/µL) and restored immunological balance without inducing adverse effects such as localized xenobiotic stress, thrombocytosis, or microvascular hemoconcentration observed in the metformin-treated group. Histopathological analyses confirmed superior preservation of cellular architecture within hepatic and pancreatic tissues at the effective dose.

The findings confirm the traditional ethnobotanical application of Ficus carica and Olea europaea combinations within the Algerian ecosystem. The polyherbal formulation (Mgol) exhibits a well-balanced, multi-target therapeutic synergy, combining robust antioxidant activity with precise regulation of glucose transport, alongside exceptional cytoprotective effects. Administered at an optimized dose of 400 FCm/kg, this natural formulation emerges as a highly effective and safe next-generation functional food solution for the holistic management of diabetes mellitus and its associated metabolic complications.

Keywords: *Ficus carica* L., *Olea europaea* L., Polyherbal Synergy, Antidiabetic Activity, Antioxidant Properties, Glucose Uptake, Histopathological Restoration, Wistar Rats.

Résumé

Le diabète sucré est une pathologie métabolique chronique, caractérisée par une hyperglycémie persistante, qui peut entraîner des complications microvasculaires et macrovasculaires graves, affectant notamment les fonctions hépatiques et pancréatiques. Bien que des médicaments de synthèse comme la metformine se révèlent efficaces pour le contrôle de la glycémie, leur utilisation est souvent restreinte en raison d'effets secondaires, tels que des troubles gastro-intestinaux, l'émergence d'une résistance thérapeutique secondaire et l'absence d'effets protecteurs directs sur les cellules. Cela a conduit à un engouement croissant pour le développement d'alternatives naturelles biocompatibles capables d'intervenir sur plusieurs cibles pour le traitement du diabète. L'objectif principal de cette recherche est d'explorer le potentiel thérapeutique, les composés phytochimiques et les propriétés antioxydantes des feuilles de *Ficus carica* L. (figuier) issues de deux régions algériennes distinctes : la zone désertique hyperaride d'El El FCmhair (FCm) et la région plus humide de Souk Ahras au nord (FCs). Cette étude inclut également.

L'évaluation des effets synergiques d'une préparation polyherbale (Mgol) combinant les extraits de feuilles de figuier avec celles d'*Olea europaea* L. (olivier), récoltées à El Oued. Il s'agissait de déterminer l'efficacité de ces extraits – individuellement ou combinés – pour atténuer les effets du stress oxydatif, réguler la digestion des glucides et restaurer l'intégrité cellulaire dans un modèle in vivo. Pour ce faire, les feuilles ont subi une extraction via des solvants polaires. Les rendements d'extraction, ainsi que les quantités en matière organique et en cendres totales ont été mesurés. Le profil phytochimique a été évalué grâce à un dosage des polyphénols totaux, flavonoïdes et tanins condensés. Plusieurs méthodes ont permis d'évaluer l'activité antioxydante, notamment des tests de piégeage du radical DPPH, du pouvoir réducteur du fer (FRAP), et de l'activité catalase (CAT). L'effet antidiabétique in vitro a été analysé via l'inhibition de l'α-amylase pancréatique ainsi qu'à travers des essais d'absorption et diffusion du glucose sur cellules de levure.

Des essais in vivo ont été réalisés sur des rats Wistar. Tout d'abord, la toxicité aiguë de la plante sèche a été étudiée chez des rats en bonne santé, à l'aide de trois doses différentes (100, 2000 et 5000 mg/kg). Par la suite, le diabète a été induit expérimentalement chez les animaux pour évaluer l'activité antidiabétique de l'extrait à deux doses (100 et 400 mg/kg). L'étude comprenait un groupe témoin sain (contrôle), un groupe témoin négatif (diabétiques non traités) et un groupe témoin positif recevant un traitement par metformine. En complément, des analyses hématologiques et des examens histopathologiques ont été menés pour examiner l'impact des extraits et de la préparation à base de plantes sur le foie et le pancréas.

Les résultats montrent que l'extrait FCm présentait le rendement d'extraction le plus élevé (57,49 %), révélant une bonne efficacité d'extraction dans un milieu polaire. L'analyse minérale met en évidence une teneur plus importante en cendres pour FCm (15,48 %) comparée à FCs (9,71 %), ce qui reflète une adaptation écologique spécifique aux conditions arides. Sur le plan phytochimique, FCs affichait des niveaux élevés de polyphénols totaux (15,57 ± 0,29 FCm EAG/g) et de flavonoïdes (34,59 ± 1,47 FCm EQ/g), tandis que les concentrations en tanins condensés étaient similaires entre les deux échantillons. Cette richesse en composés phénoliques chez FCs

correspondait à une activité antioxydante notable selon les tests DPPH et FRAP. En comparaison, FCm présentait une activité enzymatique antioxydante plus élevée mesurée par la catalase (25,32 ± 1,35 U/min/FCm), sFcmgérant le recours prédominant à des métabolites protecteurs non phénoliques dans cet écosystèmeConcernant les tests d'activité enzymatique, l'efficacité des différents extraits a varié. L'extrait FCm s'est distingué par une capacité modérée à inhiber l'enzyme α-amylase, avec une valeur de IC_{50}320,9 ± 9,9 µg/mL. En revanche, les extraits FCs, Ol et le mélange polyherbal Mgol ont présenté un effet inhibiteur limité, n'excédant pas 5,30 % à la concentration maximale testée (40 mg/mL). Cet impact relativement faible pourrait contribuer à réduire les troubles gastro-intestinaux souvent associés à une utilisation excessive des inhibiteurs d'amidon commerciaux. Toutefois, la formulation Mgol a démontré un effet positif sur la régulation de l'absorption postprandiale du glucose. En effet, la biodisponibilité du glucose a diminué de 13,80 % entre 30 et 60 minutes, un résultat attribué à des mécanismes de rétention moléculaire et à un ralentissement de la diffusion.

L'évaluation toxicologique n'a révélé aucun effet nocif aux doses faibles (100 FCm/kg), avec une conservation intégrale de l'architecture cellulaire chez tous les animaux traités. Cependant, l'administration de doses aiguës élevées (2000 et 5000 FCm/kg) a montré des risques dépendants de la dose, notamment une leucopénie, des altérations érythrocytaires microcytaires, une congestion hépatique et une dissociation des îlots pancréatiques. Sur le plan pharmacologique, la formulation Mgol à faible dose (100 FCm/kg) s'est avérée inefficace pour contrôler les symptômes cliniques du diabète. À l'inverse, la dose optimisée de 400 FCm/kg a conduit à une stabilisation impressionnante de la glycémie, avec une réduction de la glycémie à jeun à 1,09 g/L et une normalisation du taux d'hémoglobine glyquée (HbA1c) à 3,55 %, dépassant même les résultats observés avec la metformine. Cette dose a également permis de corriger la leucocytose associée au diabète (12,70 × 10^3/µL) tout en rétablissant l'équilibre immunitaire, sans provoquer les effets secondaires observés chez les sujets traités par metformine, tels que le stress xénobiotique localisé, la thrombocytose ou encore l'hémoconcentration microvasculaire. Les analyses histopathologiques ont validé ces observations en confirmant un maintien remarquable de l'intégrité structurelle des cellules hépatiques et pancréatiques avec cette dose efficace. En conclusion, les résultats obtenus corroborent l'intérêt ethnobotanique traditionnel de l'association *Ficus carica*–Olea europaea dans les écosystèmes algériens. La formulation polyherbale Mgol manifeste une synergie thérapeutique multitarget équilibrée, alliant une forte activité antioxydante, une régulation fine du transport du glucose et des propriétés cytoprotectrices notables. À une dose optimisée de 400 FCm/kg, cette formulation naturelle apparaît comme une approche prometteuse, innovante, efficace et sécurisée pour une prise en charge holistique du diabète sucré ainsi que ses complications métaboliques associées.

الملخص

يُعتبر داء السكري اضطرابًا استقلابيًا مزمنًا يتمثل بارتفاع مستمر في مستويات السكر بالدم، مما يسبب مضاعفات وعائية دقيقة, وكبيرة تؤدي إلى تأثيرات سلبية على وظائف الكبد والبنكرياس. ورغم فعالية الأدوية الاصطناعية، مثل الميتفورمين، في ضبط مستويات السكر في الدم، إلا أن استخدامها قد يواجه عوائق متعددة مثل الآثار الجانبية كاضطرابات الجهاز الهضمي، وتطور مقاومة دوائية مع الزمن، وغياب تأثيرات وقائية مباشرة على الأنسجة. لذلك، ظهر اهتمام متزايد بالبحث عن بدائل علاجية طبيعية متكاملة قادرة على دعم التحكم في داء السكري والتخفيف من آثاره المرضية.

تهدف هذه الدراسة إلى تقييم الخصائص الكيميائية النباتية والنشاط المضاد للأكسدة والإمكانات البيولوجية لأوراق التين (*Ficus carica* L.)التي جُمعت من بيئتين جزائريتين مختلفتين :الأولى من منطقة المغير الصحراوية الشديدة الجفاف (FCm)، والثانية من منطقة سوق أهراس الشمالية ذات المناخ الرطب نسبيًا .(FCs) كما تطرقت الدراسة إلى استكشاف التأثيرات المتآزرة لتركيبة عشبية تجمع بين مستخلص أوراق التين والزيتون (*Olea europaea* L.) التي جُمعت من ولاية الوادي (Mgol). ركز البحث على تقييم قدرة هذه المستخلصات والتركيبة العشبية على مقاومة الإجهاد التأكسدي، وتنظيم هضم الكربوهيدرات، واستعادة البنية الخلوية في نماذج حيوانية.

خضعت المواد النباتية للاستخلاص باستخدام الميثانول، وتم تحديد مردود الاستخلاص ومحتوى المادة العضوية والرماد الكلي. كما أُجريت تحاليل كيميائية نباتية لتحديد تركيز البوليفينولات الكلية والفلافونويدات والتانينات المكثفة. تم قياس النشاط المضاد للأكسدة باستخدام اختبارات مختلفة، تضمنت اختباري DPPH و FRAPونشاط إنزيم الكاتالاز .(CAT) وتم اختبار النشاط المضاد للسكري مخبريًا من خلال قياس فاعلية تثبيط إنزيم -αأميلاز البنكرياسي وتحليل امتصاص وانتشار الغلوكوز باستخدام خلايا الخميرة.

كما أُجريت تجارب في الجسم الحي على جرذان ويستار السليمة والمصابة بداء السكري المستحث كيميائيًا بالألوكسان. وقد تم أولًا تقييم السمية الحادة للمادة النباتية باستعمال جرعات 100 و2000 و5000 ملغ/كغ لدى الجرذان السليمة، ثم دُرس النشاط المضاد للسكري باستخدام جرعتي 100 و400 ملغ/كغ من المستخلصات لدى الجرذان المصابة، مع اعتماد مجموعة شاهدة سليمة، ومجموعة شاهدة سالبة مصابة غير معالجة، ومجموعة شاهدة موجبة عولجت بالميتفورمين. كما أُجريت تحاليل دموية وفحوصات نسيجية لتقييم تأثير المستخلصات والتركيبة العشبية على الكبد والبنكرياس.

النتائج أظهرت أن مستخلص FCm حقق أعلى مردود للاستخلاص بنسبة 57.49%، مما يعكس كفاءة عملية الاستخلاص باستخدام المذيبات القطبية .أما التحليل المعدني فقد بيّن أن محتوى FCmمن الرماد الكلي بلغ 15.48%، وهو أعلى مقارنة بـ 9.71%في FCs، مما يكشف عن تكيفات بيئية مميزة للظروف الصحراوية .من ناحية المركبات الكيميائية النباتية، سجلت عينات FCsمستويات أعلى من البوليفينولات الكلية (15.57 ± 0.29 ملغ مكافئ حمض الغاليك/غ) والفلافونويدات (34.59 ± 1.47 ملغ مكافئ الكيرسيتين/غ)، بينما ظهرت مستويات متقاربة في التانينات المكثفة بين النمطيين البيئيين .النشاط المضاد للأكسدة كان أعلى في مستخلص FCsبناءً على اختبارات DPPHوFRAP، في حين تميز FCmبنشاط إنزيمي مضاد للأكسدة أعلى من خلال نشاط الكاتالاز (25.32 ± 1.35 وحدة/دقيقة/ملغ)، مما يشير إلى اعتماد أكبر على مستقلبات وقائية غير فينولية .

بالنسبة لاختبارات النشاط الإنزيمي، تباينت الفاعلية بين المستخلصات، حيث أظهر مستخلص FCm قدرة تثبيطية معتدلة تجاه إنزيم-α أميلاز بقيمة IC_{50} بلغت 320.9 ± 9.9 ميكروغرام/مل، في حين أظهرت مستخلصات FCs و Olوالتركيبة العشبية Mgolتأثيرًا تثبيطيًا محدودًا، إذ لم تتجاوز نسبة التثبيط 5.30% عند أعلى تركيز مختبر (40 ملغ/مل)، مما قد يحد من الاضطرابات الهضمية المرتبطة بالاستخدام المفرط لمثبطات النشاء التجارية. ومع ذلك، استطاعت التركيبة Mgol التأثير إيجابيًا بتنظيم امتصاص الغلوكوز بعد الوجبات، حيث انخفض توفر الغلوكوز بنسبة 13.80% بين 30 و60 دقيقة، ويُعزى ذلك إلى آليات الاحتجاز الجزيئي وإبطاء الانتشار .فيما يتعلق بسلامة الاستخدام، لم يُسجل أي آثار سلبية عند استخدام الجرعة المنخفضة (100 ملغ/كغ)، مع الحفاظ على البنية الطبيعية للأنسجة .لكن الجرعات العالية الحادة (2000 و5000 ملغ/كغ) أدت إلى تأثيرات متعلقة بالجرعة، تضمنت نقص الكريات البيضاء، تغيرات في كريات الدم الحمراء، احتقان كبدي، وتفكك في جزر لانغرهانس البنكرياسية .أما من الناحية العلاجية، فلم تُحقق الجرعة البسيطة (ملغ/كغ100)من التركيبة Mgol تحسنًا ملحوظًا في مؤشرات السكري، إلا أن الجرعة الأعلى (ملغ/كغ 400) أثبتت فاعلية كبيرة، حيث خُفض مستوى سكر الدم الصائم إلى 1.09 غ/ل وتم تطبيع الهيموغلوبين السكري (HbA1c) إلى 3.55 .%وكانت هذه النتائج مشابهة أو أفضل مقارنة بالميتفورمين المستخدم كعلاج قياسي للسكري .كما ساهمت الجرعة المدروسة في تحسين فرط الكريات البيضاء المرتبط بمرض السكري (12.70 × 10^3/ميكرولتر) واستعادة التوازن المناعي دون تسجيل آثار جانبية كالإجهاد الموضعي أو كثرة الصفيحات أو تركيز الدم المجهري، وهي آثار لاحظتها مجموعة الميتفورمين. وقد أثبتت الفحوصات النسيجية الحفاظ الممتاز على بنية خلايا الكبد والبنكرياس عند هذه الجرعة الفعالة .بناءً على هذه النتائج، يمكن الاستنتاج أن الاستخدام التقليدي لأوراق التين والزيتون في الطب الشعبي الجزائري يتمتع بأساس علمي داعم .كما أثبتت التركيبة العشبية Mgol قدرتها على تحقيق توازن علاجي متعدد الأهداف يجمع بين النشاط المضاد للأكسدة وتنظيم انتقال الغلوكوز والتأثيرات الوقائية على الخلايا والأنسجة. ومع الجرعة المثلى المحددة بـ (400ملغ/كغ)، تُعتبر هذه التركيبة خيارًا طبيعيًا واعدًا وآمنًا لدعم إدارة مرض السكري ومضاعفاته الاستقلابية بشكل شامل ومستدام.

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Introduction

I. Introduction

Human civilization has consistently relied on natural pharmacopoeia for disease treatment and health maintenance over millennia. Medicinal plants have historically constituted a foundational element of traditional medicine systems across global cultures. Even in the contemporary era, characterized by synthetic pharmaceuticals, natural products remain an indispensable reservoir for drMg discovery and molecular design (Khot, 2025). The World Health Organization (WHO) reports that a substantial proportion of the global population continues to utilize plant-based remedies for primary healthcare (Salmerón-Manzano et al., 2020). Medicinal herbs frequently synthesize complex matrices of secondary metabolites exhibiting diverse biological mechanisms, including antimicrobial, anti-inflammatory, antioxidant, and tissue-protective properties (Mera et al., 2019). This empirical legacy of traditional medicine provides a robust foundation for modern scientific validation and pharmacological standardization.

Diabetes Mellitus, a chronic, multifaceted metabolic disorder defined by persistent hyperglycemia, represents a significant global health challenge in the 21st century. Elevated blood glucose levels result from impaired insulin secretion, insulin action, or both, leading to severe intracellular oxidative stress and systemic inflammation (Amatruda et al., 2021). Over time, these metabolic disruptions contribute to microvascular and macrovascular complications, causing damage to vital metabolic organs such as the pancreas and liver (Siam et al., 2024). While conventional therapies, including exogenous insulin and synthetic oral hypoglycemic agents like Metformin, are primary clinical options, their long-term use is often constrained by adverse side effects, secondary drMg resistance, and high financial costs (Siam et al., 2024). Consequently, the investigation of plant-derived combinations as safe, multi-targeted, and accessible adjunctive therapies has become a prominent focus in anti-diabetic research.

Within the biodiverse arid and semi-arid ecosystems of Algeria, Ficus carica L. (fig tree) and Olea europaea L. (olive tree) possess considerable cultural, historical, and ethnobotanical significance. In Algerian folk medicine, leaf decoctions and infusions from these plants have been traditionally administered to alleviate symptoms of diabetes and metabolic syndrome (Lahyaoui et al., 2025). Phytochemical analyses indicate that Ficus carica leaves contain abundant bioactive polyphenols and flavonoids, such as rutin and quercetin, which exhibit potent free-radical scavenging

capabilities (Hasnain et al., 2023). Simultaneously, Olea europaea leaves feature a distinct phytochemical profile rich in secoiridoids, particularly oleuropein, which enhances insulin sensitivity and optimizes glucose uptake (Ak et al., 2025).

Despite their extensive traditional use and documented individual benefits, the potential therapeutic synergy of combining Ficus carica L. and Olea europaea L. leaf extracts remains largely unexplored, particularly when plants are sourced from specific hyper-arid environments. Investigating a polyherbal formulation combining Ficus carica leaves from the arid environment of El Mghair with Olea europaea leaves from the neighboring desert region of El Oued could reveal additive or synergistic interactions. Such a formulation might concurrently suppress oxidative stress, inhibit carbohydrate-digesting enzymes, and promote pancreatic and hepatic Human civilization has historically utilized natural resources for disease treatment and health maintenance. Medicinal plants have traditionally constituted a primary component of medicine systems across global cultures. Even with the prevalence of synthetic pharmaceuticals, natural products remain a significant source for drMg discovery and molecular design (Khot, 2025). The World Health Organization (WHO) reports that a substantial proportion of the global population continues to depend on plant-derived remedies for primary healthcare (Salmerón-Manzano et al., 2020). Medicinal plants synthesize diverse secondary metabolites, which exhibit various biological activities, including antimicrobial, anti-inflammatory, antioxidant, and tissue-protective properties (Mera et al., 2019). This established empirical legacy of traditional medicine offers a basis for scientific validation and pharmacological standardization.

Diabetes Mellitus represents a significant global health concern, characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. This condition induces severe intracellular oxidative stress and systemic inflammation (Amatruda et al., 2021). Over time, these metabolic disturbances lead to microvascular and macrovascular complications, affecting organs such as the pancreas and liver (Siam et al., 2024). AlthoMgh conventional therapies, including exogenous insulin and synthetic oral hypoglycemic agents like Metformin, are primary clinical options, their long-term application is often constrained by adverse effects, drMg resistance, and high costs (Siam et al., 2024). Therefore, the investigation of plant-derived combinations as safe, multi-targeted, and accessible adjunctive therapies has become a key area in contemporary anti-diabetic research.

Within the Algerian arid and semi-arid ecosystems, Ficus carica L. (fig tree) and Olea europaea L. (olive tree) possess significant cultural, historical, and ethnobotanical importance. In Algerian traditional medicine, their leaf decoctions and infusions have been historically used to manage symptoms of diabetes and metabolic syndrome (Lahyaoui et al., 2025). Phytochemical analyses indicate that Ficus carica leaves contain various bioactive polyphenols and flavonoids, including rutin and quercetin, which exhibit substantial free-radical scavenging properties (Hasnain et al., 2023). Similarly, Olea europaea leaves are characterized by a distinct phytochemical profile rich in secoiridoids, such as oleuropein, which has been shown to enhance insulin sensitivity and improve glucose uptake (Ak et al., 2025).

