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THÈSE

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Étude phytochimique de *Moringa oleifera* et exploration de ses effets thérapeutiques contre la cytotoxicité induite par l'abamectine chez la rate de la souche wistar

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RÉSUMÉ

Cette étude a pour objectif d'évaluer la composition phytochimique et l'activité antioxydante de l'extrait aqueux des feuilles de *M.oleifera* (MO), ainsi que son effet protecteur contre le stress oxydatif induit par l'abamectine (ABM) chez les rats *Wistar*. L'analyse *in vitro* a montré que l'extrait est riche en polyphénols, flavonoïdes, tanins condensés et *ortho*-diphénols, avec une forte activité antioxydante démontrée par plusieurs tests (piégeage des radicaux DPPH et ABTS, le test de décoloration du β-carotène, le test FRAP, et la mesure de la capacité antioxydante totale). L'analyse HPLC et FTIR ont permis d'identifier les principaux métabolites et groupes fonctionnels. l'étude *in vivo* a été menée sur 20 rats répartis en quatre groupes : groupe 1 (témoin), groupe 2 (ABM) : abamectine (1 mg/kg) dans l'eau de boisson, groupe 3 (MO) : extrait de MO (200 mg/kg) par gavage, groupe 4 (ABM + MO) : co-administration d'ABM et de MO selon les mêmes doses, durant 3 semaines.

Les résultats ont montré que l'ABM a causé des altérations du poids des reins et du foie, des perturbations hématologiques et biochimiques (ASAT, ALAT, PAL, GGT, bilirubine, urée, créatinine, acide urique), ainsi qu'un déséquilibre du système antioxydant (GSH, GSH-Px, GST, SOD , CAT) et une augmentation des biomarqueurs de stress oxydatif (MDA, AOPPs). Ces effets étaient accompagnés de lésions histopathologiques dans les reins, le foie et le cerveau. La co-administration de MO a significativement atténué ces effets, montrant un rôle protecteur de l'extrait contre les dommages induits par l'ABM. Enfin, une modélisation moléculaire a révélé que certains composés de MO interagissent avec des enzymes antioxydantes humaines (Catalase, glutathion peroxydase, peroxyrédoxine 5), renforçant les observations *in vivo*. Ainsi, on peut conclure que l'ABM altère la fonction du foie, des reins, du cerveau et des érythrocytes conduisant a des dommages oxydant, et ces effets pourraient être prévenus par l'extrait aqueux de *M.oleifera*, probablement en raison de son activité antioxydante.

Mots clés : *M.oleifera*, abamectine, antioxydants, modélisation computationnelle, FTIR, HPLC.

ABSTRACT

This study aims to evaluate the phytochemical composition and antioxidant activity of the aqueous extract of *M.oleifera* (MO) leaves, as well as its protective effect against oxidative stress induced by abamectin (ABM) in *Wistar rats*.

In vitro analysis showed that the extract is rich in total polyphenols, flavonoids, condensed tannins, and *ortho*-diphenols, with strong antioxidant activity demonstrated through several assays (DPPH and ABTS radical scavenging, β-carotene bleaching, FRAP test, and total antioxidant capacity). HPLC and FTIR analyses help identify its main metabolites and functional groups. The *in vivo* experiment is conducted on 20 rats divided into four groups: group 1 (control), group 2 (ABM): abamectin (1 mg/kg) in drinking water, group 3 (MO): MO extract (200 mg/kg) orally, group 4 (ABM + MO): co-administration of ABM and MO at the same doses for 3 weeks. The results indicated that ABM caused alterations in kidney and liver weights, hematological and biochemical disturbances (ALAT, ASAT, PAL, GGT, bilirubin, urea, creatinine, uric acid), as well as an imbalance in the antioxidant defense system (GSH, GSH-Px, GST, SOD, CAT) and an increase in oxidative stress markers (MDA, AOPPs). These effects are associated with histopathological lesions in the kidneys, liver, and brain. Co-administration of MO significantly attenuates these effects, highlighting a protective role of the extract against ABM-induced damage. Finally, molecular modeling reveals that some MO compounds interact with key human antioxidant enzymes (CAT, GSH-Px, peroxiredoxin 5), further supporting the *in vivo* findings. Thus, it can be concluded that ABM impairs the function of the liver, kidneys, brain, and erythrocytes, leading to oxidative damage, and these effects could be prevented by the aqueous extract of *M. oleifera*, probably due to its antioxidant activity.

Key words: *M.oleifera*, abamectin, antioxydant, Computational modeling, FTIR, HPLC.

الملخص

تهدف هذه الدراسة إلى الكشف عن التركيب الفيتوكيميائي وتقييم النشاط المضاد للكسدة للمستخلص المائي لوراق المورينغا أوليفيرا، بالإضافة إلى ذلك، فحص تأثيره الوقائي ضد الجهاد التأكسدي الناجم عن التعرض للأبامكتين في الكبد والكلى وكريات الدم الحمراء والأنسجة الدماغية لدى الجرذان .أظهرت التحاليل المخبرية أن المستخلص غني بالبوليفينولات الكلية، والفلافونويدات، والتانينات المكثفة، والأورثو-دي فينولات، وأنه يتميز بنشاط مضاد للأكسدة قوي تم إثباته من خلال عدة اختبارات (اختبار التقاط الجذور الحرة DPPH وABTS، واختبار تبييض البيتا-كاروتين، واختبار FRAP، وقياس القدرة المضادة للأكسدة الكلية). وقد أسهمت تحاليل HPLC و FTIR في الكشف عن المركبات الثانوية الفعالة والمجموعات الوظيفية المميزة في المستخلص. تم إجراء دراسة حية على عشرين جرذا، تم تقسيمهم االتساوي إلى أربعة مجموعات. المجموعة الأولى، مجموعة شاهدة. أ ما المجموعة الثانية، تلقت الأبامكتين من خلال ماء الشرب (1 ملغ/كغ) . المجموعة الثالثة تلقت المستخلص المائي لأوراق *M.oleifera* (200 ملغ/كغ) .أما المجموعة الرابعة، فقد تلقت الأبامكتين و المستخلص المائي لأوراق *M.oleifera*، وذلك لمدة 3 أسابيع . مقارنة بالفوج الشاهد، أظهرت النتائج أن الأبامكتين لا يُحدث تغييرات ملحوظة في الوزن الجسمي أو في الوزن الحقيقي والنِسبي للدماغ، إلا أنه يسبب تغيرات واضحة في وزن الكلى والكبد، حيث لوحظ ضمور كلوي وتضخم كبدي. كما أدى إلى اضطرابات دموية وارتفاع في مؤشرات وظائف الكبد والكلى، مثل ناقلات الأمين، إنزيم غاما-غلوتاميل ترانسفيراز ،البيليروبين، اليوريا، الكرياتينين، حمض اليوريك، إلى جانب ارتفاع الكولسترول والدهون. كما لوحظ انخفاض في مستويات GSH في الأنسجة المدروسة وخلايا الدم الحمراء، وانخفاض في نشاط الإنزيمات المضادة للأكسدة (GSH-Px، GST، SOD، CAT)، بالإضافة إلى ارتفاع في مؤشرات الإجهاد التأكسدي (MDA(AOPP., في الدماغ، لم تتغير مستويات NO بشكل واضح، لكن نشاط الأستيل كولين إستيراز انخفض بشكل كبير.وقد اقترنت هذه التأثيرات مع تغيرات نسيجية مرضية واضحة في الكلى والكبد والدماغ .من جهة أخرى، أظهر العلاج بالمستخلص المائي لأوراق *M. oleifera* فعالية في التخفيف من هذه التأثيرات، حيث أدى إلى تحسن كبير في أغلب المؤشرات . وبالإضافة إلى ذلك، بيّنت النمذجة الجزيئية أن بعض المركبات الموجودة في المستخلص ترتبط بفعالية مع إنزيمات بشرية مضادة للأكسدة مثل GSH-Px، Catalase وPeroxiredoxin5 ، مما يدعم نتائج الدراسة الحيوية. وهكذا، يمكن الاستنتاج أن الأبامكتين يسبب خللاً في وظائف الكبد والكلى والدماغ وكريات الدم الحمراء، مما يؤدي إلى أضرار تأكسدية، وقد يكون من الممكن الوقاية من هذه التأثيرات باستخدام المستخلص المائي لنبات المورينجا ، وذلك على الأرجح بفضل نشاطه المضاد للأكسدة.

الكلمات الدالة :المورينغا أوليفيرا،الأبامكتين ، مضاد الكسدة، النمذجة الجزيئية ، HPLC، FTIR ،

Liste des abréviations

A

ABM: Abamectine

ABTS: 2,2'-azino-bis(3-éthylbenzothiazoline-6-sulfonate)

AChE: Acétycholine éstérase

ALAT: Alanine aminotransférase

AOPP: Produits de l'oxydation avancée des protéines

ASAT: Aspartate aminotransférase

B

BL: Bilirubine Libre

BT: Bilirubine Totale

C

CAT: Catalase

CDNB: 1-Chloro-2,4-dinitrobenzène.

D

DPPH: Le 2.2-diphényl 1-pycrilhydrazyle.

DTNB: 5,5'-Dithiobis(2-nitrobenzoic acid)

E

EDTA: Ethylène Diamine Tétra-acétique

F

FTIR: Spectroscopie infrarouge à transformée de Fourier

FDA: Food and Drug Administration

G

GB: Globules Blanc**s**

GGT: Gamma-glutamyl transpeptidase

GSH-Px: Glutathion peroxydase

GR: Globules Rouges

GSH: Glutathion réduit

GST: Glutathion S-Transférase

H

Hb: Hémoglobine

H_2O_2: Peroxyde d'hydrogène

HMC3: lignée cellulaire du clone 3 de la microglie humaine

HPLC: chromatographie en phase liquide à haute performance

M

MDA: Malondyaldehyde

MO: *Moringa oleifera*

Mpro: Protéase principale

N

NBT: Nitroblue Tetrazolium

nsp: Proteines non structurales

P

PAL: Phosphatase alcaline

R

ROS: Espèces réactives de l'oxygène

S

SOD: Superoxyde Dismutase

T

TNB: 2-Nitro-5-thiobenzoate

V

VGM: Volume globulaire moyen

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INTRODUCTION

INTRODUCTION

Les pesticides sont définis comme des composés chimiques utilisés pour gérer et dissuader la population de parasites, c'est-à-dire les plantes, les animaux ou les micro-organismes qui affectent l'alimentation, la santé ou le confort. Ils sont également utilisés pour contrôler d'autres êtres vivants, comme les nématodes et les arthropodes, notamment les insectes et les vertébrés, qui peuvent ruiner nos sources de nourriture et entraîner divers problèmes de santé (Zhou *et al*., 2024). Cependant, la dépendance croissante aux pesticides pour améliorer la production agricole présente un risque grandissant. En effet, les formulations de pesticides conventionnelles peuvent engendrer des problèmes écologiques et environnementaux, car la majorité des pesticides appliqués sont perdus dans l'environnement, et moins de 1 % seulement sont efficacement utilisés pour atteindre l'objectif prévu (Gungogdu *et al*., 2024). Par conséquent, des inquiétudes ont émergé quant à la contamination potentielle de l'environnement et à l'impact sur les organismes non ciblés (Dos Santos *et al*., 2023). Ces produits peuvent avoir une très large gamme d'effets néfastes sur les humains et les animaux, en particulier dans les pays en développement (Manisalidis *et al*., 2020). Selon l'Organisation mondiale de la santé, 3 millions de travailleurs dans ces pays sont soumis chaque année à des empoisonnements graves par les pesticides (Min *et al*., 2017).

L'abamectine, un insecticide largement utilisé dans le monde, est l'un des pesticides les plus répandus en Algérie (Khaldoun-Oularbi *et al*., 2013). Elle est formulée par la combinaison de deux groupes d'avermectines : l'avermectine B1a (80 %) et l'avermectine B1b (20 %).En agriculture, elle est appliquée pour protéger les cultures de légumes et de fruits contre les insectes et les acariens. En thérapie animale, elle est utilisée en raison de son efficacité et de son potentiel pharmacologique. Bien que l'abamectine soit moins nocive lorsqu'elle est utilisée à des doses thérapeutiques en raison de la présence de la barrière hémato-encéphalique, plusieurs études ont mis en évidence ses effets toxiques sur divers organes. Une recherche récente a démontré que ce composé induit des altérations significatives des fonctions hépatiques et rénales

chez les rats. Ces effets ont été attribués à une augmentation marquée des niveaux de biomarqueurs de lésions hépatiques, accompagnée d'une diminution notable de l'activité des enzymes antioxydantes (Aioub *et al*., 2022). Par ailleurs, l'administration d'abamectine a été corrélée à une production accrue d'espèces réactives de l'oxygène dans les tissus cérébraux des rats, traduisant un stress oxydatif important (Radi *et al*., 2020). En outre, l'abamectine a été identifiée comme un inducteur de dommages testiculaires, altérant le système de défense antioxydant et provoquant l'inflammation ainsi que l'apoptose (Gur *et al*., 2022).

En Algérie, bien que l'abamectine soit largement utilisée dans le domaine agricole, peu d'études ont été menées pour évaluer les risques associés à son emploi. Ce produit, disponible sur le marché local, est très demandé en raison de son efficacité contre divers nuisibles.

Les produits naturels ont toujours suscité l'intérêt de l'homme pour répondre à ses besoins quotidiens. Les plantes, en particulier, constituent une source abondante de composés bioactifs, tels que les antioxydants, qui jouent un rôle essentiel dans la prévention et le traitement de nombreuses pathologies liées au stress oxydatif. Par conséquent, les médicaments à base de plantes ont gagné en popularité, étant perçus comme plus sûrs et plus économiques que leurs équivalents synthétiques (Singh *et al*., 2020). Environ 80 % de la population mondiale dépend entièrement des plantes pour la santé et la guérison, et 25 % des produits pharmaceutiques intégrés sont dérivés de plantes médicinales et aromatiques.

Originaire de l'Inde, *M.oleifera*, ou arbre de la vie, est l'une des 13 espèces appartenant à la famille des *Moringaceae*. *M.oleifera* est reconnue comme une source de composés bioactifs (Dhakad *et al*., 2019), notamment les polyphénols. Ces derniers sont présents en abondance dans toutes les parties de la plante (Kurniawan, 2021) et représentent une grande famille de molécules antioxydantes (Chouala *et al*., 2024), dotées de propriétés thérapeutiques, notamment antipyrétiques, antioxydantes, anti-inflammatoires, anti-âge, antidiabétiques, antihypertensives, immunomodulatrices,

hépatoprotectrices et diurétiques. De récentes études ont également mis en évidence les propriétés antiparasitaires, neuroprotectrices, cérébroprotectrices et antiprolifératives contre certaines formes de cancer de *M.oleifera*. Par ailleurs, cette plante offre un potentiel prometteur en dentisterie régénérative et démontre une capacité à prévenir et atténuer l'ostéoporose (Abdelazim *et al*., 2024).

Le présent travail, réparti en trois chapitres dans ce mémoire, consiste à explorer la composition phytochimique et l'activité antioxydante de l'extrait aqueux des feuilles de *M.oleifera*, ainsi que ses effets protecteurs contre le stress oxydant induit par l'abamectine (ABM) dans les reins, le foie, les érythrocytes et le tissu cérébral des rats femelles de la souche *Wistar*.

CHAPITRE I. ETUDE BIBLIOGRAPHIQUE

I.1. ABAMECTINE

I.1. 1. Définition et applications de l'abamectine

L'abamectine, ainsi que la doramectine, l'ivermectine, l'épinomectine et la sélamectine, représente le groupe des avermectines de la famille des lactones macrocycliques. Ces dernières sont synthétisées par des microorganismes du sol du genre *Streptomyces* (El-Saber Batiha *et al*., 2020).

formulée par la combinaison de deux groupes d'avermectines : l'avermectine B1a à 80 % et l'avermectine B1b à 20 %, l'abamectine est un insecticide largement utilisé dans le monde et l'un des pesticides les plus répandus en Algérie (Khaldoun-Oularbi *et al*., 2015), et est appliquée en agriculture contre les nématodes des plantes qui se nourrissent des racines en phase de germination. Ayant un effet molluscicide, elle est également efficace contre *E. vermiculata*, protégeant ainsi les cultures de blé.

De plus, l'avermectine B1 a la capacité de réduire la population des nématodes *H. galeatus* et *T. dubius*. Plusieurs études ont confirmé l'efficacité des avermectines comme nématicides contre divers nématodes attaquant les plantes, y compris *Hoplolaimus galeatus* et *Tylenchorhynchus dubius* (El-Saber Batiha *et al*., 2020).