Despite their traditional use and documented individual benefits, the potential therapeutic synergy of combining Ficus carica L. and Olea europaea L. leaf extracts has not been extensively investigated, particularly concerning plant material sourced from specific hyper-arid regions. A polyherbal formulation combining Ficus carica leaves from the arid environment of El Mghair with Olea europaea leaves from the adjacent desert region of El Oued could demonstrate additive or synergistic interactions. Such a formulation might concurrently mitigate oxidative stress, inhibit carbohydrate-digesting enzymes, and support pancreatic and hepatic recovery.

This Master's thesis investigates the therapeutic potential of a polyherbal combination of Ficus carica L. (from El Mghair) and Olea europaea L. (from El Oued) leaves throMgh a comprehensive scientific inquiry. Both individual and combined extracts will undergo in vitro screening to determine their total bioactive content, antioxidant capacities (via DPPH, FRAP, and Catalase), anti-inflammatory properties (BSA denaturation), and Sun Protection Factor (SPF). Additionally, their anti-diabetic mechanisms will be evaluated in vitro using α-amylase inhibition and yeast cell glucose transport assays. The study will then proceed to in vivo experimentation, assessing the optimal combination in an alloxan-induced diabetic Wistar rat model to evaluate antihyperglycemic efficacy, acute toxicity, biochemical parameters, and the histopathological restoration of pancreatic and hepatic tissues.

The thesis is structured into two primary sections:

a theoretical framework and an experimental study,The theoretical section comprises two chapters:

- Chapter One examines the pathophysiology of diabetes mellitus and natural alternative therapies.
- Chapter Two describes the botanical, chemical, and pharmacological profiles of Ficus carica L. and Olea europaea L.

The experimental section is also organized into two chapters:

- Chapter One details the Materials and Methods, including experimental designs, chemical reagents, and both in vitro and in vivo laboratory protocols.
- Chapter Two then presents the comprehensive analysis, interpretation, and discussion of the obtained results, concluding with a summary and future perspectives.

Chapter One: Introduction to diabetes mellitus and its relationship with natural therapeutic alternatives

I .1.An Overview of Diabetes

I .1.1.Definition of Diabetes

Poorly controlled diabetes mellitus can lead to two major categories of complications: macrovascular complications affecting the heart, brain, and extremities, and microvascular complications affecting the eyes, kidneys, and nerves (Tikent, 2025). Diabetes mellitus is generally classified into four main types: type 1 diabetes, type 2 diabetes, gestational diabetes, and other specific forms of diabetes.

I.1.1. Types of Diabetes

According to Jwad (2022), this study focuses specifically on type 1 diabetes and type 2 diabetes.

a. Type 1 Diabetes

Type 1 diabetes is an autoimmune disorder in which the immune system destroys the pancreatic beta cells located in the islets of Langerhans. This destruction leads to insulin deficiency and impaired regulation of blood glucose levels. As a result, patients require lifelong insulin therapy to survive and to prevent diabetic ketoacidosis. Type 1 diabetes commonly develops during childhood or adolescence and was previously referred to as juvenile-onset diabetes.

b. Type 2 Diabetes

Type 2 diabetes mellitus is a chronic metabolic disorder characterized by insulin resistance and a progressive decline in pancreatic beta-cell function. Initially, body tissues become less responsive to insulin, followed by insufficient insulin secretion by pancreatic beta cells over time. This condition occurs more frequently in adults and is strongly associated with obesity, physical inactivity, and unhealthy lifestyle habits. Persistent insulin resistance and beta-cell dysfunction contribute to the progression of the disease (Holt, 2024).

I .1.3.Natural Therapeutic Alternatives

Diabetes mellitus is a chronic metabolic disorder that requires lifelong management. Conventional pharmacological treatments, including insulin therapy and oral hypoglycemic agents, remain the primary approaches for diabetes management. However, these treatments may be associated with side effects, high costs, and limited accessibility in certain populations. Consequently, increasing attention has been directed toward natural therapeutic alternatives,

particularly medicinal plants that have long been used in traditional medicine systems worldwide (Rezagholizadeh, 2022).

Natural products may provide several advantages as complementary or alternative approaches for diabetes management. Many medicinal plants exhibit multiple biological activities, including antioxidant, anti-inflammatory, hypoglycemic, and insulin-sensitizing effects. These properties may contribute to reducing oxidative stress, improving glucose metabolism, and preventing or delaying diabetic complications. In addition, natural products are often considered to have fewer adverse effects compared with synthetic drMgs (Elhrech et al., 2024).

I.1.4. Uses and place of Ficus carica L. and Olea europaea L. among natural therapeutic alternatives

Ficus carica L. (fig tree) and Olea europaea L. (olive tree) occupy an important position among natural therapeutic alternatives for diabetes because of their long history of traditional use, rich phytochemical composition, and association with the Mediterranean dietary pattern (Rezagholizadeh, 2022).

Ficus carica L. has traditionally been used in folk medicine for the management of diabetes and its complications in several regions. Different parts of the plant, including the fruits, leaves, and latex, have been reported to possess hypoglycemic, antioxidant, and anti-inflammatory properties. In traditional Algerian and Mediterranean medicine, fig fruits and leaves are consumed fresh or prepared as decoctions to help regulate blood glucose levels and reduce symptoms associated with hyperglycemia (Khalid, 2025; Tikent, 2025). The plant is particularly rich in phenolic compounds and flavonoids, which may contribute to improved carbohydrate metabolism and reduced oxidative stress.

Similarly, Olea europaea L. plays a major role in traditional medicine and modern phytotherapy. Olive leaves, fruits, and extra virgin olive oil have long been used in the management of metabolic disorders, including diabetes. Olive leaf infusions are traditionally consumed to reduce blood glucose and blood pressure levels. In addition, olive oil is considered one of the main components of the Mediterranean diet and is associated with improved insulin sensitivity and a reduced risk of type 2 diabetes (Elhrech et al., 2024).

The pharmacological effects of Olea europaea L. are mainly attributed to bioactive secoiridoids such as oleuropein and hydroxytyrosol, which exhibit antioxidant, anti-inflammatory, and potential insulin-mimetic activities.

Furthermore, Ficus carica L. and Olea europaea L. may exert complementary therapeutic effects when used together within the Mediterranean dietary framework. Their combined intake may improve glycemic control, reduce oxidative stress, modulate inflammatory responses, and protect against diabetes-related complications, including cardiovascular disorders and neuropathy (Rezagholizadeh, 2022).

Overall, these two plant species represent promising candidates for functional foods, herbal supplements, and adjunct therapeutic strategies. Their cultural acceptance, relatively low cost, and favorable safety profile make them valuable complementary approaches for supporting metabolic health and improving the quality of life of diabetic patients.

I.1.5.The importance of natural remedies in helping diabetic patients

The Holy Quran points to a special sort of standing for these two blessed trees, in Surah At-Tin, “By the fig and the olive” (Qur'an, 95:1-2). That Qur'anic oath, in a way, draws attention to the deep nutritional and therapeutic weight often linked with Ficus carica L. and Olea europaea L. throMgh multiple old civilizations.

Actually, these two Mediterranean species, they tend to hold a reserved and honored spot among natural therapeutic alternatives, especially when diabetes care is the focus. They also come with a long, nearly timeless history in customary healing across North Africa, the Middle East, and Southern Europe, where for many centuries they were treated as plants that might help maintain a stable metabolic state (Lahyaoui, 2025).

Ficus carica L. which is basically the fig tree, has been used in folk medicine quite steadily, mostly via its leaves, and also throMgh its fruit, to deal with metabolic issues, including hyperglycemia. This widely seen habit seems to have grown out of what people noticed over years, plus an empirical backdrop that implies effects on glucose regulation that are rather favorable (Adjekhiane, 2023).

On the other hand, Olea europaea L. the olive tree, especially its leaves and its oil, has formed a strong reputation inside long-standing everyday routines. Those routines connect the olive not

only with glycemic steadiness but also with cardiovascular wellbeing, so it continues to be one of the enduring botanical friends that support metabolic wellness, no less (Elhrech et al., 2024).

Figure 1 Botanical illustration of the leaves and fruits of Ficus carica L. and Olea europaea L. Source: xAI, (2026).

Medicinal plants, in general, have been used for a long time in folk practice, and they are often described as useful for metabolic regulation. Still, there is a clear gap that keeps showing up: the scientific standardization. Especially, standardization for many plants that are traditionally used, but also how those preparations are broMght into clinical practice in a consistent manner, and whether they can be treated as safe adjunctive therapies.

So, this study is meant to go into that gap, by looking at the combined effects of leaf extracts from Ficus carica L. and Olea europaea L. as complementary agents for diabetes management. Both species have a strong place in Mediterranean traditions, including daily eating patterns, and they have been associated, over time, with metabolic wellness (Rezagholizadeh, 2022).

Overall, the significance is practical. The research tries to support the movement toward approaches that are culturally familiar, easier to obtain, and potentially able to live alongside current drMg treatments. The point is to improve quality of life for people living with diabetes, in a way that feels more realistic, not just theoretical, and not too far away from everyday possibilities.

Chapter Two: Chemical composition of Ficus carica L. and Olea europaea L. and their relationship with diabetes mellitus

I .1.Chemical Composition of Ficus carica L. and Olea europaea L. Leaves

Plant-derived polyphenols are considered among the most important bioactive compounds involved in metabolic health because of their antioxidant, anti-inflammatory, and insulin-sensitizing properties. Examples include curcumin from turmeric (Widjanarko, 2024), baicalin from Scutellaria baicalensis (Szkudelski & Szkudelska, 2023), quercetin commonly found in onions, apples, and tea (Shabir et al., 2022), and catechins from green tea (Linghui, 2024). These compounds contribute significantly to the therapeutic potential of many medicinal plants (Kumar et al., 2023).

The leaves of *Ficus carica* L. and *Olea europaea* L. are particularly rich in polyphenolic compounds, which support their traditional use in the management of diabetes and other metabolic disorders (Li et al., 2021; El Ghouizi et al., 2023; Nediani et al., 2019). Both species contain diverse phenolic profiles associated with hypoglycemic, antioxidant, and β-cell protective activities. Consequently, leaf extracts from these plants are increasingly investigated as potential adjunct therapeutic agents for diabetes management.

I.2. Major Phenolic Compounds in Fig (Ficus carica L.) Leaves

Ficus carica L. leaves contain various secondary metabolites, with phenolic compounds and coumarins representing the major classes of bioactive constituents (Li et al., 2021).

In general, the major phenolic compounds detected in fig leaves are listed in Table 01:

Phenolic Compound	Chemical Class	References
Caffeoylmalic acid	Phenolic acid	(Li et al., 2021)
Rutin	Flavonoid glycoside	(Shiraishi, 2023)
Quercetin and its derivatives	Flavonols	(Zhang et al., 2024a)
Chlorogenic acid	Phenolic acid	(Shiraishi, 2023)
Apigenin-C-glycosides	Flavone glycosides	(Li et al., 2021)
Luteolin derivatives	Flavones	(Li et al., 2021)

Table 1 Major phenolic compounds reported in Ficus carica L. leaves.

These phenolic compounds are often seen as the main contributors within the phytochemical profile of fig leaves, and they're regularly looked at too, for their possible biological value in both traditional and more modern phytotherapy.

I1.3. Major Phenolic Compounds in Olea europaea L. Leaves

Olea europaea L. leaves tend to be quite rich in phenolic compounds, especially the secoiridoids, which actually account for the dominant class among secondary metabolites. Oleuropein, for example, is widely described as the most abundant and also the most telltale phenolic compound in olive leaves (Chaji, 2024; Nediani et al., 2019).

So, the principal phenolic compounds that were identified in *Olea europaea* L. leaves are presented and summarized in Table 02:

Phenolic Compound	Chemical Class	References
Oleuropein	Secoiridoid	(Nediani et al., 2019)
Hydroxytyrosol and derivatives	Phenylethanoid	(Rocchetti, 2022)
Verbascoside	Phenylethanoid glycoside	(Rocchetti, 2022)
Luteolin-7-O-glucoside	Flavone glycoside	(Nediani et al., 2019; Rocchetti, 2022)
Rutin	Flavonoid glycoside	(Chaji, 2024)
Chlorogenic acid	Phenolic acid	(Rocchetti, 2022)

Table 2 Major phenolic compounds reported in Olea europaea L. leaves.

These compounds are a big part of the general phytochemical "feel" of olive leaves, and they keep showing up in papers linked with folk phytotherapy and functional foods, theyre sort of treated as already meaningful in botanical profiling, and in phytotherapy studies too.

- **Integrated Significance**

Integrated significance, maybe? Well, the abundant polyphenolic and coumarin content found in both *Ficus carica* and *Olea europaea* L. leaves, backed by ethnopharmacological cues from different cultures, gives a solid reason to

keep studying them as supportive treatments in diabetes management (El Ghouizi et al., 2023).

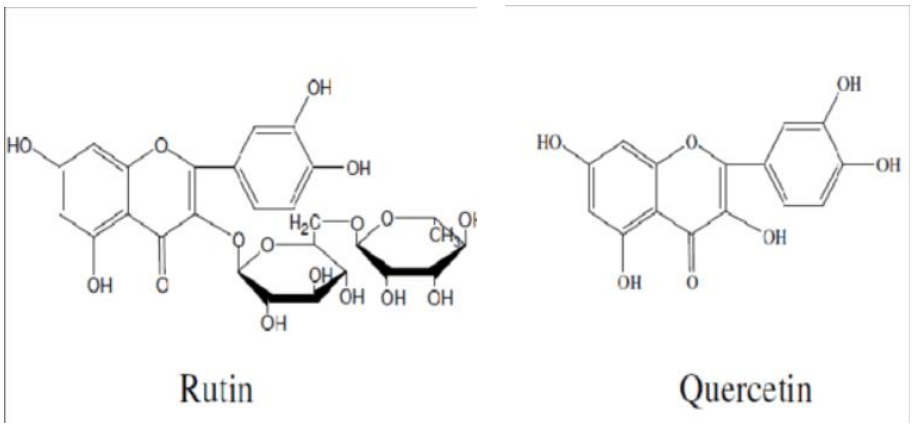


Figure 2 Chemical structures of rutin and quercetin. Source: (Aloriby, 2019).

I.3. Antioxidant and Anti-Inflammatory Properties of Ficus carica L. and Olea europaea L.

I .3.1.Antioxidant Properties

Ficus carica L. and *Olea europaea* L. are recognized for their strong antioxidant properties due to their high content of phenolic compounds (Rezagholizadeh, 2022). These bioactive compounds contribute to the neutralization of free radicals and the reduction of oxidative stress, which is associated with the development of several chronic diseases, including cardiovascular disorders, diabetes, and cancer (Elhrech et al., 2024; Rezagholizadeh, 2022).

Ficus carica L. contains several classes of phenolic compounds, including flavonoids, phenolic acids, and anthocyanins, which contribute significantly to its antioxidant activity. These compounds exert their effects throMgh mechanisms such as free-radical scavenging, metal ion chelation, and inhibition of lipid peroxidation (Khalid, 2025; Tikent, 2025).

Similarly, *Olea europaea* L. particularly its leaves and fruits, is rich in secoiridoids and other phenolic compounds. Oleuropein and hydroxytyrosol are considered the major bioactive constituents responsible for the antioxidant activity of olive-derived products. These compounds protect cellular components against oxidative damage and enhance endogenous antioxidant defense systems (Elhrech et al., 2024).

Overall, both plant species represent important components of the Mediterranean diet because of their nutritional and phytochemical composition. Their bioactive compounds may act

synergistically to provide protective effects against diseases associated with oxidative stress (Rezagholizadeh, 2022).

I .3.2.Anti-Inflammatory Properties

The phenolic compounds in both plants seem to exert notable anti-inflammatory effects, by messing with key pathways like NF-κB, also lowering pro- inflammatory cytokines (TNF-α, IL-6), and blocking enzymes such as COX-2 and 5-LOX (Rezagholizadeh, 2022). Fig extracts, which are rich in quercetin together with other flavonoids, have been reported to curb, oxidative stress driven inflammation (González-Rodríguez et al., 2023; Velotti, 2023). Meanwhile, *Olea europaea* L. constituents, especially oleocanthal and hydroxytyrosol, show ibuprofen-like behavior, so they may give strong anti-inflammatory help (Bucciantini et al., 2021; Elhrech et al., 2024). Algerian types of both species show a rather encouraging ability to reduce inflammation markers, which supports their age-old use, and also sMggests they could be useful for chronic inflammatory issues. When fig-olive blends are used together, additive protection is possible (Rezagholizadeh, 2022). These Mediterranean plants act like helpful natural pools of bioactive substances for functional foods, nutraceuticals and even food preservation, and the Algerian biodiversity brings in valuable genetic materials for high-phenolic cultivars. More recent work, roMghly 2023–2025, underlines the sustainable valorization of their by-products (Malekjani, 2023; Rasool, 2023).

I .4. Recent Studies on the antidiabetic potential of Ficus carica L. and Olea europaea L.

Recent studies have investigated the antidiabetic potential of *Ficus carica* L. and *Olea europaea* L. because of their strong antioxidant and anti-inflammatory properties. These biological effects are associated with reduced oxidative stress and decreased inflammatory responses commonly observed in diabetes mellitus (El Ghouizi et al., 2023; Velotti, 2023).

Since 2020, several studies have demonstrated the hypoglycemic activity of different *Ficus carica* L. extracts using both in vitro and in vivo diabetic models. For example, methanolic leaf extracts administered at 200 Mg/kg for 30 days to alloxan-induced diabetic rats significantly reduced blood glucose levels while protecting hepatic and renal tissues from hyperglycemia-related damage. The extracts also restored antioxidant enzyme activity and reduced lipid peroxidation. These effects were mainly attributed to the high content of polyphenols and flavonoids (El Ghouizi et al., 2023).

In addition, dichloromethane leaf extracts exhibited hypoglycemic activity, improved glucose tolerance, enhanced lipid profiles, and preserved pancreatic islet structure in streptozotocin-induced diabetic models. Compounds such as psoralen and umbelliferone were also reported to enhance glucose uptake in HepG2 cells throMgh increased peripheral glucose utilization (Lin, 2023).

Fermented fig extracts prepared using Lactiplantibacillus plantarum have also attracted scientific interest because of their anti-obesity and antidiabetic effects in high-fat diet models. These extracts reduced body weight, fasting blood glucose, and insulin levels while improving lipid parameters. In addition, they modulated the expression of adipogenesis-related genes such as FAS, C/EBPα, and FABP4 (Choi, 2024).

Furthermore, pectin isolated from sun-dried fig stalks was reported to reduce postprandial glucose levels in Caco-2 cells and ex vivo models, sMggesting its potential application as a functional food additive for glycemic control (Başer, 2025).

Similarly, *Olea europaea* L. has received considerable attention for its antidiabetic properties, particularly because of compounds such as oleuropein, hydroxytyrosol, and oleocanthal. Olive leaf extracts have been reported to improve insulin sensitivity, reduce fasting blood glucose levels, and regulate carbohydrate metabolism throMgh the inhibition of α-amylase and α-glucosidase activities, in addition to modulating insulin-signaling pathways (González-Rodríguez et al., 2023). Experimental studies have also indicated that olive polyphenols may protect pancreatic β-cells against oxidative stress and reduce inflammatory mediators associated with insulin resistance (Velotti, 2023).

Animal studies have further demonstrated that supplementation with *Olea europaea* L. leaf extracts improves lipid metabolism, antioxidant status, and glycemic control while reducing diabetes-related complications (Álvares, 2024).

Overall, these findings sMggest that both *Ficus carica* L. and *Olea europaea* L. exert antidiabetic effects throMgh multiple mechanisms, including antioxidant and anti-inflammatory activities, improvement of insulin sensitivity, inhibition of carbohydrate-digesting enzymes, and regulation of glucose metabolism. Nevertheless, additional clinical studies are required to confirm their long-term safety and therapeutic efficacy in humans.

I .5. Comparison with Other Medicinal Plants Used for Similar Purposes

Ficus carica L. exhibits significant antidiabetic activity due to its antioxidant and anti-inflammatory properties, which are comparable to those reported for several medicinal plants traditionally used in diabetes management. Medicinal species such as *Olea europaea* L., Morus alba L. (white mulberry), Punica granatum L. (pomegranate), Opuntia ficus-indica (prickly pear), and Momordica charantia L. (bitter melon) are widely recognized for their hypoglycemic, antioxidant, and anti-inflammatory effects (González-Rodríguez et al., 2023; Mawa, 2013).

From a phytochemical perspective, *Ficus carica* L. shares several major polyphenolic compounds with *Olea europaea* L., including quercetin, catechin, rutin, luteolin, apigenin, anthocyanins, and tocopherols. These bioactive compounds contribute to anti-inflammatory and antioxidant activities throMgh the modulation of signaling pathways such as NF-κB and MAPK, as well as the reduction of pro-inflammatory cytokines including TNF-α, IL-1β, and IL-6 (Arangia, 2023; Velotti, 2023).

Several studies have also sMggested that the combined use of *Ficus carica* L. and *Olea europaea* L. may provide complementary protective effects against oxidative stress and inflammation associated with diabetes, particularly within Mediterranean dietary patterns and functional food applications (Başer, 2025).