En thérapie animale, elle est utilisée en raison de son efficacité et de son potentiel pharmacologique (Mahmoud *et al*., 2021).

I.1. 2. Distribution, absorption et élimination de l'avermectine B1a

Caractérisées par leur polarité et leur lipophilie, les avermectines sont presque insolubles dans l'eau. Cette caractéristique leur confère une absorption optimale, une distribution rapide et une rétention prolongée dans l'organisme. En comparant le métabolisme et la cinétique de l'avermectine B1a avec ceux de l'avermectine B1b, ces composés ont montré le même profil toxicocinétique. L'absorption des

avermectines dépend de différents facteurs, parmi lesquels la voie d'administration et l'espèce cible (Bokreta, 2022).

Chez le rat, la biodisponibilité systémique de l'avermectine B1a a été estimée à 86 % après administration orale. L'avermectine B1a était distribuée dans tous les principaux organes. Au niveau du sang, elle se lie à l'albumine et aux lipoprotéines (Sebbag, 2011).

L'évaluation des études *in vitro* sur les microsomes humains et de rats conduit à la conclusion que le métabolisme de l'abamectine chez l'homme et le rat est comparable. Les différentes voies métaboliques des avermectines décrites sont des réactions de déméthylation, d'hydroxylation, de clivage et d'oxydation.

Au sein de l'organisme, 90 % des avermectines ingérées par voie orale sont éliminées par voie fécale et 2 % par voie urinaire. Ce taux peut changer d'une espèce à une autre et dépend de la formulation administrée.

Le processus moléculaire d'élimination implique le transporteur membranaire P-Glycoprotéine, qui est principalement exprimé dans l'intestin, le foie, la barrière hémato-encéphalique, le placenta, les reins et le cerveau (Sebbag, 2011).

I.1. 3. Mécanisme d'action des avermectines

Chez les invertébrés, les avermectines agissent en bloquant la transmission des impulsions nerveuses en ouvrant les canaux chlorure-glutamate dépendants, après fixation irréversible sur ces derniers, provoquant ainsi une hyperpolarisation des cellules nerveuses et une paralysie du parasite. De la même manière, et à des doses élevées, ces composés peuvent aussi agir sur les canaux chlorure-acide gamma-aminobutyrique dépendants (Aioub *et al*., 2022).

Connu comme un neurotransmetteur inhibiteur de l'activité cérébrale via l'augmentation de la conductance des ions chlorure au niveau des cellules nerveuses et musculaires, le GABA représente un élément clé dans le fonctionnement du système nerveux central des vertébrés.

La fixation des avermectines sur les canaux chlorure GABA-dépendants induit l'immobilisation du parasite, mais entraîne également des effets indésirables et toxiques chez l'hôte en perturbant la transmission nerveuse (Bokreta, 2022).

I.1. 4. Toxicité des avermectines

Outre leur utilisation en médecine vétérinaire, les avermectines jouent également un rôle essentiel en médecine humaine. Parmi cette famille de composés, l'ivermectine est actuellement le seul médicament autorisé pour un usage chez l'homme (Wolstenholme et Neveu, 2022). Elle est employée dans le traitement de diverses affections, notamment l'onchocercose, la strongyloïdose, la filariose lymphatique et la gale croûteuse (El-Saber Batiha *et al*., 2020). Chez l'homme, l'ivermectine altère les macrophages et interagit avec les enzymes du cytochrome P450 hépatique, entraînant ainsi une immunotoxicité et une hépatotoxicité (Zhang *et al*., 2022).

L'exposition aux avermectines, même à des doses thérapeutiques, peut induire des troubles reproductifs, hépato-rénaux, sexuels et comportementaux chez les animaux . Les avermectines induisent une neurotoxicité en endommageant le cerveau, un organe clé dans la régulation des hormones reproductives, ce qui entraîne des effets indirects sur le système reproducteur des animaux. Chez les mâles, elles provoquent des lésions testiculaires, affectant ainsi le nombre et la motilité des spermatozoïdes. Cette double action, ciblant à la fois le cerveau et les testicules, se traduit par des altérations du comportement sexuel ainsi que par une perturbation de la production hormonale. De la même manière, les avermectines affectent les organes reproducteurs féminins, entraînant des effets délétères tels que la dégénérescence et l'hémorragie des organes

reproducteurs, la détérioration des ovocytes et la formation de follicules atrétiques (Salman *et al*., 2022).

Une étude récente a montré que l'exposition à l'ABM entraîne une diminution de la viabilité des cellules HMC3 et induit leur apoptose. Une exposition prolongée à l'ABM pourrait accroître le risque de maladies neurodégénératives en raison de l'inflammation et de la réduction de la survie cellulaire (Sancer *et al*., 2025).

I.1. 5. Abamectine et stress oxydant

1.1. 5.1. Définition du stress oxydant

Le stress oxydant a été défini pour la première fois par H. Sies en 1985 comme un déséquilibre entre les pro-oxydants et les antioxydants en faveur des premiers, entraînant des dommages potentiels. Cette définition a été affinée en 1997 pour mettre en évidence l'importance de cet équilibre, précisant que le stress oxydatif correspond à un excès d'oxydants pouvant causer des altérations cellulaires. Face aux avancées scientifiques, une définition plus précise du stress oxydatif en le décrivant comme une élévation transitoire ou chronique des concentrations de ROS, perturbant le métabolisme et causant des dommages aux constituants cellulaires. La définition la plus récente élargit encore cette perspective en considérant le stress oxydatif comme une augmentation soutenue ou temporaire des niveaux de ROS, perturbant les voies métaboliques et de signalisation, et entraînant des modifications oxydatives des macromolécules, pouvant, en l'absence de compensation, conduire à la mort cellulaire par nécrose ou apoptose (Lushchak et Storey, 2021).

I.1. 5.2. Impact des pesticides sur la physiologie et les systèmes biologiques

Une exposition prolongée aux pesticides, qu'elle soit d'origine professionnelle ou environnementale, peut perturber la physiologie de divers organes du corps, y compris ceux des systèmes nerveux, endocrinien, immunitaire, reproducteur, rénal, cardiovasculaire et respiratoire. Les effets des pesticides peuvent être physiologiques ou biologiques, entraînant des modifications au niveau moléculaire, cellulaire ou

tissulaire. Bien que les mécanismes moléculaires expliquant comment les pesticides induisent des altérations biochimiques ne soient pas encore totalement élucidés, les recherches antérieures suggèrent qu'ils provoquent tous un stress oxydant (Sule *et al*., 2022).

I.1. 5.3. Mécanismes d'induction du stress oxydant par l'abamectine

Plusieurs études ont montré que l'abamectine joue un rôle dans le déclenchement du stress oxydatif par différents mécanismes. Tout d'abord, elle induit une surproduction d'espèces réactives de l'oxygène dans les cellules, ce qui entraîne des dommages cellulaires et tissulaires. Ensuite, l'exposition à cette molécule perturbe l'équilibre des enzymes antioxydantes en modifiant l'activité de la SOD, de la GST et, dans certains cas, de la GSH-Px, altérant ainsi la capacité des cellules à neutraliser l'excès de ROS. Enfin, l'abamectine favorise la peroxydation lipidique, en provoquant une élevation des niveau du MDA, un marqueur clé du stress oxydatif (Aioub *et al*., 2022; Radi *et al*., 2021).

Le stress oxydatif induit par l'abamectine entraîne des altérations biologiques majeures, affectant l'intégrité cellulaire et la fonction physiologique des organismes exposés. Il est responsable de dommages tissulaires significatifs, notamment au niveau du foie, des reins, et du cerveau, compromettant leur homéostasie et leur activité métabolique (Abdelrassoul, 2018). De plus, l'accumulation excessive d'espèces réactives de l'oxygène active des voies de signalisation impliquées dans la mort cellulaire programmée, notamment l'apoptose et la ferroptose, cette dernière étant caractérisée par une peroxydation lipidique dépendante du fer et une altération des membranes cellulaires. Par ailleurs, l'exposition à l'abamectine induit des perturbations comportementales et physiologiques chez les organismes aquatiques, se traduisant par une altération des capacités locomotrices et un déséquilibre du métabolisme énergétique, menaçant ainsi leur survie et leur adaptation aux conditions environnementales (Huang *et al*., 2024).