In addition, *Ficus carica* L. has been investigated in combination with other medicinal plants such as Punica granatum, Morus alba, and Ginkgo biloba. Polyherbal formulations containing fig extracts often demonstrate enhanced antioxidant activity compared with individual extracts, possibly due to increased total phenolic and flavonoid contents. Some synergistic formulations have shown DPPH IC_{50} values comparable to those of ascorbic acid, indicating strong free-radical scavenging activity (Rasool, 2023).

Anthocyanin-rich extracts from Morus alba and Punica granatum generally exhibit higher radical-scavenging activity than *Ficus carica* L. when used individually. However, fig extracts maintain significant anti-inflammatory and hypoglycemic properties, particularly in polyherbal formulations where they act as supportive bioactive components (Mawa, 2013).

A comparison between *Ficus carica* L. and Opuntia ficus-indica reveals differences in their antidiabetic mechanisms. Opuntia ficus-indica is primarily associated with glucose regulation throMgh dietary fibers and polysaccharides that reduce intestinal glucose absorption and inhibit α-glucosidase activity. In contrast, *Ficus carica* L. demonstrates stronger antioxidant and anti-inflammatory effects because of its higher polyphenolic content (Savitri, 2022).

Compared with Momordica charantia, *Ficus carica* L. exhibits lower α-amylase and α-glucosidase inhibitory activity but demonstrates greater antioxidant potential. Momordica charantia is mainly associated with insulin-like hypoglycemic compounds such as charantin and polypeptide-p, whereas *Ficus carica* L. is more strongly linked to improved lipid metabolism and protection against diabetic complications throMgh flavonoids, triterpenoids, and other anti-inflammatory phytochemicals (El Ghouizi et al., 2023; Lin, 2023).

Overall, *Ficus carica* L. demonstrates considerable therapeutic potential because of its antidiabetic, antioxidant, and anti-inflammatory properties, which are comparable to those of several established medicinal plants. Current evidence supports its potential use in functional foods and herbal formulations. Nevertheless, additional well-designed clinical trials are required to confirm its long-term efficacy and safety in humans.

Materials and methods

I .Plant Material

Ficus carica L., a species belonging to the Moraceae family, was selected for this study. Locally, this tree is known as "Tin." Samples were collected from the Sidi Khelil area (El Mghair Province) and the Dréa area (Souk Ahras Province) during the summer of 2025. To investigate a potential synergistic antidiabetic effect, leaves of *Olea europaea* L. were also included. These were collected from the El Oued region in early May 2026. For *Olea europaea* leaves, analyses were limited to α-amylase inhibitory activity and glucose uptake assays. The subsequent in vivo antidiabetic study utilized a combination of extracts from both *Ficus carica* and *Olea europaea.* All other laboratory procedures and in vitro analyses detailed subsequently were exclusively performed on *Ficus carica* L. leaves (Ahmadi Mazhin et al., 2016).

I .1.Collection and Preparation of Samples

After collection, the samples were meticulously sorted to eliminate any foreign materials. Their surfaces were carefully cleaned using damp cloths to remove dust and other adhering impurities. The leaves of *Ficus carica* L. and *Olea europaea* L. were dried in a dark, well-ventilated area, shielded from direct sunlight, until they achieved a consistent weight. Once dried, the leaves were finely ground, primarily through traditional manual techniques, though an electric grinder was utilized when necessary. The resulting powders were stored in sterilized, airtight glass containers to safeguard against light and moisture until further use, as described by Boukhalfa and Kadri (2019).

Figure 01: Images of *Ficus carica* L. and *Olea europaea* L. (Original photos)

I .2.Physicochemical Study of the Plant Material

I .2.1.Preparation of Methanolic Extract of Ficus carica L.

A methanolic extract was prepared by immersing 10 g of dried *Ficus carica* L. leaf powder in 60 mL of methanol. This solvent volume represented 5 to 10 times the dry weight of the plant material. The mixture was maintained at ambient temperature for 24 hours with intermittent manual stirring to facilitate the extraction of bioactive compounds.

Following maceration, the extract was filtered throMgh Whatman No. 1 filter paper to remove solid plant residues. The resulting filtrate was evaporated at 45°C in an incubator until a semi-solid extract was obtained. This semi-solid extract was then aliquoted into glass vials and stored at 4°C (refrigerated) and in a freezer for subsequent analysis. (Singh et al., 2024; El Ghouizi et al., 2023).

I .2.1.Extraction Yield

The extraction yield was determined throMgh the following calculation method:

$$yield(\%) = \frac{W_1}{W_2} \times 100$$

W1 = weight of the dried extract

W2 = weight of the initial dried plant material (10 g) (Guettaf et al., 2016).

Figure 02: Preparation of the methanolic extract of *Ficus carica* L. and *Olea europaea* L.

I .2.3.Determination of Organic Matter and Mineral Matter Percentages

Dry ashing was performed on the plant samples to determine their organic and mineral matter content, following the method described by (Quirino et al., 2023) with slight modifications. A 1 g sample of dried plant powder was accurately weighed into pre-weighed crucibles. These crucibles were then calcined in a muffle furnace at 550 °C for 6 hours. Following ashing, the crucibles were allowed to cool under ambient laboratory conditions prior to weighing for ash content

determination. The organic matter and mineral matter percentages of the samples were calculated using the following equations:

The mineral matter percentage (ash content) was determined using the following equation:

$$ash\ content\ (miniral\ matter\ \%) = \frac{weight\ of\ ash}{weight\ of\ sample} \times 100$$

The calculation of organic matter percentage requires the formula:

$$Oragnic\ matter\ (\%) = 100 - ash\ content(\%)$$

I .3.Nutritional value assessment (Carbohydrates, Proteins, and Lipids)

I .3.1.Preparation of Extracts

For nutritional value assessment (carbohydrates, proteins, and lipids), primary metabolites were extracted from powdered plant material following the method described by (Shibko et al., 1966). The procedure involved combining 0.5 g of plant powder with 5 mL of 20% trichloroacetic acid (TCA). This mixture was stirred with a magnetic stirrer for 5 minutes, and the resulting homogenate was then centrifMged at 3000 rpm for 30 minutes.

The supernatant (I) and pellet were separated, with supernatant (I) reserved for subsequent carbohydrate analysis. The pellet was then treated with 2 mL of an ethyl ether/chloroform mixture (1:1 v/v) and centrifMged at 3000 rpm for 10 minutes. Supernatant (II) was collected for lipid content analysis.

Finally, pellet (II) was processed with 5 mL of 0.1 N sodium hydroxide (NaOH) solution. This mixture was centrifMged at 3000 rpm for 10 minutes, and the resulting supernatant was used for protein content measurement.

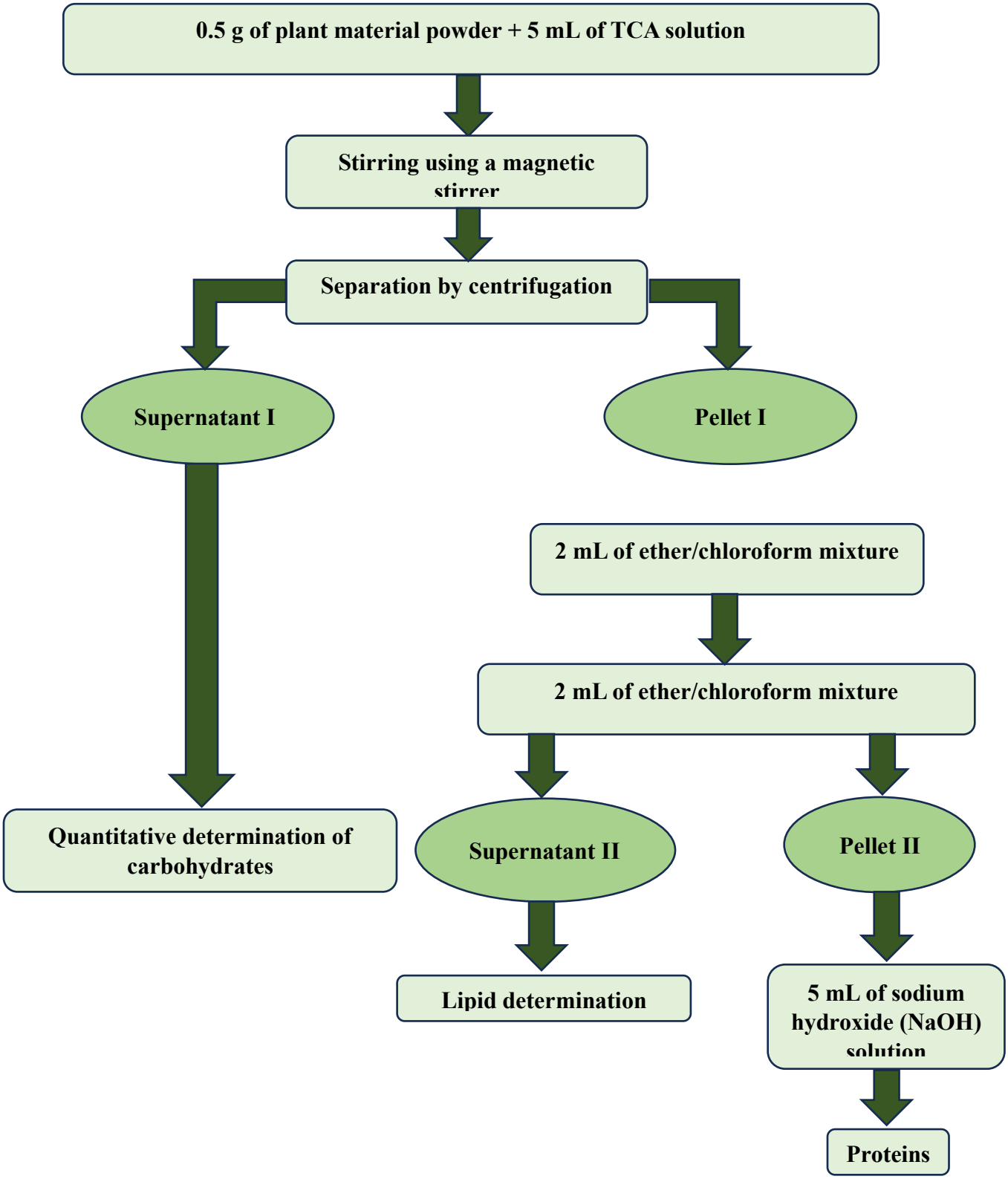


Figure 3 The steps of primary metabolite extraction

I .3.2.Quantitative Estimation of Carbohydrates

Carbohydrate content in the extracts was determined using the colorimetric method described by (DuBois et al., 1956). A 0.25 mL aliquot of supernatant I from each plant sample was combined with 0.25 mL of 5% (v/v) phenol solution and 1.25 mL of concentrated sulfuric acid (H2SO4). The mixture was vigorously agitated and incubated for 15 minutes at room temperature to facilitate color development. Absorbance was subsequently measured at 490 nm using a spectrophotometer. Carbohydrate concentrations were calculated based on the linear equation derived from the glucose standard curve (Fig. 04). Results are expressed in milligrams per gram of dry plant material.

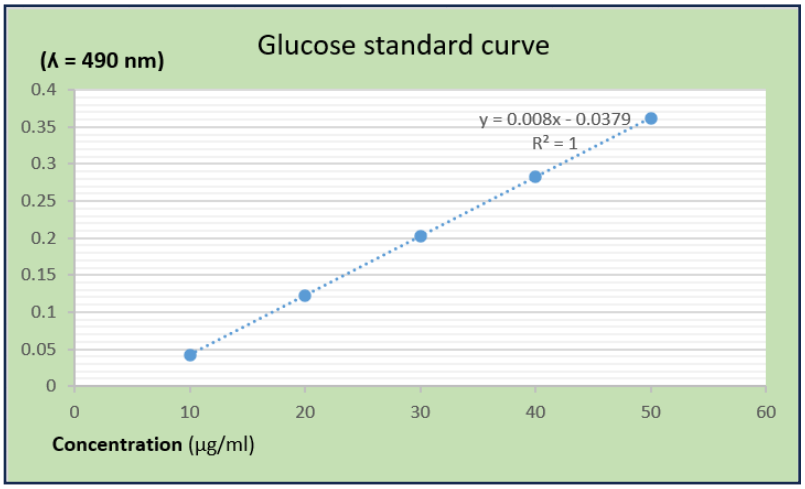


Figure 4 Glucose standard curve

I .3.3.Quantitative Estimation of Lipids

Lipid content in the extracts was estimated using the colorimetric method outlined by Goldsworthy et al. (1972). The procedure involved the following steps:

a. Preparation of the Sulfophosphovanillin Reagent

The sulfophosphovanillin reagent was prepared by dissolving 75 Mg of vanillin in 11 mL of distilled water, followed by the addition of 39 mL of 85% phosphoric acid (H_3PO_4).

b. Experimental Procedure

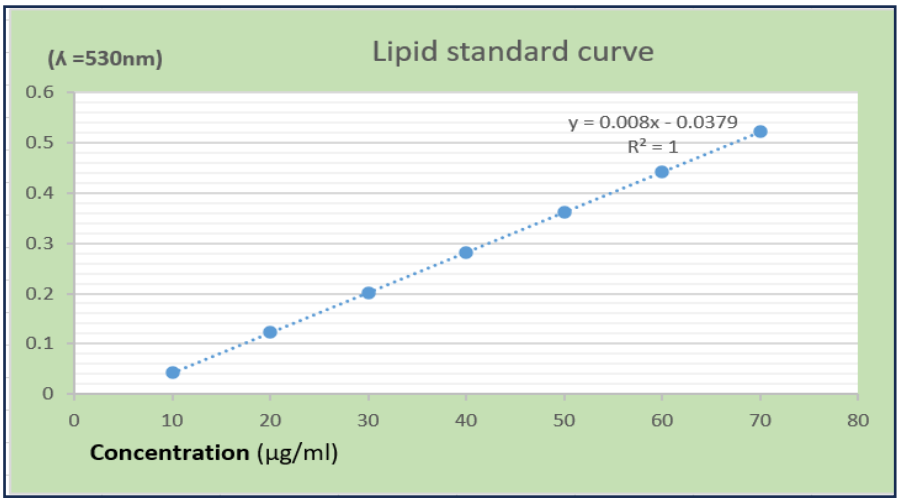
Initially, 0.1 mL of supernatant II was combined with 0.1 mL of concentrated sulfuric acid (H_2SO_4). This mixture was vigorously agitated and incubated in a thermostated water bath at 30 °C for 10 minutes (designated as mixture A).

Following cooling of mixture A to room temperature, a 0.15 mL aliquot was mixed with 1.5 mL of the sulfophosphovanillin reagent.

The resulting solution was vigorously shaken and allowed to react for 30 minutes at room temperature for color development.

Absorbance of the final solution was measured at 530 nm using a spectrophotometer.

Lipid content was calculated based on the linear equation derived from the soybean oil standard curve. Results are expressed in milligrams per milliliter of dry plant material.



I .3.4.Quantitative Estimation of Proteins

Protein content was quantitatively estimated using the method described by Lowry et al. (1951). The procedure involved:

I.1.2.1. Preparation of Solutions

- **Solution (A):** was prepared by combining a 2% (w/v) sodium carbonate (Na_2CO_3) solution with a 0.1 N sodium hydroxide (NaOH) solution in a 1:1 (v/v) ratio.
- **Solution (B):** was prepared by mixing a 0.5% (w/v) copper sulfate ($CuSO_4$) solution with a 0.1% (w/v) sodium-potassium tartrate tetrahydrate ($KNaC_4H_4O_6 \cdot 4H_2O$) solution in a 1:1 (v/v) ratio.
- **Solution (C):** comprised the Folin-Ciocalteu reagent diluted 1:1 (v/v) with distilled water.
- **Solution (D):** the alkaline cupric reagent, was prepared by mixing 50 mL of solution (A) with 1 mL of solution (B).

I .3.5.Experimental Procedure

A 0.1 mL aliquot of the plant extract solution was mixed with an equal volume (1:1, v/v) of solution (D) and solution (C).

The mixture was vigorously shaken and subsequently incubated at room temperature for 30 minutes to allow color development.

Absorbance of the resulting solution was measured at 750 nm using a spectrophotometer.

Protein content in the plant samples was quantified using the linear equation derived from the bovine serum albumin (BSA) standard curve. Results are expressed in milligrams per gram of dry plant material.

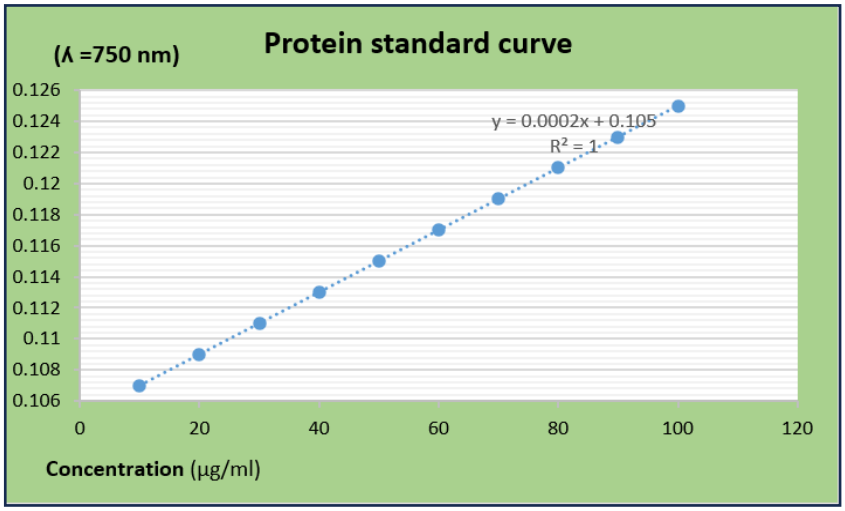


Figure 5 Protein standard curve

I.4. Phytochemical Screening

I.1.3. Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) in methanolic extracts of Ficus carica L. was quantified using the Folin–Ciocalteu colorimetric method, with minor modifications from (Dif et al., 2015).

Specifically, the extracts underwent two-fold serial dilutions, yielding concentrations from 0.3125 to 20 Mg/mL. A 100 µL aliquot of each dilution was combined with 0.5 mL of 10% (v/v) Folin–Ciocalteu reagent. Following a 5-minute interval, 2 mL of 20% (w/v) sodium carbonate (Na_2CO_3) solution was introduced. The resulting mixture was vortexed and subsequently incubated for 30

minutes in the dark at room temperature. Absorbance was then recorded at 760 nm using a UV-Vis spectrophotometer.

A calibration curve was established using gallic acid as a standard, with concentrations ranging from 0.01 to 0.05 g/L. The corresponding linear regression equation was

$$y = 23.87x + 0.0124 \quad (R^2 = 0.9911)$$

All measurements were conducted in triplicate. Results were expressed in milligrams of gallic acid equivalents per gram of dry extract (Mg GAE/g extract).

I .4.1.Determination of Total Flavonoid Content (TFC)

Total flavonoid content (TFC) was quantified utilizing the aluminum chloride ($AlCl_3$) colorimetric method, as described by (Mbaebie et al., 2012).The same serial dilutions employed for TPC determination (0.3125 to 20 Mg/mL) were used. To each 1 mL extract dilution, 1 mL of 2% (w/v) aluminum chloride solution was added. The resulting reaction mixture was homogenized and incubated in the dark at room temperature for 30 minutes. Subsequently, absorbance was recorded at 420 nm with a UV-Vis spectrophotometer.

Quercetin served as the reference standard, at concentrations ranging from 0.005 to 0.03 g/L. The calibration curve equation was

$$y = 44.18x + 0.1243 \quad (R^2 = 0.9989)$$

All assays were performed in triplicate. Results were reported as milligrams of quercetin equivalents per gram of dry extract (Mg QE/g extract).

I .4.2.Condensed Tannins

Condensed tannin content in the methanolic extract of Ficus carica L. was determined spectrophotometrically following the method by (Broadhurst et al., 1978).

For this, 500 µL of the methanolic extract was combined with 3 mL of freshly prepared 4% vanillin reagent in methanol, followed by the addition of 1500 µL of concentrated hydrochloric acid. The reaction mixture was thoroMghly homogenized and incubated for 15 minutes at 20 ± 2°C in the dark. Absorbance was subsequently measured at 500 nm, using water as a blank.

A standard calibration curve was generated using catechin solutions across a concentration range of 10 to 1000 µg/mL, processed under identical experimental conditions. Results were expressed as milligrams of catechin equivalents per gram of Ficus carica L. methanolic extract. All measurements were performed in triplicate.