I.2. *MORINGA OLEIFERA*

I.2.1. Identification, origine et répartition

Les produits naturels ont toujours suscité l'intérêt de l'homme pour répondre à ses besoins quotidiens. Les plantes, en particulier, constituent une source abondante de composés bioactifs, tels que les antioxydants, qui jouent un rôle essentiel dans la prévention et le traitement de nombreuses pathologies liées au stress oxydant. Par conséquent, les médicaments à base de plantes ont gagné en popularité, étant perçus comme plus sûrs et plus économiques que leurs équivalents synthétiques (Singh *et al*., 2020).

Environ 80 % de la population mondiale dépend entièrement des plantes pour la santé et la guérison, et 25 % des produits pharmaceutiques intégrés sont dérivés de plantes médicinales et aromatiques.

Moringa oleifera, ou arbre de la vie, est un arbre vivace à feuilles caduques, à fleurs blanches parfumées et à écorce gris argenté, mesurant 10 à 12 mètres de hauteur (Singh *et al*., 2020), appartenant à la famille des *Moringaceae*. Cette dernière est largement répartie à l'échelle mondiale, en particulier dans les régions tropicales, et comprend environ 13 espèces. Originaire de l'Inde, à l'échelle mondiale, *M.oleifera* est largement répandue en Afrique, en Asie, en Amérique centrale et dans les îles des Caraïbes (Abdelazim *et al*., 2024). Le nom "*Moringa*" est dérivé du mot tamoul "Murungai" ou "Muringa", signifiant "gousse torsadée". Quant au mot "oleifera", il dérive du mot latin "oleum", qui veut dire "huile", et de "fera", signifiant "porteur" ou "producteur". Ainsi, "oleifera" indique que la plante est connue pour contenir ou produire de l'huile. La plante est connue sous les noms de ‘’shagara al rauwaq’’ en arabe, acacia blanc, ben oléifère, moringa ailé, *Moringa oléifère* et Mouroungué en français (Singh *et al*., 2020).

I.2.2. Histoire et usages traditionnels

Les applications médicinales de la plante ont été enregistrées dans des livres médicaux anciens de nombreux pays. En Chine, le premier enregistrement de *Moringa* remonte au manuscrit de Bower (volume II), aux alentours du 4^{e}-6^{e} siècle après J.C. Avec une histoire de milliers d'années, les utilisations médicinales des parties de la plante de *Moringa* ont été documentées dans la Pharmacopée Ayurvédique de l'Inde. Les anciens Égyptiens utilisaient l'huile de *Moringa* comme écran solaire. Les anciens Grecs ont découvert de nombreuses applications médicinales pour le *Moringa*, l'introduisant aux Romains, où elle s'est répandue dans toute l'Europe. Dans l'histoire moderne, en 1817, les Jamaïcains ont introduit l'huile de *Moringa* pour divers usages alimentaires, jusqu'à ce qu'elle atteigne l'Empire britannique, d'où la Moringa s'est étendue dans le monde entier (Abdelazim *et al*., 2024).

I.2.3. Plante aux Vertus Nutritionnelles et Thérapeutiques Exceptionnelles

Avec une riche histoire d'utilisations traditionnelles et médicinales, *M.oleifera* a suscité un intérêt croissant dans le monde scientifique moderne, devenant aujourd'hui l'objet de nombreuses études visant à explorer et à confirmer ses propriétés biologiques et ses applications potentielles.

Dans le monde actuel, marqué par des modes de vie accélérés, les individus se voient souvent contraints de consommer des aliments riches en calories, malgré une sensibilisation accrue aux enjeux de santé. Ces habitudes alimentaires déséquilibrées contribuent à l'augmentation des problèmes de santé tels que l'obésité, l'hypertension, le diabète et diverses maladies chroniques. Un régime équilibré, intégrant des niveaux optimaux de vitamines, minéraux et acides gras polyinsaturés, est essentiel pour prévenir ces affections (OMS, 2025).

Les feuilles de *M.oleifera* se distinguent par leur valeur nutritionnelle exceptionnelle. Elles sont non seulement riches en protéines facilement digestibles, mais contiennent

également des quantités significatives de composés essentiels, tels que les vitamines, le calcium, le fer, l'acide ascorbique et les antioxydants. Ces caractéristiques confèrent à *M.oleifera* un rôle potentiel majeur dans la promotion d'une alimentation équilibrée et la prévention des maladies liées à la nutrition (Islam *et al*., 2021).

M.oleifera est reconnue comme une source de composés bioactifs (Dhakad *et al*., 2019), notamment les polyphénols. Ces derniers sont présents en abondance dans toutes les parties de la plante (Kurniawan, 2021) et représentent une grande famille de molécules antioxydantes (Chouala *et al*., 2024), dotées de propriétés thérapeutiques, notamment antipyrétiques, antioxydantes, anti-inflammatoires, anti-âge, antidiabétiques, antihypertensives, immunomodulatrices, hépatoprotectrices et diurétiques. De récentes études ont également mis en évidence les propriétés antiparasitaires, neuroprotectrices, cérébroprotectrices et antiprolifératives contre certaines formes de cancer de *M.oleifera*. Par ailleurs, cette plante offre un potentiel prometteur en dentisterie régénérative et démontre une capacité à prévenir et atténuer l'ostéoporose (Abdelazim *et al*., 2024).

I.2.3.1. Propriété Antidiabétique

L'activité antidiabétique de *M.oleifera* a été rapportée dans plusieurs études menées à travers le monde. Des tests biologiques menés sur des rats diabétiques ont montré que l'administration d'extraits aqueux de feuilles de *M.oleifera* à des doses de 250 et 800 mg/kg entraîne une activité hypoglycémique satisfaisante ainsi qu'une amélioration de la fonction pancréatique (Omodanisi *et al*., 2017; Aja *et al*., 2013). De plus, l'administration orale de l'extrait de feuilles a permis de réduire les déséquilibres lipidiques plasmatiques associés au diabète, un mécanisme qui pourrait favoriser l'utilisation du glucose sanguin en inhibant la néoglucogénèse hépatique ou en stimulant son absorption par les muscles et les tissus adipeux (Oyedepo *et al*., 2013). Des études ethnobotaniques menées en Afrique sur l'utilisation des plantes médicinales dans la gestion du diabète ont révélé que *M.oleifera* figure parmi les espèces les plus utilisées en raison de son efficacité. Les feuilles de *M.oleifera* constituent un légume vert

bénéfique pour atténuer les complications du diabète chez les patients diabétiques. L'extrait aqueux de cette plante inhibe l'activité des enzymes α-amylase et α-glucosidase, améliore la capacité antioxydante, la tolérance au glucose et le taux d'absorption du glucose par les cellules de levure. Son utilisation en tant que phytopharmaceutique, seul ou en complément d'autres traitements, pourrait ainsi contribuer à la prise en charge du diabète (Fatoumata *et al*., 2020).

I.2.3.2. Propriété anticancéreuse

Les espèces réactives de l'oxygène sont directement impliquées dans la mort cellulaire. Divers stress environnementaux entraînent une production excessive de ROS, provoquant des dommages oxydatifs progressifs pouvant aboutir à l'apoptose ou à la nécrose des cellules. Les composés bioactifs présents dans les feuilles de *M.oleifera* et responsables de ses propriétés anticancéreuses comprennent les glucosinolates, la niazimicine et l'isothiocyanate de benzyle. La niazimicine, un composé bioactif extrait des feuilles de *Moringa*, a démontré un potentiel significatif en tant qu'agent anticancéreux. L'isothiocyanate de benzyle est particulièrement étudié pour son lien avec l'activité anticancéreuse. Des recherches antérieures ont montré que le l'isothiocyanate de benzyle induit une production intracellulaire de ROS, entraînant ainsi la mort cellulaire. Ce mécanisme pourrait expliquer l'efficacité du *M.oleifera* en tant qu'agent anticancéreux potentiel. De plus, le *Moringa* contient la zéatine, une cytokinine naturelle, connue pour ses propriétés anti-âge et antitumorales, qui s'est révélée efficace contre le cancer de la prostate et le cancer de la peau et possède également de puissantes propriétés antioxydantes. Des études ont mis en évidence un effet cytotoxique significatif des feuilles de MO sur des lignées cellulaires du myélome humain, suggérant ainsi un potentiel thérapeutique dans le traitement des cancers hématologiques (Islam *et al*., 2021).