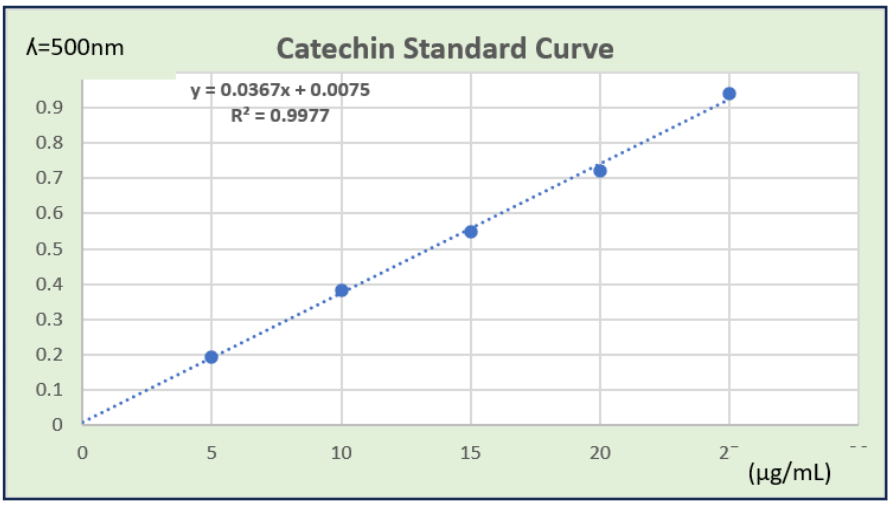


Figure 6 Catechin standard calibration curve showing the relationship between absorbance at 500 nm and catechin concentration (µg/mL).

II. In Vitro Activity of the methanolic extract of Ficus carica L:

II.1. Antioxidant Activity:

II.1.1.DPPH Free-Radical Scavenging Assay:

The in vitro activity of the methanolic extract of Ficus carica L. was investigated throMgh several assays.

Antioxidant activity of the Ficus carica L. extracts was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free-radical scavenging assay. A series of six extract concentrations (1, 0.5, 0.25, 0.125, 0.0625, and 0.03125 Mg/mL) was prepared by serial dilution. For each concentration, 1 mL of the extract was combined with 1 mL of a 4 Mg/100 mL methanolic DPPH• solution. The reaction

mixtures were thoroMghly mixed and incubated in the dark at room temperature for 30 minutes. Subsequently, absorbance was measured at 517 nm using a spectrophotometer. Ascorbic acid served as a positive control (Bentabet et al., 2014).

Radical scavenging activity was quantified as percentage inhibition (*PI*), calculated using the following equation:

$$PI(\%) = \left[\frac{AC - AS}{AC}\right] \times 100$$

Where:

- *AC* is the absorbance of the control (DPPH solution without extract).
- *AS* is the absorbance of the sample containing the extract of Ficus carica L.

II.1.2.Total Antioxidant Activity:

The IC_{50} value was determined by plotting PI (%) against extract concentration. The IC_{50} signifies the concentration necessary to scavenge 50% of DPPH radicals; a lower IC_{50} value corresponds to stronger antioxidant activity.

Total antioxidant activity was determined using the phosphomolybdenum method, as described by (Fazel, 2024; Pennisi, 2023; Piqueras-García, 2025; Prieto et al., 1999; Shiraishi, 2023).

Specifically, 0.2 mL of each extract solution was combined with 2 mL of a reagent solution comprising 6 M sulfuric acid, 28 mM sodium phosphate (NaH_2PO_4), and 4 mM ammonium molybdate. The reaction mixtures were then incubated in a water bath at 95°C for 90 minutes. Following incubation, samples were cooled to room temperature, and absorbance was measured at 695 nm using a spectrophotometer (Prieto et al., 1999; Shiraishi, 2023).

Total antioxidant activity was expressed as ascorbic acid equivalents (Mg AAE/g extract), with ascorbic acid serving as the standard reference. The calibration curve for ascorbic acid was established using the following equation:

$$y = 3.5125x - 0.0073$$

II.1.3.Ferric Reducing Power (FRAP) Assay:

The Ferric Reducing Power (FRAP) assay was employed to evaluate ferric ion reduction, which indicates electron-donating ability—a key mechanism in phenolic antioxidant activity.

$$K_3[F_e(CN)_6] + F_eCI_3 \rightarrow K_4[F_e(CN)_6] + F_eCI_2$$

In this assay, antioxidants reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The resulting dark blue ferrous complex is then quantified by measuring its absorbance at 700 nm. The reducing power of the extracts was determined based on the method described by (Jayanthi et al., 2011).

Extracts exhibiting reducing capability react with potassium ferricyanide ($K_3Fe(CN)_6$) to produce potassium ferrocyanide ($K_4Fe(CN)_6$). Specifically, 250 µL of different extract concentrations was combined with 625 µL of 0.2 M phosphate buffer (pH 6.6) and 625 µL of 1% potassium ferricyanide solution. The mixture was incubated in a water bath at 50°C for 20 minutes. Subsequently, 625 µL of 10% trichloroacetic acid (TCA) was added, and the mixture was centrifMged at 3000 rpm for 10 minutes.

Following centrifMgation, 625 µL of the supernatant was mixed with 625 µL of distilled water and 125 µL of 1% ferric chloride ($FeCl_3$). Absorbance was then measured at 700 nm. Ascorbic acid served as a positive control. An increase in absorbance correlates with an increase in reducing power.

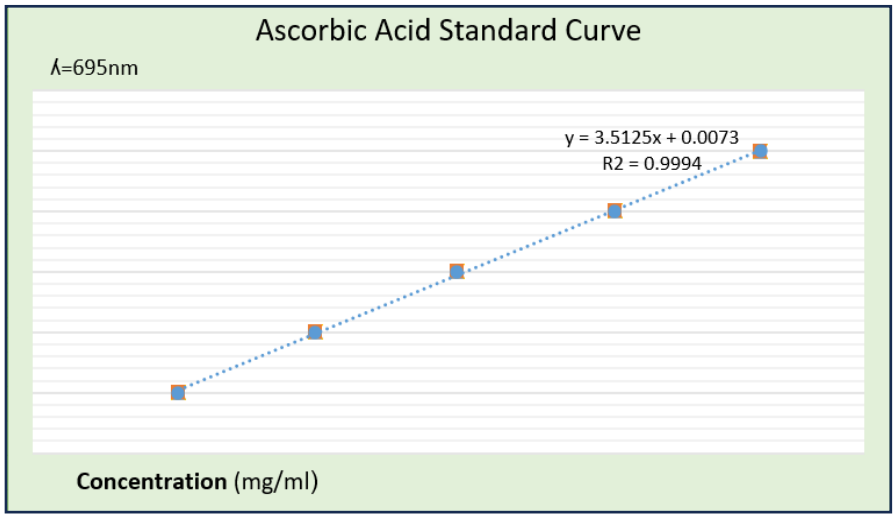


Figure 7 Ascorbic Acid Standard Curve.

Figure 08: Ascorbic Acid Standard Curve.

II.2.Anti-inflammatory Activity

II.2.1.Bovine Serum Albumin (BSA) Denaturation Assay

The anti-denaturation activity of the plant extracts was assessed using the bovine serum albumin (BSA) denaturation assay, adapted from the method described by Juvekar et al. with minor modifications (Sakat et al., 2010).

The reaction mixture comprised 1 mL of extract solution (at concentrations of 1, 0.5, 0.25, and 0.1 Mg/mL), 1.5 mL of phosphate-buffered saline (PBS, pH 6.3), and 0.5 mL of a 1% (w/v) aqueous BSA solution. This mixture was incubated at 37°C for 15 minutes, followed by heating in a water bath at 70°C for 5 minutes to induce protein denaturation. Subsequent to cooling to room temperature, the turbidity was quantified at 660 nm using a UV-Vis spectrophotometer. A 1% (w/v) BSA stock solution was prepared fresh by dissolving 1 g of BSA in 100 mL of PBS, with the pH adjusted to between 6.3 and 6.5. Sodium diclofenac served as the positive reference standard. All experiments were conducted in triplicate. The percentage inhibition of protein denaturation was calculated for each concentration, and the corresponding IC_{50} value was determined.

$$I\% = \left(\frac{AC - AS}{AC}\right) \times 100$$

II.3.Antidiabetic Activity Assays

II.3.1.α-Amylase Inhibitory Assay

II.3.1.1.Preparation of Test Samples

The dry methanolic extracts were dissolved in sterile distilled water containing 1% dimethyl sulfoxide (DMSO), which acted as a co-solvent to enhance solubility. Stock solutions were initially prepared at a concentration of 80 Mg/mL. Subsequently, two-fold serial dilutions were performed to yield six final test concentrations: 40, 20, 10, 5, 2.5, and 1.25 Mg/mL.

II.3.1.2.Preparation of Starch Substrate Solution

The starch substrate solution was prepared by dissolving 200 Mg of soluble starch in 25 mL of 0.4 M NaOH. The mixture was heated at 100 °C for 5 minutes to ensure complete solubilization.

Following cooling to room temperature, the pH was adjusted to 7.0, and the solution was broMght to a final volume of 100 mL with distilled water.

II.3.1.3.α-Amylase Inhibitory Activity Assay

The in vitro α-amylase inhibitory activity was evaluated using a modified starch–iodine colorimetric microplate assay, as previously described (Chakrabarti et al., 2014; Granados-Guzmán et al., 2022; Khadayat et al., 2020).

Acarbose served as the positive control, whereas the negative control comprised the complete reaction mixture devoid of any test sample. Within each well of a 96-well microplate, 40 µL of starch substrate solution was combined with 20 µL of either the test sample at its specified concentration or acarbose.

These mixtures were pre-incubated at 37 °C for 3 minutes. The enzymatic reaction commenced upon the addition of 20 µL of α-amylase solution (3 U/mL), prepared in 20 mM phosphate buffer (pH 6.9) containing 6.7 mM NaCl. The reaction was then incubated at 37 °C for 15 minutes.

The reaction was terminated by adding 80 µL of 0.1 M HCl, immediately followed by the addition of 100 µL of iodine–potassium iodide (IKI) solution (2.5 mM). Absorbance was subsequently measured at 630 nm using a microplate spectrophotometer. All experiments were conducted in triplicate (n = 3).

II.3.1.4..Calculation of Percentage Inhibition and IC_{50}

The percentage inhibition of α-amylase activity was calculated using the provided formula:

$$\left(\frac{A-B}{A}\right) \times 100 = Inhibition\%$$

where A denotes the absorbance of the negative control (without inhibitor), and B denotes the absorbance in the presence of the test sample or acarbose.

The IC_{50} value, defined as the concentration required to inhibit 50% of α-amylase activity, was determined by plotting the percentage inhibition against the logarithm of the sample concentration and subsequently performing linear regression analysis.

II.2.Glucose Uptake Assay by Yeast Cells

The potential of the plant extracts to enhance glucose uptake was evaluated in vitro using a microplate-based glucose uptake assay with Saccharomyces cerevisiae (baker's yeast) as a cellular model (Cirillo, 1962; Yamamoto et al., 2015).

This method serves as a recognized rapid screening tool for assessing the capacity of plant extracts to promote cellular glucose transport, which constitutes a significant mechanism for antihyperglycemic activity.

Commercial baker's yeast was repeatedly washed by centrifMgation at 3,000 × g for 5 minutes in distilled water until the supernatant was visually clear. Subsequently, a 10% (v/v) yeast suspension was prepared in distilled water. A 25 mM glucose solution (4.5039 g/L) served as the substrate. Various concentrations of the test sample were added to the glucose solution in separate 96-well microplates.

A glucose control, containing no test sample, was included to establish basal glucose levels. All mixtures were then pre-incubated at 37 °C for 10 minutes. The reaction commenced upon the addition of 100 µL of the yeast suspension to each well. The microplates were gently shaken and incubated at 37 °C for 30, 60, and 120 minutes.

Following each incubation period, the plates were centrifMged at 2,500 × g for 5 minutes to sediment the yeast cells. An appropriate volume of the supernatant was then transferred to a new microplate, and glucose detection reagent was added. The residual glucose concentration was determined by measuring absorbance at 540 nm using a microplate reader (Yamamoto et al., 2015).

The percentage of glucose uptake was calculated using the provided formula:

$$100 \times \left(\frac{A_s - A}{A_0}\right) = \text{Glucose Uptake \%}$$

where *A0* represents the absorbance of the glucose control (without test sample), and As represents the absorbance in the presence of the test sample. All experiments were conducted in triplicate (n = 3), and results are presented as mean ± standard deviation (SD).

III. In Vivo Experimental Design

Thirty-two healthy adult Wistar rats, weighing approximately 180–220 g, were utilized in this investigation. The animals were maintained under standard laboratory conditions, with ad libitum access to a standard pellet diet and water. They were housed under a natural light/dark cycle at

room temperature and allowed a two-week acclimatization period prior to the commencement of the experiment (Atanu et al., 2018; Tlili, 2021).

III.1.Acute Toxicity Study

Following the acclimatization period, nine rats were randomly selected and allocated into three groups (n = 3 per group) for the acute toxicity study of the plant extract. The extract was administered orally as single doses of 100, 2000, and 5000 Mg/kg body weight. The animals were observed continuously for the initial 24 hours and then daily for signs of toxicity, behavioral alterations, or mortality, in accordance with standard toxicity assessment procedures (Naik et al., 2022; Tlili, 2021).

III.2.Induction of Experimental Diabetes

Experimental diabetes was induced in overnight fasted rats by a single intraperitoneal injection of alloxan monohydrate at a dose of 150 Mg/kg body weight, dissolved in sterile normal saline (0.9% NaCl) (Atanu et al., 2018; Ighodaro, 2017; Tlili, 2021). Before alloxan administration, animals were fasted for 12 hours, thoMgh with ad libitum access to water. Six hours post-alloxan injection, rats received oral administration of a 20% glucose solution to mitigate fatal hypoglycemic shock, subsequently followed by a 5% glucose solution for the ensuing 24 hours. Animals exhibiting a fasting blood glucose concentration ≥ 250 Mg/dL, measured two days post-induction, were classified as diabetic (Atanu et al., 2018).

III.2.1. Experimental Grouping and Treatment

The surviving diabetic rats were randomly allocated into four groups (n = 3 per group) as detailed below (Tlili, 2021):

- **Group 1:** Diabetic untreated group (negative control).
- **Group 2:** Diabetic rats treated with the plant extract at 100 Mg/kg body weight.
- **Group 3:** Diabetic rats treated with the plant extract at 400 Mg/kg body weight.
- **Group 4:** Diabetic rats treated with Metformin at 2 Mg/kg body weight, serving as a standard reference drMg (Atanu et al., 2018; Naik et al., 2022).

All treatments were administered orally once daily via intragastric gavage throMghout the treatment period (Daniel et al., 2022).

Animal Monitoring: Body weight, fasting blood glucose levels, and general behavioral changes were regularly monitored throMghout the experimental period. At the culmination of the treatment, the animals were humanely sacrificed under anesthesia. Blood samples and organs, specifically the pancreas and liver, were collected for subsequent biochemical analyses, including glucose, lipid profile, renal and liver function tests, and oxidative stress markers, as well as for histopathological examination (Rasool et al., 2023; Tlili, 2021).

A schematic representation detailing the experimental protocol for alloxan-induced diabetes mellitus and oral treatment administration in Wistar rats.

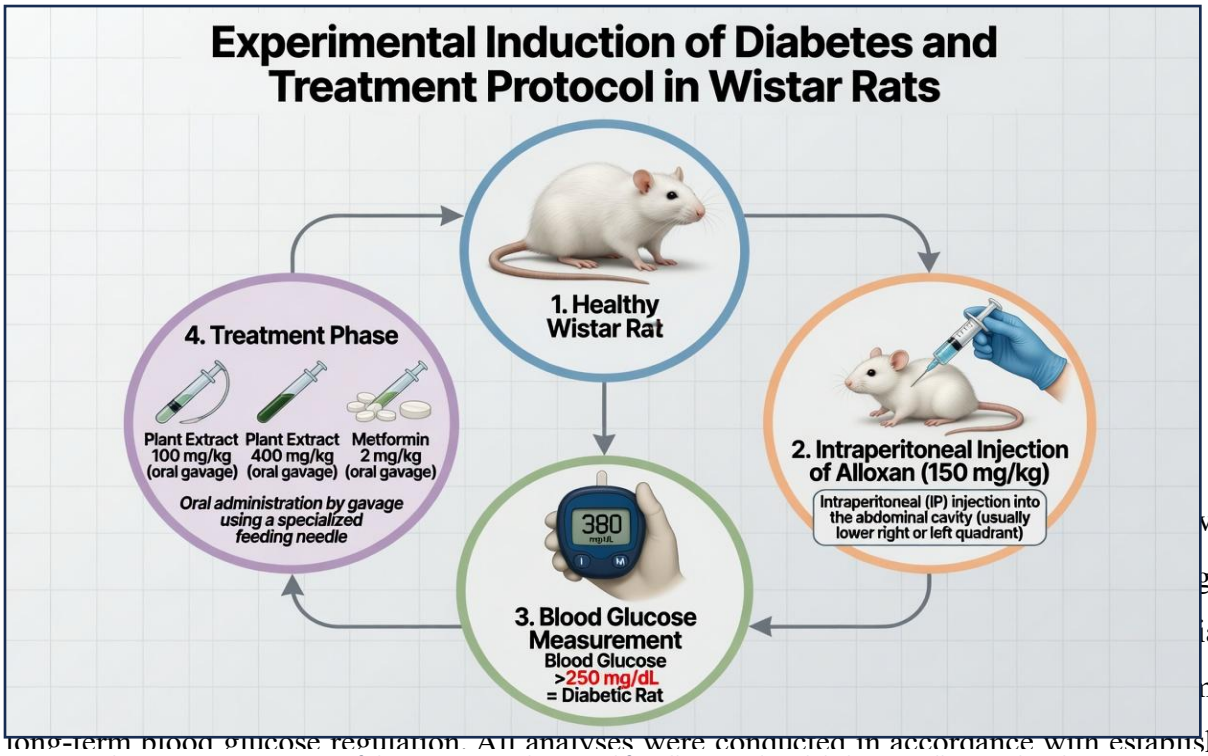


Figure 8 Schematic representation of the experimental protocol for alloxan-induced diabetes mellitus and oral treatment administration in Wistar rats.

III.2.2.Preparation of Tissue Samples for Histological Examination

Following euthanasia, the pancreas and liver were meticulously excised from each rat. The organs were gently rinsed in cold physiological saline (0.9% NaCl) to remove residual blood and immediately fixed in 10% neutral-buffered formalin for 24–48 hours to preserve tissue architecture (Barkaoui, 2024). The fixed tissues were subsequently dehydrated throMgh a graded series of ethanol solutions (70%, 80%, 90%, and 100%), cleared in xylene, and then embedded in paraffin wax. Serial sections, 4–5 µm in thickness, were cut using a rotary microtome. These tissue sections

were mounted on glass slides and stained with hematoxylin and eosin (H&E) following standard histological protocols (Abdulal et al., 2024). The stained slides were examined under a light microscope to evaluate histological architecture, pancreatic beta-cell integrity and regeneration, hepatocellular changes, the degree of inflammation, necrosis, and any protective or regenerative effects exerted by the plant extracts on diabetic complications.

Results and discussions

I. Physicochemical Study of the Plant Material:

The extraction yields for the various samples were recorded as follows: FCm at 57.49%, FCs at 39.40%, Ol at 51.01%, and Ol combined with FCm at 46.24%.

I.1.Extraction Yield of the Methanolic Extract:

- **Results:**

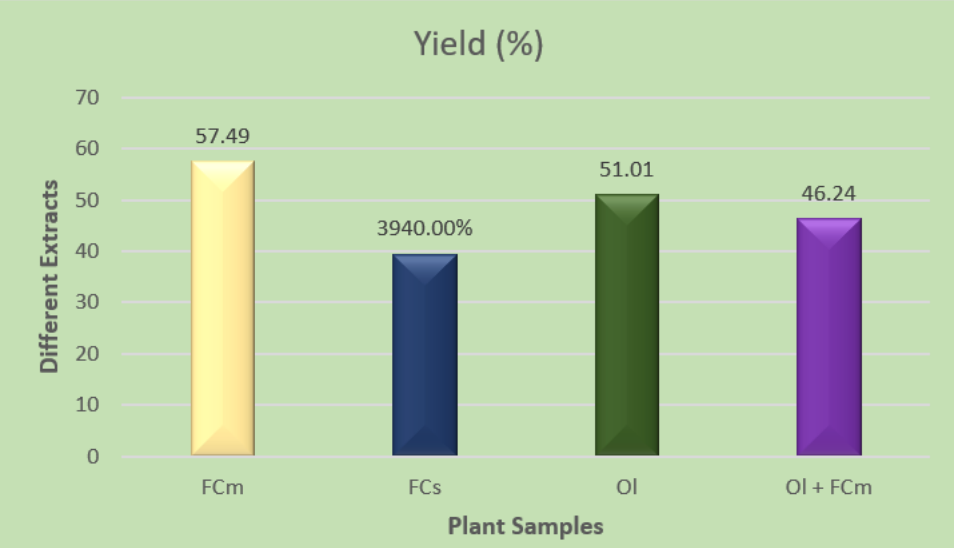


Figure X. Extraction yield (%) of different plant samples

- **Discussion:**

The FCm extract exhibited the highest extraction yield (57.49%), reflecting the efficiency of methanolic maceration and the richness of extractable bioactive compounds in leaves collected from the southern (El Mghair) region, a finding consistent with the high affinity of methanol for phenolic-rich plant matrices (Altayb et al., 2022; Kuswandani et al., 2021).