Enfin , une étude menée sur l'investigation de l'effet anticancéreux de l'extrait aqueux de feuilles de MO a montré que le traitement par ce dernier possède un fort potentiel pour induire l'apoptose des cellules cancéreuses en modifiant le potentiel de la

membrane mitochondriale. Les tests *in vivo* de la même étude ont révélé que l'administration de l'extrait aqueux de *M.oleifera* chez des souris porteuses de tumeurs solides entraîne une réduction significative de la croissance tumorale ainsi qu'une augmentation de la durée de vie des souris allogreffées porteuses de tumeurs (Barhoi *et al*., 2020) .

I.2.3.3. Propriété anti-inflammatoire

Les composés bioactifs de *M. oleifera* sont censés exercer leurs effets anti-inflammatoires par plusieurs mécanismes : (a) inhibition des enzymes pro-inflammatoires : la quercétine et le kaempférol sont des composés de *M. oleifera* qui inhibent l'activité des enzymes pro-inflammatoires (cyclooxygénase et lipoxygénase), qui sont des enzymes clés impliquées dans la production de médiateurs inflammatoires tels que les prostaglandines et les leucotriènes ; (b) régulation de la production de cytokines : il a été démontré que les isothiocyanates (une classe de composés de *M. oleifera*) modulent les voies de signalisation impliquées dans l'inflammation, tout comme la voie du facteur nucléaire kappa B (NF-kappa B) ; cela assure la modulation des voies de signalisation. Il a également été démontré que les isothiocyanates inhibent la production de cytokines pro-inflammatoires facteur de nécrose tumorale α (TNF-α) et interleukine-1β (IL-1β) et augmentent la production de cytokines anti-inflammatoires telles que l'interleukine-10 (IL-10) ; et (c) une activité antioxydante : les flavonoïdes et les polyphénols contribuent à réduire le stress oxydatif et l'inflammation. Ces composés peuvent également inhiber l'activité des enzymes pro-inflammatoires et moduler la production de cytokines (Chiș *et al*., 2023).

I.2.3.4. Propriété neuroprotectrice

L'arbre miracle est reconnu pour atténuer les symptômes des maladies neurodégénératives et neuroinflammatoires telles que la maladie d'Alzheimer et la maladie de Parkinson (Igado et Olopade., 2016). Il peut également agir comme antidépresseur, anxiolytique et protéger contre l'ischémie cérébrale. Cette propriété

neuroprotectrice de *M.oleifera* est liée à ses caractéristiques anti-inflammatoires et antioxydantes.

Plusieurs composés bioactifs présents dans le MO, notamment l'épigallocatéchine, l'acide gallique, l'acide palmitique, la lutéoline, l'acide oléique, l'acide salicylique, ainsi que des alcaloïdes, des saponines, des tanins, des stéroïdes, des glucosinolates et des flavonoïdes, exercent diverses propriétés neuroprotectrices. Leur mécanisme d'action commun repose principalement sur la prévention de la formation des ROS, la réduction de l'inflammation et l'amélioration de la cognition et de la mémoire. Cela explique pourquoi *M.oleifera* pourrait être une plante médicinale prometteuse pour le traitement de l'anxiété, de la migraine, de la démence et de la maladie de Parkinson (Camilleri et Blundell 2024).

En effet, des études ont révélé que le MO peut inhiber, de manière dose-dépendante, le développement et la progression de l'accident vasculaire cérébral ischémique aigu. Il permet de diminuer la perte neuronale, la mauvaise répartition et la vacuolisation des corps cellulaires neuronaux, ainsi que la rétraction des noyaux cellulaires et la nécrose des tissus cérébraux et des neurones. Aussi, MO favorise de meilleures performances motrices et une activité cholinergique accrue tout en réduisant le volume de l'infarctus cérébral dans le cortex et le sous-cortex (Ghimire *et al*., 2021) .

I.2.3.5. *M.oleifera* et maladie liée au coronavirus (COVID-19)

L'infection par le COVID-19 entraîne une inflammation sévère due à la tempête de cytokines, qui peut rapidement provoquer une défaillance organique. Cet effet est initialement causé par des signaux chimiques induits par la chimiotaxie des neutrophiles via des substances comme l'interleukine-6/8. Une fois activées, ces substances sécrètent d'autres composants inflammatoires, déclenchant une cascade de réactions amplifiant la réponse inflammatoire. Malheureusement, les protéines non structurales 9 et 10 (nsp9, nsp10) présentes dans le COVID-19 favorisent cette réponse inflammatoire (Muhammad *et al*., 2021).

Dans une étude utilisant des méthodes de chimie quantique, de docking moléculaire et de dynamique moléculaire menée par Muhammad *et al*., 2021, il a été conclu que les phytocomposés, l'acide ellagique et l'apigénine, isolés du *M.oleifera*, ont présenté les plus fortes affinités de liaison, avec des valeurs de −7,1 kcal/mol et −6,5 kcal/mol contre nsp9 et de −6,9 kcal/mol et −7,1 kcal/mol contre nsp10, respectivement. De plus, ces deux composés ont montré une absorption intestinale élevée et un taux d'élimination rapide grâce à leur solubilité dans l'eau, tout en étant capables d'inhiber la croissance virale.

La réplication virale du COVID-19 est largement facilitée par la protéase principale (Mpro), ce qui en fait une cible prometteuse pour les médicaments anti-COVID-19 (Sen *et al*., 2021). Sen *et al*., 2021, ont étudié l'interaction entre MO et Mpro, révélant que les flavonoïdes isorhamnétine, kaempférol et apigénine présentaient une affinité de liaison remarquable et formaient des complexes protéine-ligand stables avec Mpro. Il en était de même pour les formes glycosidiques des phytocomposés, telles que la quercétine-3-rhamnoside, la myricétine-3-rutinoside et la rutine. Cette interaction permet de supprimer Mpro et, par conséquent, d'inhiber la réplication virale. Étant donné que ces phytoconstituants agissaient de manière similaire à l'agent antiviral baicaléine, cela renforce encore davantage le potentiel du *M.oleifera* comme agent préventif et antiviral contre le COVID-19.

De même, Mathpal *et al*., 2021, ont rapporté que le kaempférol-3-O-rutinoside et la vitexine présents dans MO avaient une forte affinité de liaison avec Mpro et formaient des complexes stables. En outre, ces composés étant hydrosolubles, ils pouvaient être facilement absorbés par les intestins et traverser la barrière hémato-encéphalique, ce qui confirme leur potentiel en tant que candidats médicamenteux.

Par ailleurs, Nair et James (2020), ont analysé l'affinité de liaison de divers phytocomposés présents dans MO avec Mpro et ont comparé leurs affinités de liaison ainsi que d'autres critères, tels que l'absorption intestinale, avec des antiviraux approuvés par la FDA (Food and Drug Administration). La majorité des

phytocomposés étudiés présentaient une affinité de liaison égale ou légèrement inférieure à celle des antiviraux approuvés par la FDA. Cependant, contrairement à ces derniers, qui sont tous des substrats de la P-gp et présentent un faible taux d'absorption intestinale (à l'exception d'un seul medicament: le Maraviroc), certains phytoconstituants de MO ne partageaient pas cette caractéristique. Cette étude *in silico* a révélé le potentiel de développement d'un médicament à base de MO pouvant prévenir la résistance aux antiviraux contre le COVID-19, tout en inhibant Mpro de manière similaire aux médicaments approuvés par la FDA.

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CHAPITRE II. MATERIEL ET METHODES

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II.1. Plante et préparation de l'extrait

Les feuilles en poudre de *M. oleifera* ont été obtenues auprès de BIOGATRANA (Gatrana Sidi Bouzid 9100, Tunisie), une société spécialisée dans l'extraction de produits naturels. La figure. 3 illustre le protocole expérimentale globale de l'analyse phytochimique.