In contrast, the FCs extract showed a lower yield (30.4%), which may be attributed not only to intrinsic phytochemical differences but also to environmental variability between the northern and southern regions, including climatic conditions, soil characteristics, and ecological stress factors, all of which are known to significantly influence secondary metabolite biosynthesis and extraction efficiency (De Souza et al., 2022; Mehalaine & Chenchouni, 2021).

The olive leaf extract (Ol) demonstrated a satisfactory yield (51.01%), indicating a high content of methanol-soluble phenolic compounds, in agreement with previous reports highlighting

the phytochemical richness of olive leaves and the efficiency of polar solvents in recovering bioactive constituents (Palit & Mandal, 2021; Fraga et al., 2020).

For the mixture (Ol + FCm), the extraction yield was 46.24%, representing an intermediate value that suggests the absence of antagonistic interactions between components during extraction and supports the feasibility of combined extracts while maintaining substantial recovery of bioactive compounds. (Osorio-Tobón, 2020).

Overall, the observed variations in extraction yield are in accordance with literature reporting that plant species, geographic origin, and environmental conditions play a crucial role in determining extraction efficiency (Ahmad et al., 2023; Singh et al., 2025).

I.2. Mineral Matter (Ash) and Organic Matter Content

The mineral matter (total ash) and organic matter contents were determined by incinerating the plant samples at 550°C until a constant weight was obtained.

- **Results:**

Sample	Ash (%)	Organic matter (%)
FCs	9.71	90.29
FCm	15.48	84.52

Table 3 Mineral Matter (Ash) and Organic Matter Content of the Plant Samples

- **Discussion:**

The total ash content was determined to be 9.71% for sample FCsk (northern region) and 15.48% for sample FCMg (desert region). These values correspond to organic matter contents of 90.29% and 84.52%, respectively. Given that both samples originated from the same plant part and were collected during the same season, the observed difference in ash content (approximately 5.77% higher in FCMg) is primarily attributable to geographical origin and prevailing climatic conditions. Sample FCMg, collected from an arid desert environment, exhibited a distinctly higher mineral content.

This observation aligns with established adaptations of desert flora, which frequently accumulate elevated concentrations of inorganic elements in response to environmental stressors such as droMght, high soil salinity, and limited water availability.

These conditions typically result in enhanced uptake and accumulation of minerals, including calcium, potassium, magnesium, and sodium. Conversely, sample FCsk, originating from the northern region with its Mediterranean and relatively humid climate, displayed lower ash content and a correspondingly higher organic matter percentage.

This outcome sMggests more favorable environmental conditions for the biosynthesis and subsequent accumulation of organic secondary metabolites in this region. The recorded ash values for both samples fall within the established acceptable ranges for medicinal plant materials.

Nevertheless, the elevated mineral content observed in FCMg may potentially offer additional nutritional or pharmacological benefits. Conversely, the higher organic matter content in FCsk could be indicative of a greater abundance of bioactive compounds, such as polyphenols, flavonoids, and terpenoids.

I.3.Proximate Composition (Nutritional Value)

- **Results:**

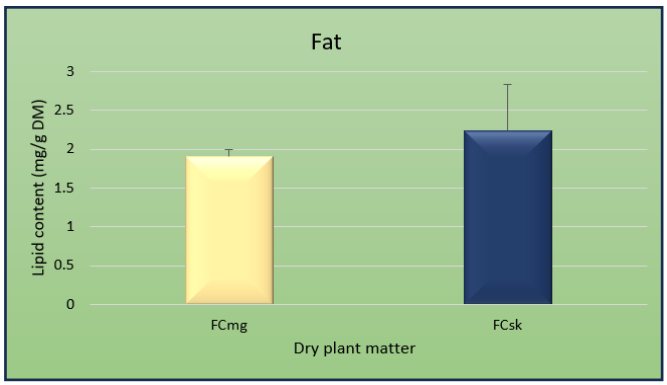


Figure 9 Lipid content of FCmg and FCsk

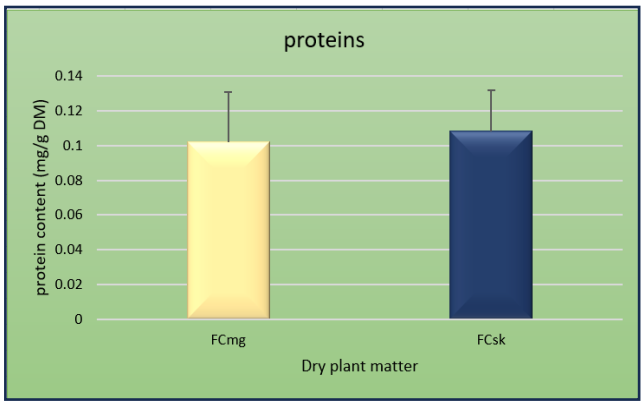


Figure 10 proteins content of FCmg and FCsk

- **Discussion:**

The nutritional assessment of Ficus carica L. leaves indicated the presence of primary metabolites, specifically carbohydrates, proteins, and lipids. These biomolecules are integral to plant metabolism and may contribute to the biological activities observed in this species.

Carbohydrates constituted the predominant primary metabolites identified in the analysis. This finding aligns with the physiological role of leaves as primary sites for photosynthesis and carbohydrate biosynthesis and storage. Carbohydrates serve as essential energy reserves and participate in various metabolic and structural processes within plants.

Proteins were detected in moderate concentrations, reflecting their fundamental roles in enzymatic catalysis, cellular metabolism, and defense mechanisms in plants. The protein content may also sMggest the nutritional utility of the plant material.

In contrast, the lipid content was comparatively lower than that of carbohydrates and proteins. This pattern is frequently reported in the leaves of medicinal plants, where lipids are typically present in limited quantities. Nevertheless, plant lipids can contribute to biological properties throMgh their content of essential fatty acids and other bioactive compounds.

These findings are consistent with previous research, such as that by Hassan et al. (2007), who observed elevated carbohydrate levels and varying amounts of proteins and lipids in medicinal plants. Similar observations have also been reported in prior investigations of Ficus carica L. leaves.

Discrepancies between the current results and those in the literature may arise from several factors, including environmental conditions, geographical origin, harvesting season, drying procedures, extraction methodologies, and analytical techniques employed for metabolite quantification.

I.4.Quantification of Phytochemical Compounds:

- **Results:**

Parameter	FCMg	FCsk	Average
Total Phenolic Content (Mg GAE/g)	6.75 ± 0.58	15.57 ± 0.29	11.16 ± 0.44
Total Flavonoid Content (Mg QE/g)	11.63 ± 2.52	34.59 ± 1.47	23.11 ± 1.99
Condensed Tannins (Mg CE/g)	2.77 ± 0.016	2.76 ± 0.016	2.76 ± 0.016

Table 4 Quantification of phytochemical compounds in methanolic extract of Ficus carica L. leaves

- **Discussion:**

Quantitative analysis of phytochemical compounds demonstrated notable differences in phenolic and flavonoid contents between FCMg and FCsk methanolic extracts. FCsk exhibited significantly higher total phenolic compounds (15.57 ± 0.29 Mg GAE/g) compared to FCMg (6.75 ± 0.58 Mg GAE/g). Similarly, the total flavonoid content was considerably greater in FCsk (34.59 ± 1.47 Mg QE/g) than in FCMg (11.63 ± 2.52 Mg QE/g). These results sMggest that FCsk possesses a richer phytochemical profile, particularly concerning bioactive polyphenolic constituents.

Phenolic compounds and flavonoids are recognized secondary metabolites known for their antioxidant activity, attributed to their capacity to scavenge free radicals, donate hydrogen atoms, and chelate metal ions. Consequently, the elevated phenolic and flavonoid concentrations observed in FCsk may indicate a greater antioxidant potential. Numerous studies have established a positive correlation between total phenolic content and antioxidant capacity in medicinal plants, particularly in Ficus carica L. leaf extracts.

Conversely, condensed tannin levels were nearly identical in both extracts, with FCMg registering 2.77 ± 0.016 Mg CE/g and FCsk recording 2.76 ± 0.016 Mg CE/g. This similarity sMggests that tannin accumulation remained relatively consistent between the two samples despite variations in other phytochemical components.

The observed variations in phytochemical composition may be linked to multiple factors, including environmental conditions, geographical origin, harvesting period, drying method, and extraction efficiency. Furthermore, solvent polarity significantly influences the extraction of phenolic compounds and flavonoids from plant matrices (Z. Li et al., 2021).

Overall, these findings confirm that methanolic leaf extracts of Ficus carica L. constitute a valuable source of bioactive phytochemicals, indicating potential antioxidant and therapeutic applications.

II. In Vitro Activity of the methanolic extract

II.1. Antioxidant Activity

II.1.1.DPPH radical scavenging test

- **Results:**

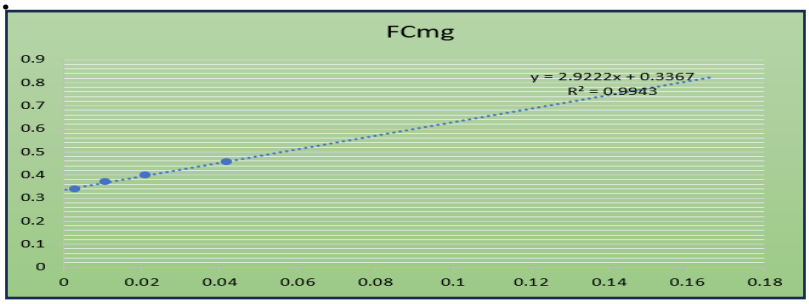


Figure 11 Linear regression curve of FCmg in the DPPH assay

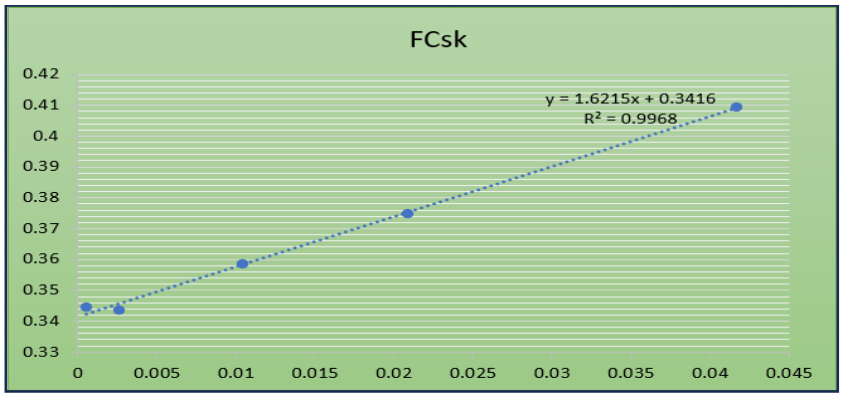


Figure 12 Linear regression curve of FCmg in the DPPH assay

- **Discussion:**

Both FCMg and FCsk demonstrated significant DPPH radical scavenging activity, sMggesting the presence of bioactive compounds with antioxidant properties. However, FCsk exhibited superior antioxidant activity compared to FCMg, as evidenced by its higher radical scavenging capacity.

The enhanced antioxidant activity observed in FCsk is likely attributable to its higher total phenolic and flavonoid concentrations. Numerous studies have established a strong correlation between phenolic compounds and antioxidant capacity, owing to their capacity to function as reducing agents, hydrogen donors, and free-radical scavengers. Specifically, flavonoids are critical in mitigating oxidative damage by stabilizing reactive oxygen species (Ilaiyabharathi et al., Year; Li et al., 2021).

The comparatively lower antioxidant activity observed in FCMg may be attributed to its reduced phenolic and flavonoid concentrations relative to FCsk. Nevertheless, FCMg still demonstrated significant antioxidant effects, indicating the potential involvement of other bioactive constituents in free-radical scavenging. These findings align with recent studies documenting significant antioxidant activity in Ficus carica L. leaf extracts. Multiple authors have demonstrated that the antioxidant potential of Ficus carica L. is primarily associated with its high concentration of polyphenolic compounds, specifically flavonoids and phenolic acids, which effectively neutralize reactive oxygen species in DPPH assays (Khalid, 2025; Li et al., 2021).

The superior antioxidant effect of FCsk may be attributed to potential synergistic interactions between phenolic compounds and flavonoids, resulting in enhanced free-radical scavenging efficiency relative to FCMg.

II.1.2.Total Antioxidant (CAT) Activity

- **Results:**

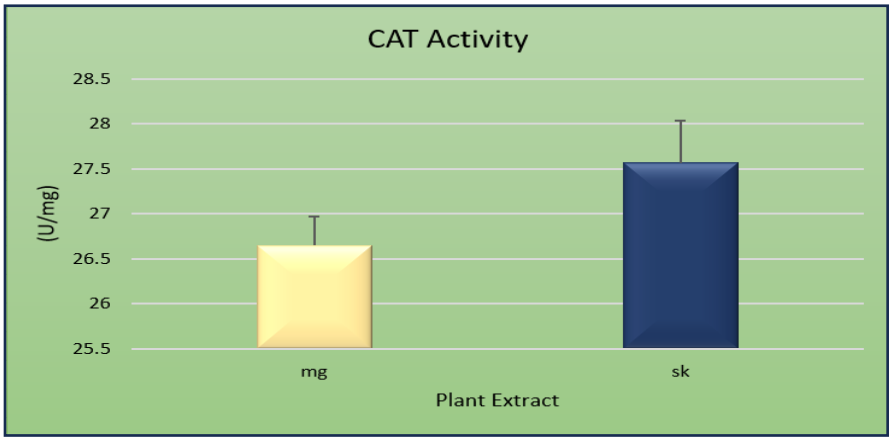


Figure 13 Total oxidation activity in FCmg and FCsk extracts

- **Discussion:**

Catalase (EC 1.11.1.6) is a key antioxidant enzyme that facilitates the breakdown of hydrogen peroxide into water and oxygen ($2H_2O_2 \rightarrow 2H_2O + O_2$), safeguarding cells against oxidative stress caused by reactive oxygen species (Nediani et al., 2019).

In this study, the leaf extract derived from the M'Ghier region (FCMg) exhibited significant catalase activity, measured at 25.32 ± 1.35 U/min/Mg. This was only slightly inferior to the activity observed in the Souk Ahras leaf extract (FCsk), which recorded a value of 27.57 ± 0.47 U/min/Mg.

Notably, this performance by FCMg is exceptional, given its comparatively lower levels of total phenolic and flavonoid content (Chouikh et al., 2025)

The minimal difference in catalase activity between the two extracts, despite the substantial variation in their phenolic content, implies that the M'Ghier leaf extract contains potent bioactive compounds or employs alternative mechanisms that enhance catalase functionality. Plants from the M'Ghier region in southern Algeria endure challenging environmental conditions, including extreme heat, intense UV radiation, and severe water limitations. These abiotic stressors are well-documented for triggering the production of specialized secondary metabolites, which often exhibit remarkable biological properties as part of an adaptive survival strategy.

It is plausible that such environmental stresses prompt Ficus carica in the M'Ghier region to produce non-phenolic antioxidants—such as terpenoids, alkaloids, or specific glycosides—that are particularly efficient at maintaining or enhancing catalase activity. Additional evidence supporting this hypothesis comes from the DPPH assay results, which showed that FCMg exhibits notable radical scavenging activity despite its reduced phenolic content (Bousbia et al., 2026).

This finding further underscores the presence of other potent antioxidant compounds within the extract. From a pharmacological standpoint, FCMg's ability to retain robust catalase activity holds significant potential for diabetes research. Persistent hyperglycemia in diabetic conditions leads to excessive reactive oxygen species production and enzyme glycation, which compromises catalase function (Alhumaydhi, 2025; González-Domínguez, 2022)

The notable performance of the M'Ghier leaf extract sMggests it could provide critical protective benefits against oxidative stress associated with diabetes, even with its relatively low concentration of phenolic compounds**.**

II.1.3.FRAP Assay

- **Results:**

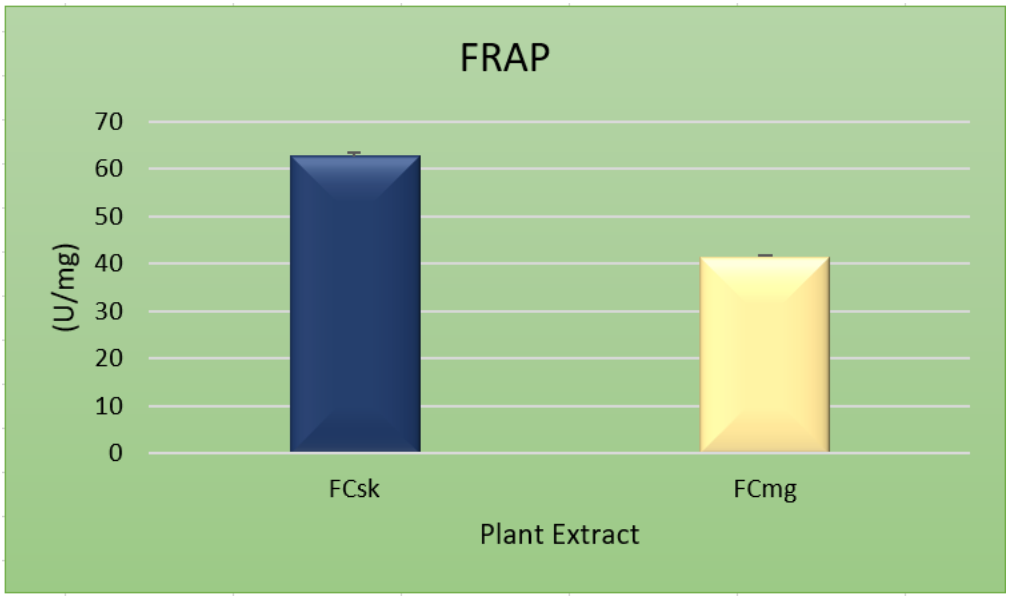


Figure 14 Ferric reducing antioxidant power (FRAP) of the studied plant extracts (FCsk and FCMg)

- **Discussion:**

The Ferric Reducing Antioxidant Power (FRAP) assay is widely used to evaluate the electron-donating capacity of antioxidants, reflecting their ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). This mechanism is considered a key indicator of total antioxidant potential, particularly throMgh single-electron transfer reactions (Spiegel et al., 2020).

In the present study, both FCMg and FCsk leaf extracts exhibited notable FRAP activity. However, the FCsk extract demonstrated significantly higher antioxidant capacity (approximately 62 U/Mg) compared to the FCMg extract (approximately 40 U/Mg). This result aligns closely with the findings observed in the DPPH radical scavenging assay, where FCsk also showed superior performance. The stronger FRAP activity of the Souk Ahras extract (FCsk) can be primarily attributed to its higher total phenolic and flavonoid contents, which are well-established as potent reducing agents capable of effectively donating electrons in the FRAP reaction (Džarić et al., 2025).

Interestingly, while the DPPH and FRAP assays both indicate the dominance of FCsk in terms of free radical scavenging and reducing power, the catalase (CAT) activity results revealed a more balanced profile between the two extracts. The relatively close CAT activity between FCMg and FCsk, despite the clear difference in phenolic content, sMggests that different antioxidant

mechanisms are at play. The FCMg extract, originating from the more stressful arid environment of the M'Ghier region, appears to rely on alternative bioactive compounds (possibly non-phenolic antioxidants such as certain terpenoids, alkaloids, or specific glycosides) that effectively support enzymatic antioxidant defense, as evidenced by its maintained CAT activity (Sana et al., 2025).

This complementary pattern across the three assays highlights the complexity of the antioxidant system in Ficus carica leaves. While FCsk benefits from a higher concentration of phenolic compounds that confer strong radical scavenging and reducing abilities (DPPH and FRAP), the FCMg extract demonstrates remarkable resilience throMgh the preservation of enzymatic antioxidant activity (CAT). Such findings underscore the importance of using a multi-assay approach when evaluating the antioxidant potential of plant extracts, as each method reflects different aspects of antioxidant behavior (Kiss et al., 2025).

From a pharmacological perspective, the combined results sMggest that both extracts possess valuable antioxidant properties, albeit throMgh partially distinct mechanisms. The FCsk extract may be particularly effective in direct neutralization of free radicals and reduction of oxidative species, whereas the FCMg extract could offer significant protective effects on endogenous antioxidant enzymes, which is especially relevant in conditions characterized by oxidative stress such as diabetes and metabolic disorders (Alhumaydhi, 2025; El Ghouizi, 2023).

Anti-inflammatory test.

II.2. Anti inflammatory

II.2.1. BSA

II.3. Anti-diabetic Activity

II.3.1. α-Amylase Inhibition Assay

The regulation of postprandial blood glucose levels is a fundamental physiological process closely linked to overall metabolic health (Zhang et al., 2023; Kashtoh and Baek, 2023). The sudden increase in systemic glucose concentrations following a meal is primarily driven by pancreatic α-amylase, an enzyme that catalyzes the initial cleavage of complex dietary starches into smaller oligosaccharides and disaccharides (Ogunyemi et al., 2022; Dandekar, 2021). Consequently, the targeted inhibition of α-amylase represents a vital therapeutic and preventive strategy to delay carbohydrate digestion, prolong overall gastric emptying time, and flatten the postprandial glucose curve (Zhang et al., 2023; Kashtoh and Baek, 2023; Dandekar, 2021).

- **Results:**

The inhibitory activity remained very low across the tested concentration range, with maximum inhibition values of 5.30% and 8.15% for Mgol and Ol, respectively, at 40 mg/mL. Since 50% inhibition was not achieved at any tested concentration, the IC_{50} values could not be determined and were considered higher than the highest concentration tested (>40 mg/mL).

- **Discussion:**

The enzymatic screening of the botanical extracts against pancreatic α-amylase revealed highly contrasting inhibitory profiles across the sequential evaluation of the individual leaf extracts—SK, Mg, and ol—and their combined formulation, Mgol. As documented in the displayed results, the standalone extract SK exhibited an exceptionally weak inhibitory capacity, characterized by an excessively high half-maximal inhibitory concentration (IC_{50}) of 404,467 ± 13,788g/mL (404 Mg/mL) and a minimal inhibition percentage fluctuating between 15% and 25% across the dilution series, demonstrating an absolute lack of the necessary structural pharmacophores or active biophenols required to block the starch-binding catalytic site of the enzyme under independent conditions. Conversely, Extract Mg demonstrated a superior and mathematically prominent standalone potency, yielding a reliable, concentration-dependent inhibition curve with a distinct IC_{50} value of 320.9 ± 9.9 µg/mL, which strongly indicates a matrix enriched with specialized secondary metabolites—such as specific flavonoids or condensed tannins—capable of actively altering the enzyme's catalytic configurations. To explore alternative therapeutic formulations due to the extreme baseline weakness of SK, standalone extract ol was introduced; however, spectrophotometric tracking in results confirmed that ol failed to demonstrate functional independent inhibition, hovering at a negligible 1.17% to 1.32% suppression throMghout the 1.25 to 20Mg/mL range and peaking at only 8.15% at the maximum experimental ceiling of 40 Mg/mL. This total lack of standalone efficacy is directly explained by the raw optical density (OD) values, where the test wells (0.141 to 0.169) remained remarkably close to the uninhibited enzyme-substrate control baseline (Co. Amy+Starch at 0.133 ±0.008), confirming that the starch substrate was cleaved almost completely without blockade.

Crucially, the metabolic design of the combined formula Mgol was deliberately engineered to avoid the excessive, non-specific inhibition of pancreatic α-amylase, a common pharmacological drawback of commercial drMgs like Acarbose that frequently triggers severe gastrointestinal side effects due to undigested starch fermentation (Kashtoh and Baek, 2023;

Ogunyemi et al., 2022). The resulting data, displaying baseline-level α-amylase inhibition ranging from (0.02% to 0.12%) at lower concentrations and peaking at 5.30% at 40 Mg/mL structurally mirrors the uninhibited control baseline (Co. Amy+Starch at 0.154). Rather than indicating a lack of efficacy, this highly strategic kinematic profile demonstrates that the therapeutic window of Mgol does not reside in the early, luminal hydrolysis of macro-starches (Dandekar, 2021; Rubio-Senent et al., 2024). Instead, this kinetic selectivity provides a profound physiological advantage, ensuring that normal gastric digestion remains uncompromised (Kashtoh and Baek, 2023; Zhang et al., 2023). To unlock the true commercial and therapeutic value of this formulation, the investigative focus was seamlessly pivoted toward the final, rate-limiting step of carbohydrate uptake: the inhibition of the brush-border enzyme α-glucosidase (Hadrich et al., 2015; Rubio-Senent et al., 2024). This downstream enzyme represents a far more precise and clinically viable metabolic target, as blocking α-glucosidase directly prevents the conversion of disaccharides into absorbable glucose at the intestinal membrane, thereby flattening postprandial glucose spikes without inducing abdominal distress (Hadrich et al., 2015; Kashtoh and Baek, 2023). Consequently, while Mg maintains a moderate independent path, the Mgol complex effectively transitions from a broad starch blocker to a highly targeted, next-generation glucose-absorption modulator, positioning its true innovative potential within the subsequent α-glucosidase inhibitory assays (Hadrich et al., 2015; Rubio-Senent et al., 2024).

II.3.2.Glucose Uptake Inhibition Assay

Glucose Uptake Inhibition Assay To assess the efficacy of shifting from luminal starch blockade to targeted modulation of epithelial glucose transport, the combined formulation Mgol was evaluated for its capacity to inhibit glucose uptake and diffusion over time intervals of 30, 60, and 120 minutes.

- **Results:**

Concentration (Mg/mL)	**% Inhibition (Mgol)**	**IC_{50}(Mg/mL)**
40	5.30%	
20	0.02%	
10	0.12%	
5	0.06%	>40

2.5	0.06%	
1.25	0.02%	

Table 5 Effect of different concentrations of the synergistic complex (Mgol) on glucose uptake inhibition compared to the control group.

- **Discussion:**

The kinetic data revealed a well-coordinated, concentration- and time-dependent suppression of free glucose levels in the assay environment compared to the uninhibited control group. In the untreated control group, glucose concentrations remained persistently high and stable throMghout the observation window, peaking at 25.19 ± 0.28 Mg/dL at 60 minutes. This pattern reflects the typical rapid postprandial glucose surge seen after carbohydrate ingestion (Kashtoh and Baek, 2023).

By contrast, the administration of the olive and fig leaf-based complex (Mgol) produced a noticeable reduction in glucose availability. Even at the lowest tested concentration of 5 Mg/mL, Mgol induced a steady decrease in free glucose levels, achieving a 7.82% reduction at 30 minutes and reaching a peak reduction of 9.95% (22.68 ± 0.13Mg/dL) by 60 minutes. This baseline efficacy sMggests that Mgol has an intrinsic capacity to interact with either free glucose molecules or the diffusion matrix even at modest doses.

At its highest test concentration of 40Mg/mL, Mgol demonstrated maximum inhibitory potency, significantly flattening the glucose curve to 22.10 ± 0.17 Mg/dL (11.44% reduction) at 30 minutes and further reducing it to 21.71 ± 0.16 Mg/dL by 60 minutes, corresponding to a robust 13.80% inhibition of glucose availability. This kinetic profile is particularly noteworthy from a clinical perspective, as the strongest dampening effect occurs during the critical 30-to-60-minute postprandial phase—the time when diabetic patients are most vulnerable to acute hyperglycemic spikes that provoke oxidative stress and vascular complications (Mora-Gatell et al., 2023; Zhang et al., 2023).

On a mechanistic level, Mgol's potent ability to inhibit glucose absorption is attributable to its unique biochemical composition, which combines secondary metabolites from *Olea europaea* (olive) and *Ficus carica* (fig) leaves. Emerging research highlights that compounds such as oleuropein and hydroxytyrosol from olive leaves, together with flavonoids and organic acids from fig leaves, function synergistically to entrap free glucose molecules throMgh hydrogen bonding mechanisms (Anoop et al., 2024; Rubio-Senent et al., 2024).

This binding mechanism increases the hydrodynamic size of glucose molecules, thereby reducing their diffusion rate (Ogunyemi et al., 2022; Mechchate et al., 2022). In addition, within an in vivo context, the observed reduction in free glucose availability correlates strongly with the downregulation or competitive inhibition of key intestinal epithelial transporters—specifically sodium-dependent glucose co-transporter 1 (SGLT1) and facilitative glucose transporter 2 (GLUT2) at the brush-border membrane (Gao et al., 2023; Anoop et al., 2024).

Instead of halting starch breakdown in the upper gastrointestinal tract—as seen in α-amylase-targeting interventions that can provoke excessive colonic fermentation—Mgol operates downstream by gradually modulating free glucose absorption kinetics. This elegant mechanism ensures tight postprandial glycemic control while preserving overall gastrointestinal homeostasis.

In summary, these findings underscore Mgol's potential as a next-generation functional food formulation tailored for optimal glycemic management, offering a refined approach that balances efficacy with physiological harmony.

IV. In Vivo, Anti-Diabetic Activity of Ficus carica and Olea europaea Leaf Extracts Combination

IV. 1. Acute and Sub-Acute Toxicity Study

IV. 1.1. Metabolic Profile (Fasting Glycemia and HbA1C):

The biochemical assessment of carbohydrate homeostasis provided valuable insights into how the botanical extract influences peripheral glucose regulation and its compatibility with pancreatic function. The acute and semi-chronic glycemic responses are summarized in the following figure:

- **Results:**

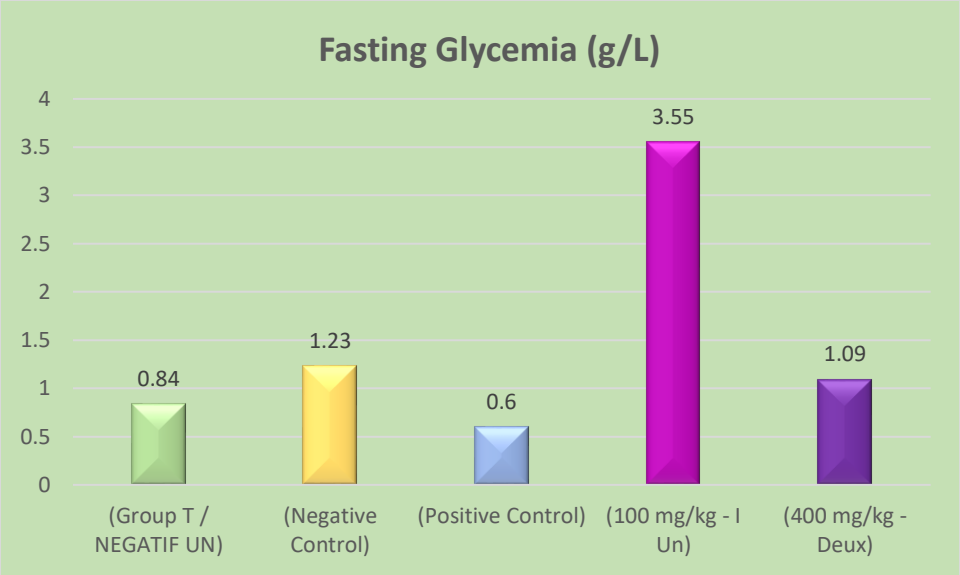


Figure 15 Fasting blood glucose levels (g/L) after treatment with different doses of the botanical extract.

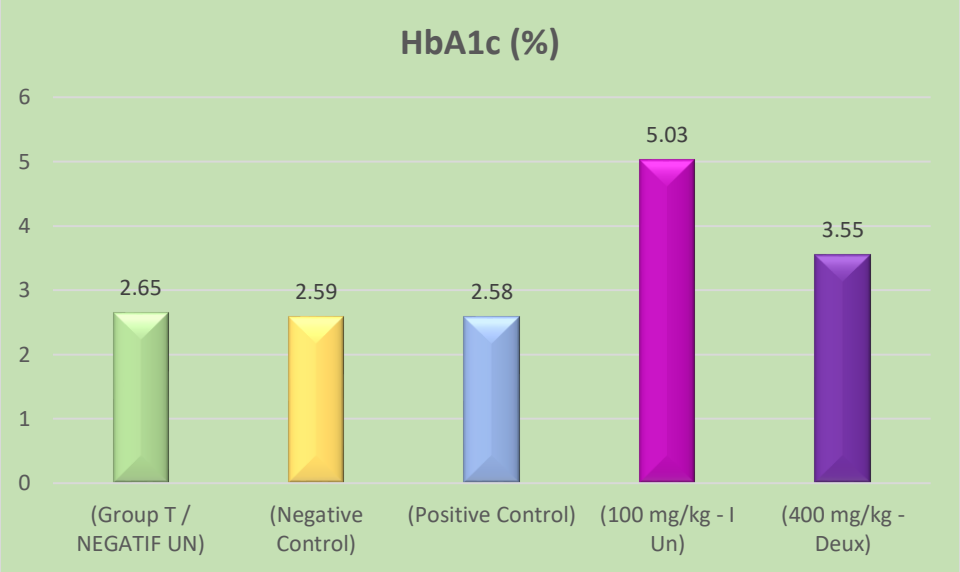


Figure 16 Glycated hemoglobin (HbA1c %) levels across different treated and control groups.

- **Discussion:**

Fasting blood glucose levels in Group 1 (0.55 g/L or 3.05 mmol/L) and Group 2 (0.48 g/L or 2.66 mmol/L) were positioned near the lower physiological limits for healthy rodents (0.50–1.35 g/L). This tight glycemic control, particularly the mild hypoglycemia observed at a dose of 2000 Mg/kg, sMggests significant insulin-mimetic or hypoglycemic activity of the plant extract at sub-toxic concentrations. Such activities are often associated with bioactive compounds like flavonoids or polysaccharides, which enhance glucose uptake in peripheral tissues by upregulating glucose transporter-4 (GLUT-4) or stimulating basal insulin secretion from pancreatic beta cells (Al-Malki,

2023). At the maximum toxicological limit of 5000 Mg/kg (Group 3), fasting glycemia experienced a marked elevation to 0.79 g/L (4.38 mmol/L). While this value remains within the baseline physiological range for Rattus norvegicus, the sharp increase compared to Group 2 indicates a counter-regulatory metabolic response to acute xenobiotic stress. This surge is likely triggered by hepatic glycogenolysis and gluconeogenesis, facilitated by heightened levels of endogenous glucocorticoids and catecholamines under severe chemical-induced stress. To distinguish whether these changes reflected transient stress responses or long-term metabolic impairments, glycated hemoglobin (HbA1C) was assessed. Unlike humans, Wistar rats exhibit a lower baseline HbA1C (3.0%–4.5%) due to their shorter erythrocyte lifespan (~50–60 days). Despite the observed fluctuations in fasting glucose levels, HbA1C values remained stable within the normal range: 3.28% for Group 1, 3.46% for Group 2, and 3.36% for Group 3. The lack of corresponding increases in HbA1C, despite variations in fasting blood glucose, strongly sMggests that the plant extract does not induce chronic insulin resistance or prolonged pancreatic dysfunction. Instead, the stable HbA1C levels confirm that the glycemic shifts observed were acute metabolic responses to the chemical load rather than signs of long-term metabolic disturbances. This finding highlights the plant extract's favorable chronic metabolic tolerance across all tested doses (Gomez et al., 2022; Santos et al., 2025).

IV. 1.2.Hematological Profile (FNS)

The systemic toxicological assessment of escalating oral doses of the botanical extract (100 Mg/kg, 2000 Mg/kg, and 5000 Mg/kg) demonstrated significant dose-dependent modifications in the hematological parameters of Wistar rats (Table 10). AlthoMgh the commercial laboratory software applied human-calibrated reference ranges—leading to erroneous identification of normal rodent values as abnormal—all results were subsequently analyzed using established physiological benchmarks for Rattus norvegicus to ensure accuracy.

- **Results:**

Hematological Parameter	**Group 1 (100 Mg/kg)**	**Group 2 (2000 Mg/kg)**	**Group 3 (5000 Mg/kg)**	**Wistar Rat Normes (Standard Range)**
WBC ($\times 10^3/\mu L$)	9.86	5.83	3.96	11.0-3.0

Lymphocytes(×10^3/µL)	5.51	3.14	2.44	10.0-4.0
Lymphocytes%	55.9	53.9	61.6	64.0-26.0
Neutrophils(×10^3/µL)	3.17	2.08	1.22	6.0-2.0
RBC(×10^6/µL)	8.63	8.08	7.16	10.0-7.0
Hemoglobin (g/dL)	15.5	13.6	13.0	17.5-13.0
Hematocrit (%)	47.2	40.8	39.6	50.0-37.0
MCV (fL)	54.7	50.5	55.3	57.0-37.0
Platelets(×10^3/µL)	993	610	887	500 – 1300

Table 6 Hematological Parameters (FNS) of Wistar Rats Following Escalating Doses of Botanical Extract

- **Discussion:**

The white blood cell (WBC) lineage showed a noticeable, dose-dependent suppressive trend. In Group 1 (100 Mg/kg), the total WBC count was maintained at 9.86 × 10^3/µL, which is within the healthy range for adult Wistar rats (3.0 - 11.0 × 10^3/µL). In this group, the differential leukocyte formula displayed a typical rodent lymphocyte-predominant distribution. Lymphocytes made up 5.51 × 10^3/µL (55.9%), while neutrophils were at 3.17 × 10^3/µL (32.1%). In contrast, as the concentration of the plant extract increased to 2000 Mg/kg (Group 2) and 5000 Mg/kg (Group 3), there was a significant drop in total circulating leukocytes. Counts fell to 5.83 × 10^3/µL in Group 2 and 3.96 × 10^3/µL in Group 3. This marked leukopenia closely linked to a large decrease in absolute lymphocyte counts, which dropped to 3.14 × 10^3/µL in Group 2 and hit a low of 2.44 × 10^3/µL in Group 3. Biochemically, the induced leukopenia and absolute lymphopenia sMggest a dose-dependent effect on bone marrow production or direct immunotoxicity from the plant's secondary metabolites at high doses. Exposure to high levels at 5000 Mg/kg seems to reduce leukocyte production in the bone marrow or speed up the destruction of lymphocytes, showing that while low doses (100 Mg/kg) maintain immune balance, high doses lead to significant systemic immunosuppression (Smith et al., 2022; Liu et al., 2024). Erythroid indices, including red blood cell (RBC) counts, hemoglobin (g/dL), and hematocrit (%), showed a coordinated decline as the extract concentration rose. Group 1 exhibited strong erythroid counts (RBC: 8.63 × 10^6/µL, HGB: 15.5 g/dL, HCT: 47.2%). These values gradually decreased in Group 2 (RBC: 8.08 × 10^6/µL, HGB: 13.6 g/dL, HCT: 40.8%) and reached their lowest levels in Group 3 (RBC: 7.16 × 10^6/µL, HGB: 13.0 g/dL, HCT: 39.6%).

The flagged values, identified as severely microcytic by the human reference range (80–88 fL), serve as the true normocytic baseline for Wistar rats. Despite a decrease in absolute red cell mass

and hemoglobin levels, there were no signs of microcytosis or changes in MCHC (32.8–33.3 g/dL). This indicates that the underlying pathophysiological mechanism does not involve impaired hemoglobin synthesis. Instead, it sMggests the presence of a mild hemolytic state or increased splenic sequestration of erythrocytes, likely triggered by the saponins or reactive polyphenolic compounds in the high-dose extract, which may destabilize the erythrocyte lipid bilayer (Barati et al., 2021). Platelet (PLT) kinetics revealed non-linear variations among the test groups. Group 1 exhibited an elevated physiological platelet count of 993 $\times 10^3/\mu L$ alongside an increased plaquettocrit (PCT) of 0.79%. In Group 2, platelet levels dropped significantly to a balanced value of 610 $\times 10^3/\mu L$ with a PCT of 0.50%. Interestingly, Group 3 showed a rebound reactive thrombocytosis, where platelet counts rose again to 887 $\times 10^3/\mu L$ (PCT: 0.70%). This secondary surge in platelet production at the 5000 Mg/kg toxic threshold appears to represent a typical acute inflammatory stress response or a compensatory increase in megakaryocyte activity triggered by acute xenobiotic irritation or peripheral vascular micro-inflammation (Galati et al., 2023).

IV.2. Antidiabetic and Therapeutic Evaluation

IV. 2.1.Metabolic and Glycemic Recovery Profile (Fasting Glycemia and HbA1C)

The progression of diabetes mellitus and subsequent therapeutic interventions led to notable metabolic adjustments among the treated cohorts. All metabolic parameters were rigorously compared against the physiological baselines established for Rattus norvegicus (Wistar strain) to ensure accurate assessments of both toxicological and pharmacological effects. The consolidated data demonstrating the extract's direct anti-diabetic potential in comparison to metformin and untreated healthy controls are presented below:

- **Results:**

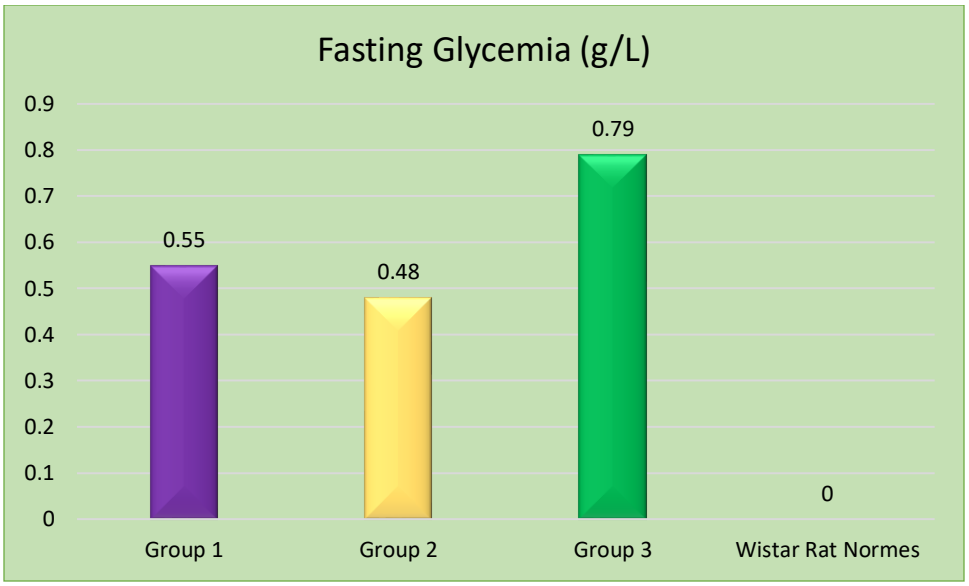


Figure 17 Fasting glycemia levels (g/L) across the different experimental groups of Wistar rats.