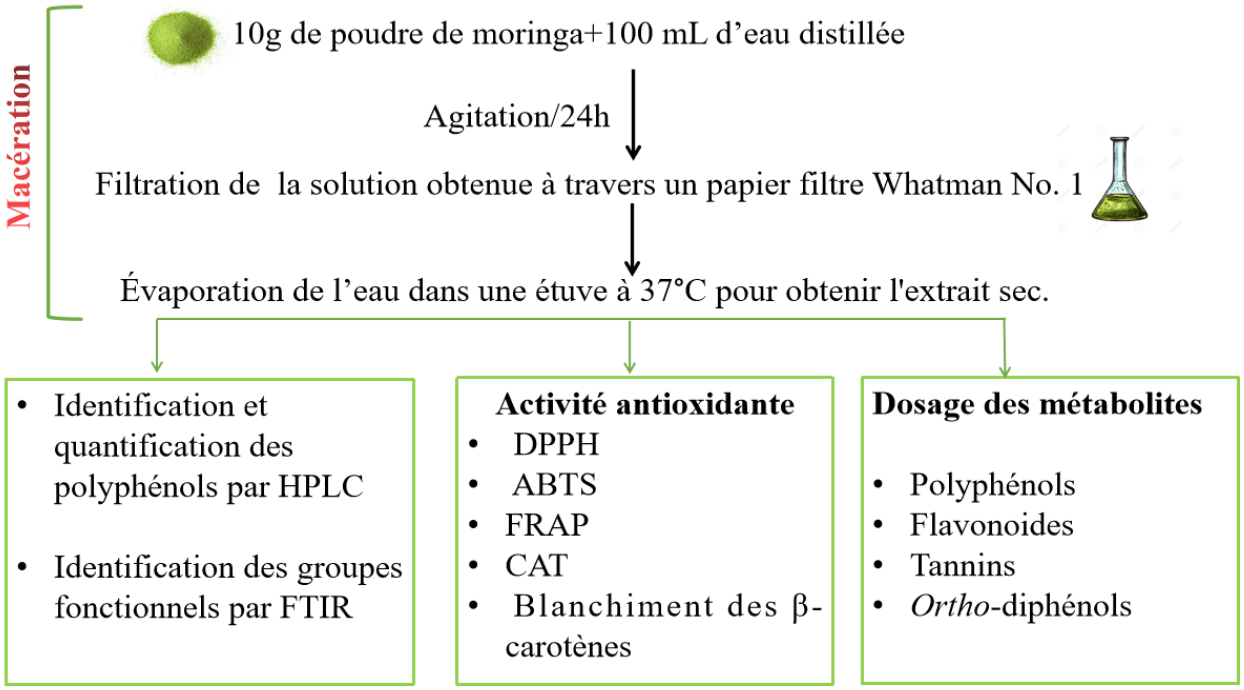


Figure 3. Illustration du protocole expérimental globale de l'étude phytochimique.

II.2. Dosages phytochimiques

II.2.1. Quantification des polyphénols totaux

Le dosage des polyphénols totaux, selon la méthode de Wolfe *et al.*, (2003), repose sur une réaction d'oxydo-réduction entre les polyphénols présents dans l'échantillon et les composés chimiques du réactif de Folin-Ciocalteu. Le protocole suivant décrit les étapes nécessaires à sa mise en œuvre.

200 µL de l'échantillon
1mL du réactiof de Folin Ciocalteu dilué (10×)
Incubation/ 4 min
800 µL de carbonate de sodium 7.5%
Incubation/2h
Mesure de DO á 765 nm

- **Courbe de calibration**:Acide gallique
- **Expression des résultats**: Equivalent mg acide gallique/g d'extrait.

II.2.2. Quantification des tanins condensés

Le dosage des tanins par la méthode de Julkunen-Tiitto (1985). Le dosage des tanins condensés selon Julkunen-Tiitto repose sur une réaction colorimétrique entre les proanthocyanidines (tanins condensés) et la vanilline en milieu acide. Cette réaction forme un complexe coloré rouge dont l'intensité est proportionnelle à la concentration en tanins. L'absorbance du mélange est mesurée à 500 nm à l'aide d'un spectrophotomètre. Une courbe d'étalonnage établie avec un standard, généralement la catéchine, permet de quantifier les tanins condensés dans l'échantillon, exprimés en équivalent catéchine. Le protocole qui suit décrit les étapes nécessaires à sa mise en œuvre.

50 µL de l'éxtrait
3mL de vanilline (4%)
Agitation
1.5 mL HCL ⟶ Mesure de DO á 550 nm.

- **Courbe de calibration:** Catéchine.
- **Expression des résultats:** Equivalent catéchine/g d'éxtrait.

II.2.3. Quantification des flavonoïdes

Le dosage des flavonoïdes par la méthode de Zhishen *et al*., (1999) repose sur la formation d'un complexe coloré entre les flavonoïdes présents dans l'échantillon et le chlorure d'aluminium ($AlCl_3$), qui réagit spécifiquement avec les groupes hydroxyles des flavonoïdes. L'intensité de la couleur jaune générée est proportionnelle à la concentration en flavonoïdes totaux. Le protocole qui suit décrit les étapes nécessaires à sa mise en œuvre.

1 mL de l'éxtrait
1 mL de chlorure d'aluminium 2% → 15 min → Mesure de DO á 430 nm.

- **Courbe de calibration**: Quercétine.
- **Expression des résultats** :Equivalent mg quercétine /g d'éxtrait.

II.2.4. Quantification des *ortho*-diophénols

Le dosage des *ortho*-diphénols par la méthode de Mateos *et al.*, (2011), repose sur une réaction colorimétrique spécifique entre les *ortho*-diphénols présents dans l'échantillon et le réactif d'acide chlorhydrique-molybdate. Cette réaction conduit à la formation d'un complexe coloré dont l'intensité est proportionnelle à la concentration en *ortho*-diphénols. Le protocole qui suit décrit les étapes nécessaires à sa mise en œuvre.

4 mL de l'échantillon
1mL de molybdate de sodium → Agitation , incubation/15 min → Mesure de DO á 370nm

- **Courbe de calibration**: Acide caféique
- **Expression des résultats**: Equivalent mg acide caféique /g d'extrait

II.3. Activité antioxydante *in vitro*

II.3.1. Test du piégeage du radical DPPH

Le dosage de l'activité antioxydante par la méthode du DPPH (2,2-diphényl-1-picrylhydrazyle) selon Kirby et Shmid (1997), repose sur la capacité des antioxydants présents dans l'échantillon à réduire le radical libre DPPH, ce qui entraîne un changement de couleur de la solution, de violet à jaune. Cette réduction est mesurée spectrophotométriquement, et l'intensité de la décoloration est inversement proportionnelle à la concentration d'antioxydants dans l'échantillon. Le protocole qui suit décrit les étapes nécessaires à sa mise en œuvre.

950 µL d'une solution DPPH (0.1mM)
50 µL de l'éxtrait a différentes concentration

Incubation a l'obscurité/30 min

Lecture des DO á 515 nm.

- **Expression des résultats:**

Inhibition (%) = (Ac − Ae)/Ac) × 100

Ac: Absorbance du blanc.
Ae: Absorbance de l'extrait.

II.3.2. Test du piégeage du radical cationique ABTS$^{\bullet+}$

La méthode de RE et al., (1999) repose sur l'utilisation du radical ABTS$^{\bullet+}$ pour évaluer l'activité antioxydante des échantillons. Ce radical est généré par réaction entre l'ABTS$^{\bullet+}$ et un oxydant puissant, le persulfate de potassium, et une fois formé, il peut être réduit par les antioxydants présents dans l'échantillon. La réduction du radical ABTS•+ entraîne un changement de couleur de la solution, du bleu-vert à incolore, dont l'intensité est mesurée par spectrophotométrie.Le protocole qui suit décrit les étapes nécessaires à sa mise en œuvre.

88 µL de persulfate de potassium (2.45mM)
5mL s d'une solution ABTS (7 mM)

Incubation a l'abri de la lumière/12h

Dilution de la solution (ABTS$^{\bullet+}$) pour atteindre une absorbance de 0.7 a 734 nm.

2.9 mL

100 µL de l'echantillon.

Lecture des DO á 734nm.