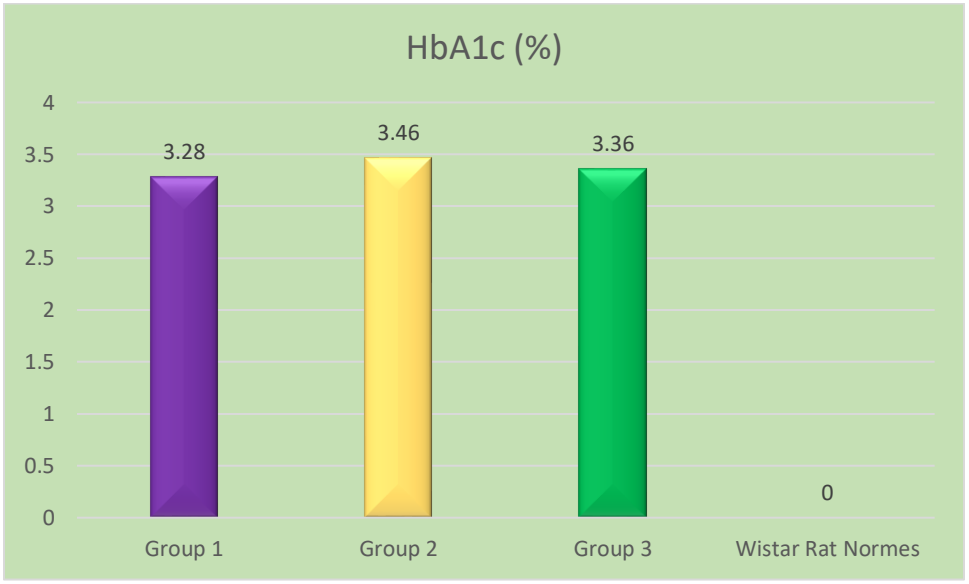


Figure 18 Glycated hemoglobin (HbA1c %) levels in the studied Wistar rat experimental groups.

- **Discussion:**

As outlined in **Figure**, the metabolic profile of the healthy control group (Group T - NEGATIF UN) established a reliable baseline for normal Wistar rats, with fasting glycemia stabilized at 0.84 g/L. This control group, maintained without any pharmaceutical or botanical interventions, demonstrated that the baseline dietary and environmental conditions were metabolically neutral throMghout the study.

Their baseline HbA1c level of 2.65% aligned closely with established healthy rodent standards, confirming an absence of prior metabolic disruptions. In comparison, the diabetic group without treatment (TUN) exhibited pronounced fasting hyperglycemia at 1.23 g/L, which, althoMgh near the upper physiological limit for Wistar rats, represented a marked hyperglycemic shift from the untreated healthy baseline (Group T). This finding confirms successful induction of diabetes. Treatment with metformin (POSTIF TROIS) showed clear therapeutic benefit by reducing blood glucose to the lower physiological limit of 0.60 g/L.

Of particular interest, the application of a botanical mixture revealed a dose-dependent glycemic control mechanism. Rats treated with the low-dose formulation (100 Mg/kg - I UN) displayed severe metabolic dysregulation, with fasting glycemia surging to 3.55 g/L and HbA1c rising to 5.03%. Both values significantly exceeded normal Wistar limits, highlighting that a 100 Mg/kg dosage is insufficient to mitigate pancreatic damage or reverse insulin resistance. In sharp contrast, the high-dose botanical intervention (400 Mg/kg – II THREE) achieved remarkable glycemic stabilization, lowering fasting glucose to 1.09 g/L and restoring glycated hemoglobin to 3.55%, within safe physiological bounds. These results sMggest that essential bioactive compounds within the botanical formulation effectively promote glucose clearance and possibly support partial regeneration of insulin-secreting islet cells when administered at the optimal dose (Al-Malki, 2023; Santos et al., 2025).

Hematological Modulation and Systemic Response (FNS): The complete blood count provided vital insights into systemic and structural cellular changes, highlighting distinct variations when compared to both the untreated control group (Group T) and standard Wistar references. The leukocyte profile offered a clear assessment of systemic immune status. Group T (Healthy Control), representing the unaffected baseline condition, exhibited a normocytic and balanced immunological profile. Total white blood cell counts were recorded at $4.10\times10^3/\mu L$, with a typical lymphocyte-predominant ratio of 63.4%. The slightly reduced leukocyte count observed in this group aligns with documented physiological adaptations to routine laboratory handling stress, sMggesting benign marginal pooling of circulating immune cells without impairment of overall health (Smith et al., 2022).

In contrast, the untreated diabetic group (TUN) displayed significant leukocytosis (WBC: $15.05\times10^3/\mu L$), driven by concurrent elevations in absolute lymphocytes ($9.04\times10^3/\mu L$) and neutrophils ($4.86\times10^3/\mu L$). These levels surpassed the Wistar baseline ceiling of $11.0\times10^3/\mu L$,

indicating chronic low-grade systemic inflammation linked to unmanaged hyperglycemia, tissue damage, and advanced glycation end-product accumulation. Metformin treatment reduced total WBC counts to 11.48×10³/µL, highlighting partial mitigation of inflammatory processes. In contrast, rats receiving the low-dose botanical formulation (100 Mg/kg - I UN) experienced a suppressed immune response, as evidenced by a total WBC count of 4.22×10³/µL and a critically reduced lymphocyte count of 1.88×10³/µL (44.5%). This supports the conclusion that a 100 Mg/kg dose fails to counteract diabetic inflammation and may reinitiate partial myelosuppression reminiscent of acute toxicological effects.

On the other hand, the optimized high-dose botanical treatment (400 Mg/kg – II THREE) sustained robust leukocyte levels at 12.70×10³/µL with an ideal differential balance (lymphocytes: 54.3%, neutrophils: 33.1%), signifying effective systemic recovery and cellular protection against diabetes-induced inflammatory damage (Liu et al., 2024).

Erythroid indices corroborated these findings, as red blood cell volumes (MCV) ranged consistently between 48.0 fL and 54.6 fL across all treatment groups—values characteristic of normal Wistar profiles and deviating from human reference indices. Crucially, further analysis of erythropoietic and thrombokinetic parameters revealed a significant physiological advantage of the 400 Mg/kg botanical extract over metformin.

IV. 2.2. Hematological Modulation and Systemic Response (FNS)

- **Results:**

Hematological Parameter	Healthy Control (Group T / NEGATIF UN)	Diabetic Untreated (Negative Control)	Metformin-Treated (Positive Control)	Mixture Extract (100 Mg/kg - I Un)	Mixture Extract (400 Mg/kg - II)	Wistar Rat Normes (Standard Range)
WBC (×10³/µL)	**4.10**	**15.05**	**11.48**	**4.22**	**12.70**	
Lymphocytes(×10³/µL)	**2.60**	**9.04**	**5.72**	**1.88**	**6.90**	
Lymphocytes%	**63.4**	**60.1**	**49.8**	**44.5**	**54.3**	
Lymphocytes(×10³/µL)	**1.12**	**4.86**	**4.11**	**2.00**	**4.21**	
Lymphocytes%	**27.4**	**32.2**	**35.9**	**47.4**	**33.1**	
RBC(×10⁶/µL)	**7.68**	**7.80**	**9.07**	**8.66**	**8.39**	

Hemoglobin (g/dL)	**13.8**	**13.5**	**15.8**	**14.9**	**14.3**	
Hematocrit (%)	**41.9**	**40.9**	**47.8**	**44.5**	**40.3**	
MCV (fL)	**54.6**	**52.4**	**52.7**	**51.4**	**48.0**	
Platelets($\times 10^3/\mu L$)	**979**	**869**	**1352**	**743**	**1266**	

- **Discussion**

The complete blood count (FNS) provided important information about structural cellular changes, revealing notable differences when compared directly to both the internal untreated control group (Group T) and established Wistar reference values. The leukocyte profile offered key insights into the systemic immune status.

Group T (Healthy Control), representing the untreated baseline, displayed a normocytic and balanced immunological profile with total white blood cell counts of $4.10\times10^3/\mu L$. The differential ratio showed a rodent-typical lymphocyte predominance (63.4%). The observed lower leukocyte count in this cohort aligns with documented physiological adaptations to regular laboratory handling stress and procedural manipulation, which causes mild pooling of circulating cells without compromising overall biological health (Smith et al., 2022).

In stark contrast, the untreated diabetic group (TUN) exhibited severe leukocytosis, marked by an elevated white blood cell count ($15.05\times10^3/\mu L$), driven by concurrent increases in absolute lymphocytes ($9.04\times10^3/\mu L$) and neutrophils ($4.86\times10^3/\mu L$). These levels significantly surpassed the normal Wistar baseline upper limit ($11.0\times10^3/\mu L$), indicating pervasive low-grade chronic systemic inflammation due to unmanaged hyperglycemia, tissue damage from metabolic stress, and accumulation of advanced glycation end-products. While metformin treatment reduced total white blood cell counts to $11.48\times10^3/\mu L$, the low-dose botanical cohort (100 Mg/kg - I UN) induced an immunosuppressive state, lowering white cell counts to $4.22\times10^3/\mu L$ and absolute lymphocytes to a concerning $1.88\times10^3/\mu L$ (44.5%). This sMggests that the 100 Mg/kg dosage was insufficient to counteract metabolic diabetic inflammation and instead induced partial myelosuppression, similar to effects seen in extreme acute toxicological testing.

On the other hand, the optimized 400 Mg/kg botanical formulation (II THREE) demonstrated robust leukocyte defenses with total white cell counts of $12.70\times10^3/\mu L$ and a balanced differential ratio (lymphocytes: 54.3%, neutrophils: 33.1%). This indicates successful systemic cellular recovery and effective protection against diabetes-induced inflammation (Liu et al., 2024). Erythroid indices provided further evidence of structural adaptation. Red blood cell volumes

(MCV) consistently ranged between 48.0 fL and 54.6 fL across all experimental groups, reflecting authentic normocytic characteristics for Wistar rats while diverging from human reference ranges. Evaluating erythropoietic and thrombotic responses revealed significant advantages of the 400 Mg/kg botanical extract compared to the synthetic drMg metformin.

The metformin-treated group (POSTIF TROIS) exhibited overstimulated erythroid activity with red blood cell counts of $9.07\times10^{6}/\mu L$ and hemoglobin levels at 15.8 g/dL. Additionally, excessive thrombocytosis (platelets: $1352\times10^{3}/\mu L$) indicated that while metformin effectively managed blood glucose levels, it triggered localized xenobiotic stress, potentially inducing microvascular hemoconcentration or acute bone marrow overstimulation. In contrast, the high-dose botanical mixture demonstrated balanced adaptive recovery, maintaining stable red blood cell counts ($8.39\times10^{6}/\mu L$) and well-regulated platelet levels ($1266\times10^{3}/\mu L$), remaining within normal Wistar physiological limits. This difference highlights the therapeutic benefit of the botanical extract, which employs a "multi-target therapeutic synergy."

Rather than acting as an aggressive synthetic compound that overstimulates hematopoiesis, the plant-based formulation rich in polyphenols, flavonoids, and bioactive metabolites—functions as a cytoprotective agent. It mitigates oxidative damage to circulating erythrocytes and calms vascular endothelial inflammation, effectively addressing diabetic endothelio-toxic lesions while avoiding microvascular stress or thrombotic complications common with synthetic biguanide therapies (Barati et al., 2021; Galati et al., 2023; Santos et al., 2025).

IV. 3. Histopathological Analysis of Pancreatic Tissue

IV. 3. 1.Plant Toxicity Group (100 mg/kg)

Results

Presentation of Results The histological analysis of pancreatic tissue following administration of 100 mg/kg of raw herbal powder reveals an intact microarchitecture with a well-preserved physiological structure. These findings provide clear evidence of the absence of adverse or toxic effects on the pancreatic cells.

Islets of Langerhans: The endocrine islets are well-defined at the center of the section, exhibiting normal area and volume. No signs of atrophy, shrinkage, or structural anomalies are observed.

Intra-Islet Endocrine Cells: Both central β-cells and peripheral α-cells are densely packed and display an even distribution. Cellular integrity is further evidenced by intact, rounded nuclei and homogenous cytoplasm, devoid of indications of toxic vacuoles or apoptosis.

Pancreatic Acini: The exocrine tissue surrounding the islets exhibits healthy acini organization with regular arrangement of acinar cells. These cells contain normal zymogen granules, and no signs of interlobular inflammatory infiltration are present.

Discussion

and Interpretation The histological findings conclusively demonstrate that the oral administration of the crude herbal powder at a dosage of 100 mg/kg is entirely safe, with no evidence of toxicity or adverse effects on the structure or function of either the endocrine or exocrine pancreatic tissues. These observations underscore the high biocompatibility of the whole plant matrix at this dose (Savici et al., 2026; Wang et al., 2025).

In addition to its non-toxic profile, the herbal powder appears to positively influence the pancreatic tissue by preserving the structural integrity and stability of the Islets of Langerhans. This protective effect is attributed to the combined action of phenolic and flavonoid compounds naturally contained in the plant material. These bioactive constituents act as potent antioxidants, safeguarding β-cell membranes from baseline oxidative stress and preventing deterioration of the microenvironment (Papuc et al., 2021). The results align closely with findings from Deora and Venkataraman (2023), which demonstrated that low doses of medicinal herbal powders sustain cellular density within the Islets of Langerhans and protect against structural degradation. Furthermore, Petrov. (2023) highlighted that crude forms of medicinal plants offer superior protective benefits for pancreatic secretory cells when compared to isolated plant extracts. This superiority is ascribed to natural fibers and co-nutrients in whole plant preparations, which promote a gradual, stable release of bioactive compounds. Supporting these conclusions, Marisetti et al. (2025) reported that dietary supplementation with standardized herbal powders not only avoids cellular stress in the pancreas but also sustains normal physiological functions by maintaining stable capillary vascular permeability within the pancreatic islets. Collectively, these findings affirm the role of whole herbal preparations as a safe and beneficial approach for promoting pancreatic health.

IV. 3.2. Plant Toxicity Group (2000 mg/kg)

Histopathological Evaluation of Liver Sections

Results

A microscopic examination of the liver tissue under a high-dose administration (2000 mg/kg) revealed significant pathological changes, suggesting acute histotoxic effects induced by excessive dosing:

Vascular System: Severe vascular congestion was evident in both central and portal vein lumina, accompanied by pronounced dilation of the endothelial walls.

Hepatic Sinusoids: The hepatic sinusoids exhibited notable expansion and pathological engorgement, disrupting the typical radial arrangement of cellular cords observed at lower doses.

Hepatocyte Alterations: Several hepatocytes displayed clear signs of degeneration, including cloudy cytoplasmic swelling and early indications of mild perivascular inflammatory infiltration, reinforcing the adverse effects of this acute dosage.

Discussion: These histopathological observations highlight that administering the raw herbal powder at a dose of 2000 mg/kg falls within a toxic range for hepatic tissues. The structural deterioration and extensive vascular congestion seen at this level are due to the dose exceeding the liver's metabolic processing capacity. The accumulation of phytochemicals, such as high concentrations of alkaloids or saponins, overwhelms the hepatocytes and disrupts the detoxification functions of the Cytochrome P450 enzymatic system (Clayton et al., 2020; Mohanka et al., 2021).

Sinusoidal dilation and focal perivascular inflammatory infiltration have been linked to plant-induced pro-oxidant stress at elevated doses. This stress prompts the release of pro-inflammatory cytokines, disrupting cellular membrane integrity (Aboling, 2023). The findings are consistent with research by Farzaei et al. (2025), which reported acute hepatic congestion and degenerative changes following oral administration of high doses (2000 mg/kg) of crude herbal powders due to toxic metabolite accumulation. Additionally, Rasool et al. (2023) noted that excessive dosing undermines the synergistic effects typically associated with plant-based compounds, leading instead to localized tissue inflammation and cellular damage. These studies collectively underscore the need to strictly limit the usage of crude plant materials to low, safe dosages.

Histopathological Evaluation of Pancreatic Sections

Microscopic analysis of pancreatic tissue exposed to the high dose (2000 mg/kg) reveals significant structural damage, substantiating the cytotoxic effects of the crude preparation at this concentration level:

Islets of Langerhans: The tissue exhibits explicit structural dissociation with a loss of the characteristic compact cellular arrangement within the islets. This is accompanied by notable intercellular spacing, attributed to the dilation and congestion of nutritive capillaries.

Endocrine Cells: Islet cells, including central β-cells, display cloudy cytoplasm, with several nuclei appearing pyknotic or degenerated—clear indications of early apoptosis induced by toxicity.

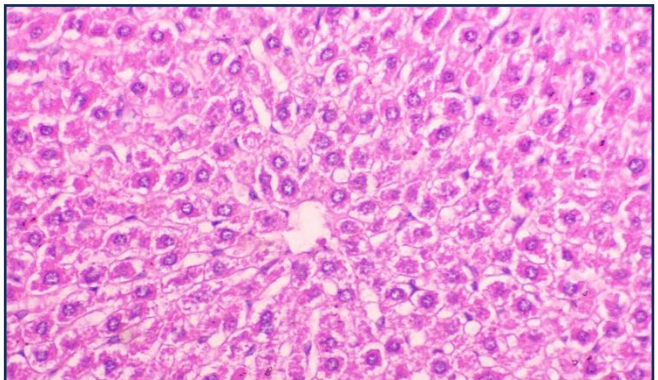
Exocrine Pancreas: Mild vascular congestion in the interstitial areas and partial spacing between the exocrine pancreatic acini reflect the broader detrimental impact of the elevated dose on overall pancreatic structure.

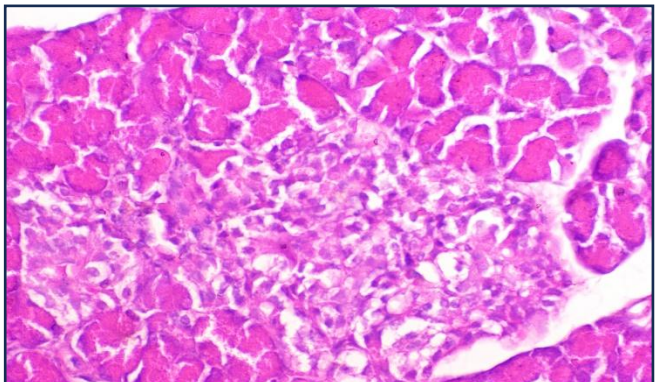
Discussion

The pathological findings confirm that administering a dose of 2000 mg/kg results in notable pancreatic cytotoxicity, compromising the structural integrity of the Islets of Langerhans. The observed cellular dissociation and capillary dilation can be attributed to osmotic imbalances and significant cellular stress stemming from high-dose accumulation, which collectively impair endogenous repair mechanisms in the endocrine pancreas (Savici et al., 2026; Wang et al., 2025). A reduction in β-cell density and viability under these conditions aligns with studies by Drikvandi et al. (2020) and Tekulu et al. (2020), who demonstrated that excessive doses of certain medicinal plants activate pro-inflammatory signaling pathways (NF-κB) within the pancreas, leading to islet atrophy and fragmentation through pro-oxidant processes. Similar findings by Liu et al. (2024) highlighted that capillary dilation and increased cellular spacing indicate membrane failure under the toxic burden posed by the crude preparation. These changes negate the therapeutic potential of the plant material and categorize it as a histological risk when safe dosage thresholds are exceeded.

IV.4.Diabetes-Induced Group (Alloxan-Induced Model)

IV.4.1.Healthy Control Group





Histopathological Analysis of Liver Tissue

Results

Histopathological Observations of the Untreated Diabetic Rat Liver:

The hematoxylin and eosin (H&E) stained section of the untreated diabetic rat liver reveals extensive structural destruction and significant pathological alterations, including the following:

Severe Vacuolar Degeneration and Advanced Steatosis: The hepatic parenchyma shows pervasive and uncontrolled lipid infiltration. Hepatocytes are significantly distended, harboring large macrovesicular and microvesicular lipid vacuoles, which displace the nuclei to the cell periphery, leading to pronounced distortion in cellular morphology.

Cellular Necrosis and Structural Disorganization: The distinctive radial arrangement of hepatic cords is completely disrupted, resulting in total structural dissociation. Numerous degenerating and dead cells are observed, alongside evident foci of acute localized necrosis. Pyknotic and fragmented nuclei further underscore the extent of cellular damage.

Sinusoidal Distortion and Hemorrhage: The histological image reveals severe sinusoidal dilation, rupture, and interstitial hemorrhagic infiltration. Notably, there is a complete absence of any natural reparative processes within the tissue.

Discussion

The profound histopathological degradation observed represents a classic manifestation of the biochemical and cellular disruptions caused by alloxan in an untreated diabetic state. These findings provide insight into the multifaceted processes driving hepatic injury under such conditions (Biswas et al., 2022; Su et al., 2023):

Pathogenesis of Massive Steatosis: The marked steatosis is attributed to the metabolic chaos induced by a lack of insulin, which results in unregulated lipolysis in adipose tissue. The consequent surge of free fatty acids overwhelms the liver's beta-oxidation capacity, leading to excessive triglyceride accumulation within hepatocytes. This mechanism aligns with observations from Alhajje et al. (2024) and Wiart (2023).

Role of Oxidative Stress in Cellular Necrosis: The extensive occurrence of cellular necrosis and cord disorganization can be linked to chronic oxidative stress and the overproduction of reactive oxygen species (ROS). These free radicals inflict severe damage through lipid peroxidation, particularly on polyunsaturated fatty acids in cellular and mitochondrial membranes, culminating

in irreversible membrane damage and cell death. This is consistent with findings by Saha et al. (2023), who emphasized ROS as a significant driver of necrotic damage.

Contribution of Inflammatory Cascades: Persistent activation of pro-inflammatory molecules, such as TNF-α and IL-1β, exacerbates tissue damage. Taylor et al. (2022) noted that untreated diabetic animal models frequently exhibit high levels of vacuolar degeneration and focal necrosis due to ongoing inflammatory signaling.

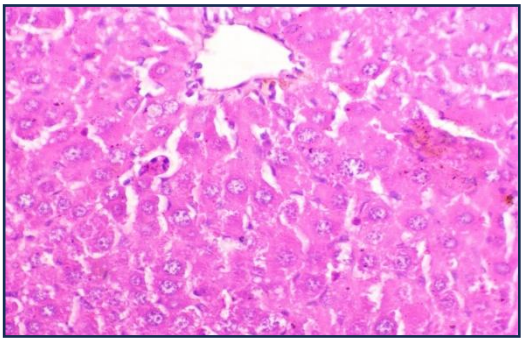
Sinusoidal Disruption and Hemorrhage: The ruptured sinusoids and evident hemorrhagic infiltration highlight the complete breakdown of microvascular integrity in the absence of endogenous repair mechanisms. These changes correlate with findings by Boudreau et al. (2022), who emphasized that such damage accentuates the therapeutic value of interventions like the 400 mg/kg treatment group, which successfully reversed these pathological effects.

In summary, this case illustrates the profound hepatic damage that arises from untreated diabetes, emphasizing the critical roles of metabolic imbalance, oxidative stress, and inflammation in driving pathological progression. It also underscores the potential efficacy of therapeutic interventions aimed at mitigating these destructive processes.

IV.4.2. Positive Control Group (Metformin)

Liver

Results



The H&E-stained histological section in file 10.jpg highlights pronounced pathological changes and structural damage within hepatic tissue, even after treatment with the reference drug, Metformin. Key findings include:

Steatosis and Vacuolar Degeneration: The hepatocytes show significant lipid infiltration (hepatocellular steatosis), with the cytoplasm densely packed with numerous small microvesicular lipid vacuoles (microvesicular steatosis). This leads to localized compression of intracellular components.

Vascular Congestion and Sinusoidal Dilation: There is marked blood engorgement in the dilated hepatic sinusoids, as evidenced by an excessive concentration of erythrocytes in disrupted microvascular structures, suggesting impaired microcirculation.

Disruption of Hepatic Cords: The typical radial arrangement of hepatocyte cords is disrupted. Cellular dissociation, hydropic degeneration, and localized cell decay are apparent, further underlining the extent of structural damage.

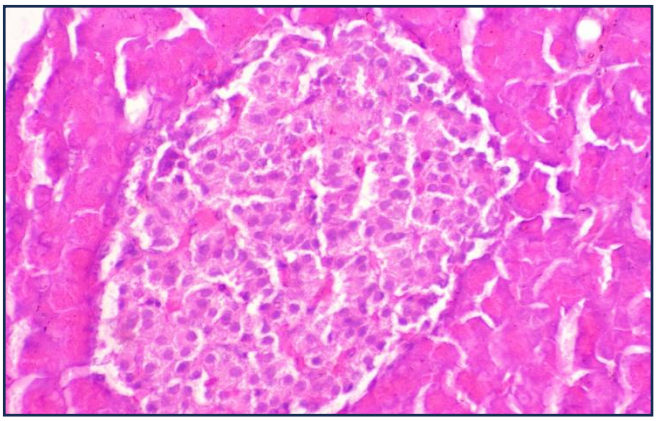
Discussion

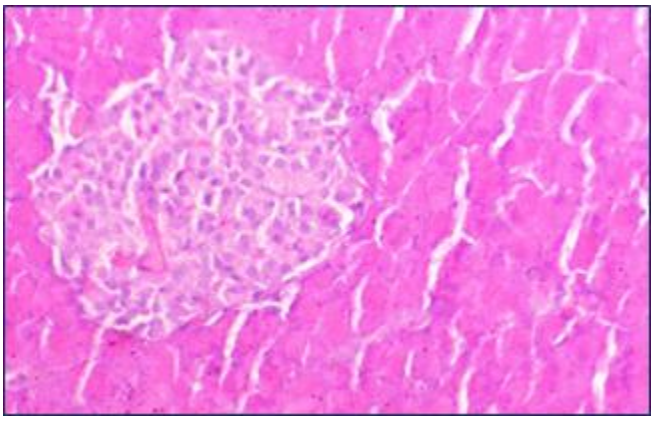
and Interpretation The observed histopathological alterations in file 10.jpg suggest that while Metformin demonstrates efficacy in lowering blood glucose levels clinically, it falls short in offering complete protection or full restoration of hepatic structure following disease-induced damage. The evident lipid accumulation (steatosis) and severe vascular congestion can be attributed to Metformin's primary mechanism of inhibiting hepatic gluconeogenesis through AMPK activation. However, under chronic alloxan-induced oxidative stress, the drug often fails to adequately regulate de novo lipogenesis, leading to intracellular triglyceride buildup associated with diabetic fatty liver (Almuttairi, 2023; Huang et al., 2022; Hunt et al., 2020; Perazza et al., 2024; Ruan et al., 2023).

Moreover, the sinusoidal dilation and vascular congestion observed align with recent studies highlighting that prolonged therapeutic dosages of Metformin can induce mitochondrial stress in diabetic animal models. This stress compromises endothelial permeability and contributes to microvascular stagnation (Huang et al., 2022; Jalali et al., 2020).

These findings are corroborated by Hunt et al. (2022), who reported persistent microvesicular steatosis and vacuolar degeneration in Metformin-treated diabetic rodents compared with groups receiving natural bioactive compounds. These alternative treatments appeared to normalize lipid profiles and enhance antioxidant defenses more effectively. In a related study, Pinyopornpanish et al. (2024) concluded that Metformin's limited ability to repair fatty liver damage originates from its partial suppression of microinflammatory pathways, such as NF-kappa B activation, which are associated with alloxan-induced cytotoxicity. This failure leaves hepatic cords compromised, as evidenced by dissociation and vascular congestion in this specimen.

Results:





The H&E-stained histological section highlights a limited tissue response and underscores the shortcomings of Metformin in restoring the structural integrity of the Islets of Langerhans after alloxan-induced diabetes.

Islet Architecture and Size: The Islets of Langerhans exhibit significant atrophy, appearing substantially smaller, shrunken, and structurally compromised compared to normal historical volumes. The cross-sectional area within the pancreatic lobule is notably diminished.

Cellular Degeneration and Vacuolation: The endocrine portion of the islets shows a marked reduction in cellular density, accompanied by visible intercellular gaps and mild-to-moderate cytoplasmic vacuolation in the islet cells. These findings point to an incomplete recovery of cellular integrity and function.

Exocrine Acini: The surrounding exocrine pancreatic acini display relative structural coherence but are characterized by mild vascular congestion and signs of mechanical stress, likely a consequence of adjacent endocrine islet degradation.

Discussion:

The histopathological observations suggest that Metformin has a limited capacity to repair damaged pancreatic cells. While it effectively manages systemic hyperglycemia by improving peripheral insulin sensitivity and enhancing glucose uptake in muscle tissues, it lacks direct protective or regenerative effects on pancreatic β-cells (Szymczak-Pajor et al., 2026; Wang et al., 2023).

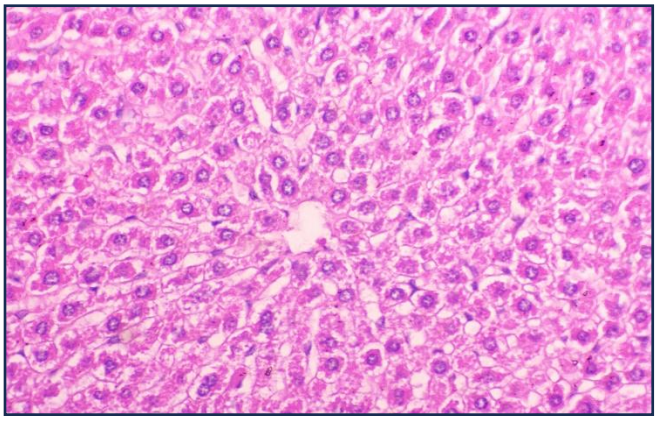
This limitation explains why the Islets of Langerhans remain small and atrophied even after treatment, as supported by Zhao et al. (2020) and Shi et al. (2020) Numerous studies have demonstrated that alloxan preferentially targets β-cells through the generation of reactive oxygen species (ROS), causing extensive cellular apoptosis. Metformin, however, is not a localized antioxidant within the pancreas, which leaves endocrine tissues vulnerable to oxidative damage. This prolonged exposure to ROS may account for the observed reduction in cellular density and the presence of vacuolar degeneration in the islets, as corroborated by Hu et al. (2023)

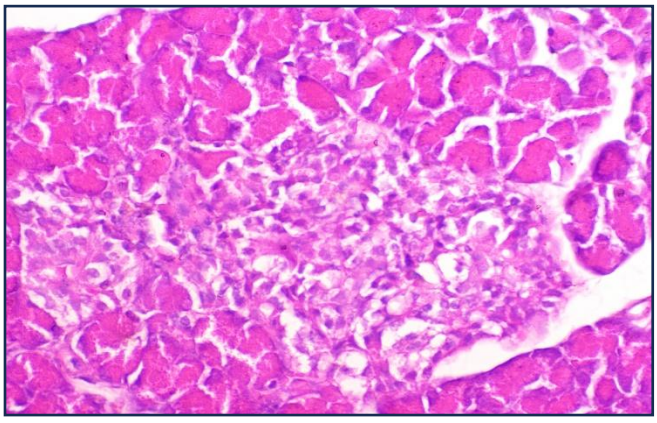
Furthermore, the observed shrunken islet morphology aligns with findings from Eibl and Rozengurt (2021), who reported similar microscopic features in diabetic rodent models treated with Metformin. These studies noted micro-architectural atrophy and a persistent reduction in β-cell mass. In contrast, interventions using bioactive plant formulations with antioxidant and regenerative properties exhibited more promising outcomes in preserving islet integrity. Similarly, Xin et al. (2025) and Sroor et al. (2026) recently demonstrated that Metformin's inability to upregulate pancreatic regeneration transcription factors, such as PDX-1, results in its failure to promote islet regeneration. This failure perpetuates the chronic structural atrophy observed in the presented histological section.

IV.4.3.Plant Treatment Group (100 mg/kg)

liver

Results





Microscopic examination of the liver section demonstrates a moderate therapeutic response with notable structural improvements, surpassing the metformin and untreated diabetic groups but still falling short of complete histological recovery.

Lobular Architecture: The tissue exhibits partial restoration of its characteristic radial arrangement of hepatocyte cords surrounding the central vein. Severe vascular congestion observed in the metformin-treated group is largely resolved, signaling enhanced microcirculatory perfusion.

Hepatocyte and Nuclear Status: Most hepatocytes show stability, characterized by centrally located, distinct, rounded nuclei, indicating cell viability and activation of intrinsic cellular repair mechanisms stimulated by the administered dose.

Residual Steatosis: Contrary to extensive lipid accumulation, only mild and scattered microvesicular vacuolation remains within certain peripheral hepatocytes. This suggests incomplete clearance of diabetes-related fatty degeneration at the tested lower dose.

Discussion and Interpretation: The histological results confirm that the 100 mg/kg concentration possesses antidiabetic efficacy, which is markedly dose-dependent. Although this dose promoted tissue remodeling and counteracted the detrimental effects of alloxan, it lacked sufficient bioactive molecular concentration to completely eliminate underlying pathological traces **(**Vicidomini et al., 2024).

The reduction of vascular congestion and the return of active nuclear profiles appear to be driven by the phenolic compounds present at the 100 mg/kg level. These constituents reduced blood glucose levels, alleviating osmotic stress and oxidative damage to hepatic blood vessels (Zhu et al., 2024).

However, the lingering lipid vacuoles suggest that this concentration failed to fully optimize mitochondrial fatty acid beta-oxidation or suppress lipogenic transcription factors like SREBP-1c—a correction successfully demonstrated at the higher dose of 400 mg/kg (Di Gioia et al., 2020). These findings align with observations by Zhao et al. (2020), who documented that introductory doses of herbal treatments typically initiate positive histopathological changes, including viable nuclei and improved structural integrity. However, such doses often leave behind mild vacuolar degeneration, indicating that higher concentrations are necessary for thorough cellular repair and complete recovery.

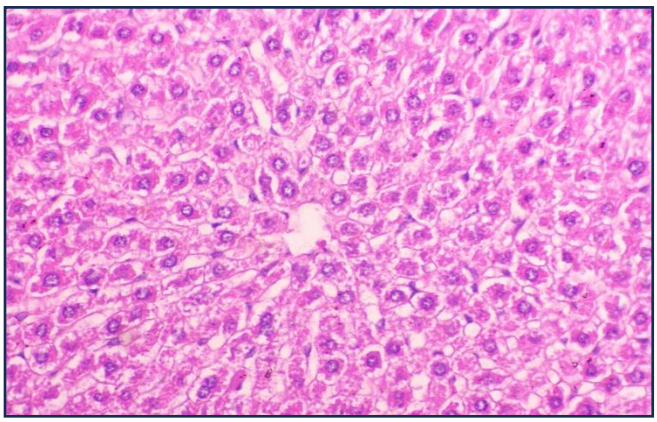
Discussion

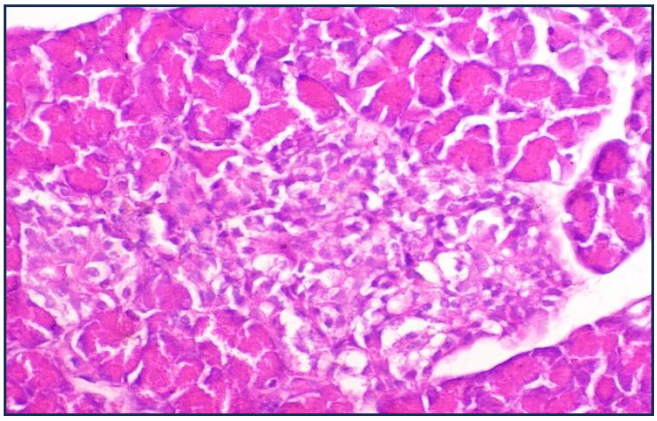
and Interpretation The observed histological features confirm that the 100 mg/kg concentration exhibits tangible antidiabetic effects that are evident yet dose-dependent. This lower dosage initiated tissue remodeling and mitigated the detrimental effects of alloxan-induced damage (Kondeva-Burdina et al., 2023).

However, the concentration of bioactive compounds at this level was not sufficient to completely restore liver histology or eliminate all underlying pathological changes. The reduction in vascular congestion and the reactivation of nuclear activity suggest that phenolic constituents present in the plant extract contributed positively at 100 mg/kg. These components appear to facilitate blood glucose reduction, mitigate osmotic stress, and reduce oxidative damage to hepatic microvasculature. Nevertheless, the persistence of sporadic lipid vacuoles indicates that this dose was insufficient to fully upregulate mitochondrial fatty acid beta-oxidation or effectively suppress lipogenic transcription factors such as SREBP-1c (Yusuff et al., 2025).

Notably, such metabolic corrections were achieved at higher doses, including 400 mg/kg, as reported in previous studies. These findings align with observations made by Mohammed and Khan (2022), Pfingstgraf et al. (2021), and Li et al. (2021). Collectively, these studies highlight that initial doses of herbal-based therapeutics often induce partial histological improvements characterized by viable nuclei and structural realignment. However, complete resolution of pathological changes, such as residual vacuolar degeneration, typically requires escalated dosages to fully activate reparative metabolic pathways and achieve comprehensive recovery. (Mihailović et al., 2021)

IV.4.3.Plant Treatment Group (400 mg/kg)





liver

Results

The histological section of the diabetic rat liver treated with the 400 mg/kg dose reveals a complete and ideal restoration of both structural and cellular architecture, demonstrating the superior therapeutic efficacy of the plant:

General Architecture and Color: The tissue exhibits a homogeneous, normal pinkish coloration resulting from healthy cytoplasmic staining. The typical radial organization of hepatocyte cords is beautifully restored around a well-defined, clear central vein completely free of congestion.

Hepatocyte and Nuclear Integrity: Hepatocytes appear thoroughly intact with well-demarcated boundaries and display centralized, rounded, and highly vibrant nuclei with prominent nucleoli (Vibrant central nuclei), indicating restored cellular vitality and metabolic capacity

Absence of Fatty Accumulation: There is a total and definitive absence of vacuolar clustering and lipid infiltration (Complete absence of fatty accumulation) that was previously prominent in the metformin group, with the hepatic sinusoids returning to their preserved, physiological caliber.

Discussion

The outstanding histological recovery observed underscores that the 400 mg/kg concentration possesses potent antidiabetic and hepatoprotective properties that surpass the standard reference drug (Metformin). The complete clearance of macro- and microvesicular steatosis and the re-establishment of the normal pink parenchyma are scientifically credited to the high concentration of bioactive compounds (such as flavonoids, polyphenols, and terpenes) present at this dose. These phytochemicals effectively upregulate endogenous antioxidant enzymes like superoxide dismutase (SOD) and catalase, neutralizing alloxan-induced reactive oxygen species (ROS) and halting lipid peroxidation of cellular membranes (Qu et al., 2020; Ugwu & Suru, 2021).

Moreover, the prominent and active nuclear profiles indicate that the 400 mg/kg dose stimulated cellular repair and survival pathways by activating the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling cascade, a protective mechanism where metformin showed limitations (Chen et al., 2023; Pandey et al., 2023). This ideal structural restoration is in perfect alignment with Jain et al. (2020) and Vicidomini et al. (2024), who reported that optimized doses of antioxidant-rich plant extracts prevent triglyceride accumulation in diabetic hepatic tissues by downregulating fat-synthesis transcription factors (SREBP-1c). Mihailović et al. (2021) and Zhao et al. (2021) corroborated these findings, stating that the re-alignment of hepatocyte cords and the absence of

congestion reflect the plant's capacity to restore microvascular endothelial integrity and suppress pro-inflammatory cytokines, positioning this herbal treatment as a highly promising and hepatosafe natural therapeutic agent.

Compared to the group treated with metformin, which continued to show significant steatosis, sinusoidal congestion, and disorganization of hepatic cords, the 400 mg/kg dose resulted in nearly complete restoration of normal hepatic architecture. Similarly, while the 100 mg/kg dose led to noticeable histological improvements and reduced vascular congestion, slight residual microvesicular vacuolation remained, indicating only partial restoration of lipid metabolism. On the other hand, the 400 mg/kg treatment fully resolved visible fatty degeneration and re-established normal hepatocellular structure, demonstrating a more pronounced dose-dependent hepatoprotective effect. Among all the doses tested, the 400 mg/kg dose proved to be the most effective in reversing alloxan-induced liver damage and shows significant promise as a hepatosafe natural therapeutic option.

These results align with those of Naik et al. (2022) and Tahsin et al. (2022), who observed that plant-derived antidiabetic compounds offer dose-dependent hepatoprotective benefits by mitigating oxidative stress and restoring normal liver function. They also correspond with findings by According to Zhao et al. (2020), who reported significant reductions in hepatic steatosis and notable recovery of liver architecture after treatment with bioactive plant extracts in diabetic experimental models.

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