- **Expression des résultats:**

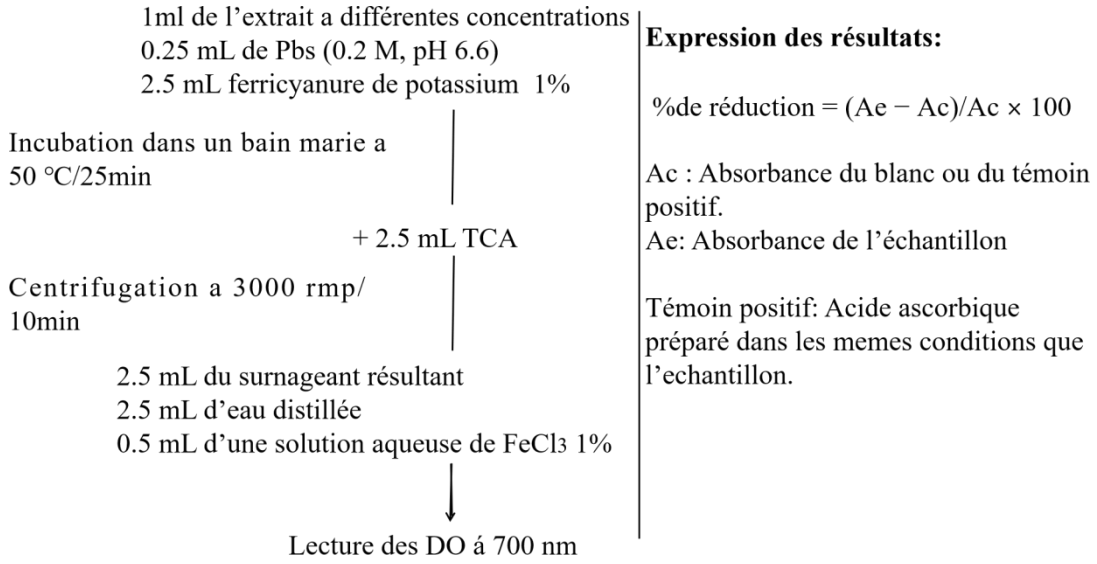
Inhibition (%) = (Ac − Ae)/Ac) × 100

- Ac: Absorbance du blanc (Solution diluée de l'ABTS$^{\bullet+}$)
- Ae: Absorbance de l'extrait.

II.3.3. Test de réduction du fer

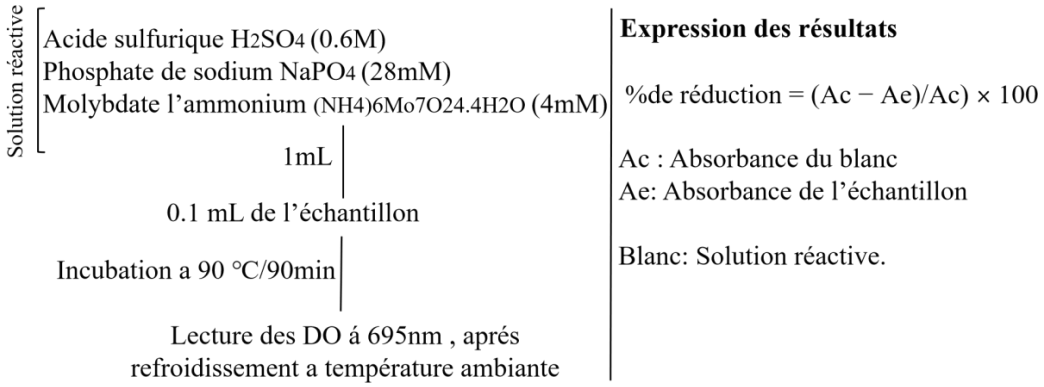
Le dosage de l'activité antioxydante par la méthode FRAP (Ferric Reducing Antioxidant Power) selon Pan *et al*., (2008) repose sur la capacité des antioxydants présents dans l'échantillon à réduire le fer ferrique (Fe^{3+}) en fer ferreux (Fe^{2+}). Cette réduction entraîne la formation d'un complexe coloré bleu, dont l'intensité est mesuré

spectrophotométriquement à 700 nm. L'absorbance est proportionnelle à la quantité de fer réduite, et donc à la capacité antioxydante de l'échantillon. Le protocole qui suit décrit lesétapes nécessaires à sa mise en œuvre.



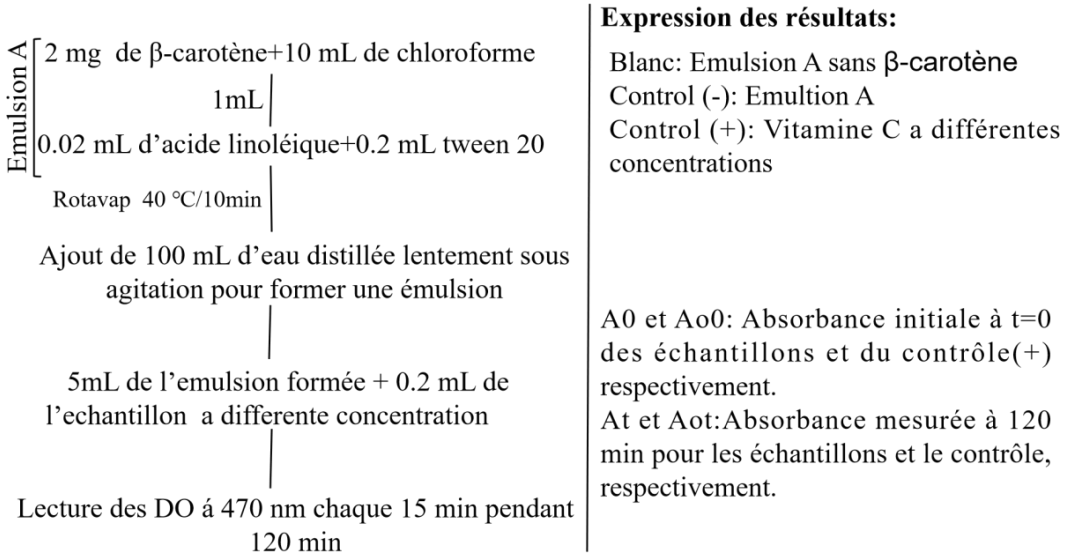
II.3.4. Capacité antioxydante totale

L'évaluation spectrophotométrique de la capacité antioxydante totale a été réalisée selon la technique décrite par Prieto *et al.*, (1999) basée sur la formation d'un complexe de couleur verte après la réduction des ions molybdate en molybdène en présence de l'échantillon.Le protocole qui suit décrit les étapes nécessaires à sa mise en œuvre.



II.3.5. Test de blanchiment du β-carotènes

La méthode adoptée par Ismail *et al*., (2004) pour le test de blanchiment du β-carotène repose sur l'évaluation de la capacité des antioxydants à inhiber l'oxydation du β-carotène, provoquée par les radicaux libres générés par l'acide linoléique. En présence d'antioxydants, la décoloration du β-carotène est limitée, et l'intensité de cette inhibition est mesurée par spectrophotométrie. Le protocole qui suit décrit les étapes nécessaires à sa mise en œuvre.



II.4. Identification et quantification des polyphénols par HPLC

Un système Shimadzu (LC 20 AL) équipé d'un détecteur UV-VIS SPD 20A (Shimadzu), d'une colonne analytique Shim-pack VP-ODS C18 (4,6 mm × 250 mm, 5 µm) et d'un injecteur universel (Hamilton 25 µL) a été utilisé pour l'analyse HPLC. La phase mobile, composée d'acide acétique à 1 % et d'acétonitrile, a été acheminée vers la colonne à un débit de 1 millilitre par minute à l'aide d'une pompe, après filtration à travers une membrane de 0,45 µm. Le résultat a été obtenu à une longueur d'onde de 268 nm.

II.5. Identification des groupements fonctionnels par FTIR

La spectroscopie infrarouge à transformée de Fourier (FTIR, Thermo Fisher Scientific, Waltham, MA, USA) a été utilisée pour caractériser les différents groupes fonctionnels présents dans les feuilles en poudre de *Moringa oleifera*. Les spectres FTIR ont été enregistrés dans la plage de 4000 à 500 cm^{-1}

II.6. Modélisation moléculaire et tests d'interactions

Les activités biologiques de certains antioxydants étudiés ont été évaluées d'avantage par modélisation moléculaire et tests d'interaction. Dans ce contexte, les cibles PRDX5 (1H2D), CAT (1QQW) et GSH-Px (1GP1) ont été sélectionnées. Les structures cristallines de ces macromolécules ont été récupérées à partir des bases de données RCSB, puis préparées en supprimant les molécules d'eau cristallisées et en ajoutant les hydrogènes polaires et les charges de Kollman. Par la suite, les macromolécules ciblées ont été traitées selon le champ de force CHARMm, comme décrit par Badraoui *et al.,* 2023. Les catégories de liaisons prédites, les interactions moléculaires et les ancrages serrés ont également été évalués conformément aux méthodes décrites par Rahmouni *et al*., 2024.

II.7. Etude *in vivo*

II.7.1. Expérimentation animale et traitement

Cette étude a été réalisée sur vingt rats femelles de la souche *Wistar*, pesant environ 180–200 g, obtenus de l'Institut Pasteur (Alger, Algérie). Les animaux ont été acclimatés pendant 2 semaines dans des conditions environnementales standard (25 ± 3°C, cycle lumière/obscurité de 12 heures), logés dans des cages en plastique avec un accès libre à une nourriture standard pour rongeurs (**Tableau 01**) et à de l'eau. Toutes les procédures expérimentales ont été réalisées conformément aux directives internationales relatives aux soins et à l'utilisation des animaux de laboratoire (Réf. : Instructions du Conseil sur la protection des animaux vivants utilisés dans les recherches scientifiques,

Journal officiel des Communautés Européennes, 1986 (JO 86/609/CEE) L358, pp. 1-18).

Groupes expérimentaux :

Les rats ont été répartis en quatre groupes égaux :

1. **Groupe témoin**, ayant reçu de l'eau distillée par gavage.
2. **Groupe ABM**, traité par l'ABM dissous dans l'eau de boisson, en tenant compte de la consommation quotidienne d'eau des animaux (24 mL/200 g de poids corporel) à une dose de 1 mg/kg de poids corporel (Meligi et Hassan, 2017).
3. **Groupe MO**, ayant reçu l'extrait aqueux de M. oleifera à une dose de 200 mg/kg de poids corporel par gavage (Albrahim et Binobead, 2018).
4. **Groupe ABM-MO**, ayant reçu une combinaison d'ABM et de MO administrée de la même manière et aux mêmes doses que celles des groupes précédents, pendant 3 semaines.

Tableau 01. Formulation de l'aliment pour 1 kg de ration.

Composants alimentaires	**Quantité en g/Kg d'aliment**	**Pourcentage %**
Maïs	620	62
Soja	260	26
Phosphate	16	1.6
Calcaire	09	0.9
Cellulose	10	1.0
Minereaux	10	1.0
Vitamines	10	1.0

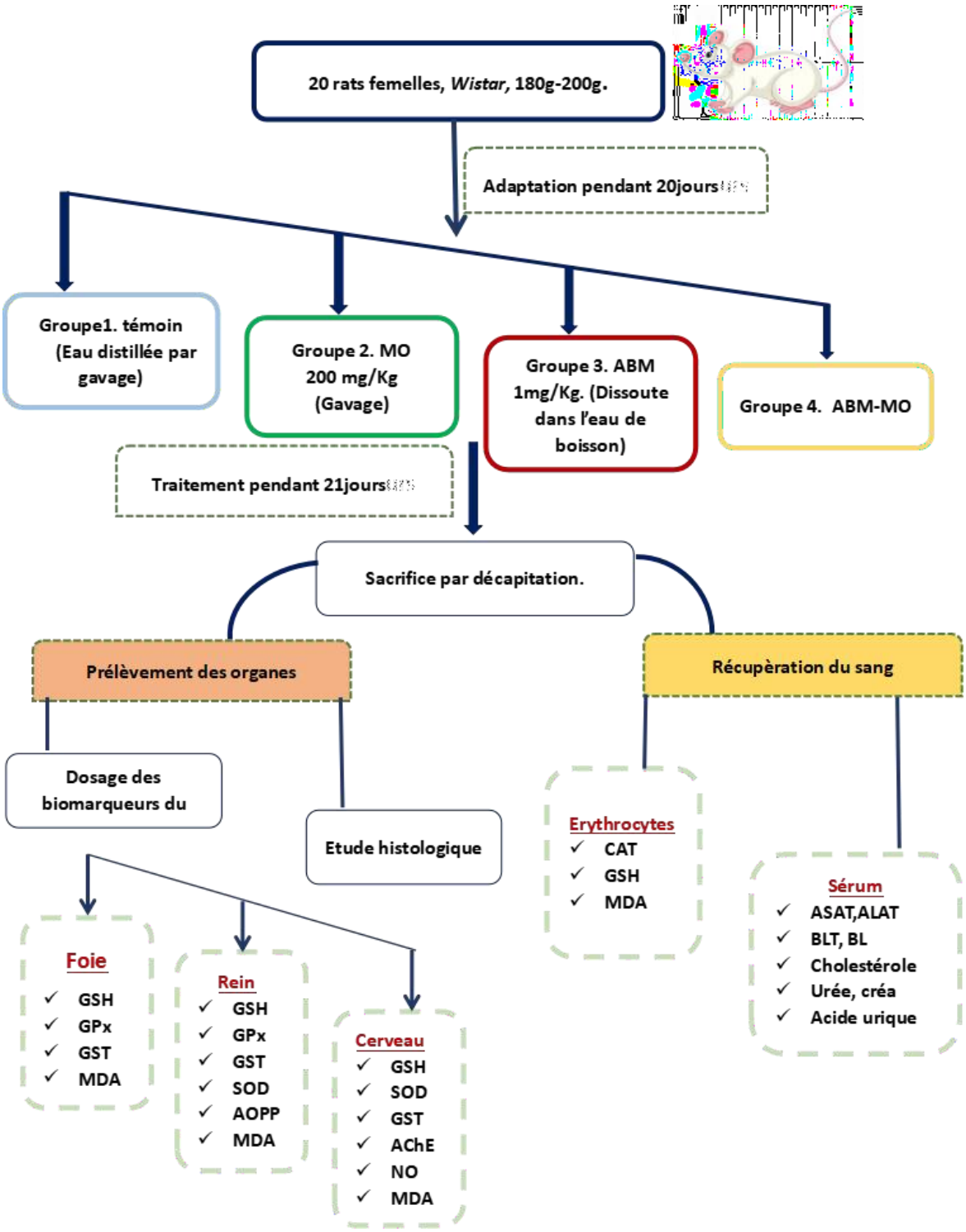


Figure 4. Illustration du protocole expérimentale pour l'étude *in vivo.*

II.7.2. Sacrifice des animaux et prélèvements

Le jour suivant la dernière administration, les rats ont été sacrifiés par décapitation cervicale. Le sang été récupéré afin d'isoler les érythrocytes et de mesurer certains paramètres biochimiques dans le sérum, et les organes ont été prélevés : une partie a été utilisée pour l'étude histologique, tandis qu'une autre partie a été homogénéisée pour le dosage des biomarqueurs de stress oxydant et l'acétylcholine.

II.7.3. Prélèvement du sang

Afin d'obtenir du sérum pour la mesure de l'urée, de la créatinine, de l'acide urique, des transaminases, du cholestérol, des triglycérides, de la bilirubine, ainsi que de la phosphatase alcaline, des échantillons de sang collectés sans EDTA ont été centrifugés à 3000 tours/ min pendant 15 minutes à 4°C. Les échantillons de sérum ont ensuite été conservés à –20°C, le culot des même tubes a servi pour l'obtention du lysat érythrocytaire pour le dosage des indicateurs du stress oxydant au niveau des érythrocytes. Le sang destiné à l'analyse hématologique (GR, GB, hématocrite, VGM, Hb, Plaquettes) a été collecté dans des tubes contenant de l'EDTA.

II.7.4. Indices hématologiques

Les indices hématologiques ont été quantifiés à l'aide d'un compteur automatisé de cellules sanguines, ERMA PCE - 210N (Japon).

II.7.5. Dosage biochimique

Les paramètres biochimiques ont été mesurés à l'aide de kits commerciaux de la marque ERBA (Espagne).

II.7.6. Homogénéisation des organes

Les échantillons de foie, de reins et de cerveau ont été rapidement prélevés, rincés avec une solution de NaCl à 0,9 % et pesés. Un gramme de tissu de chaque organe a été homogénéisé dans 2 mL de solution tampon Tris (TBS : 50 mM Tris, 150 mM NaCl, pH 7,4) dans des conditions froides sur glace. La suspension cellulaire a ensuite été centrifugée à 9000 tours/min pendant 15 minutes à 4 °C. Les surnageants obtenus

ont été divisés en aliquotes, puis stockés à –20 °C. Ces aliquotes ont été utilisées pour la détermination des indicateurs du stress oxydant ainsi que pour le dosage de l'acétylcholinestérase dans le cerveau.

II.7.7. Préparation des échantillons pour l'étude histologique

Conformément à la méthode de Hould (1984), des fragments de foie, de reins et de cerveau ont été lavés avec une solution de NaCl à 0,9 %, fixés dans du formol à 10 %, puis inclus dans de la paraffine. Les coupes en paraffine ont été sectionnées en tranches de 5 µm d'épaisseur et colorées avec de l'hématoxyline-éosine. Les observations ont été réalisées à l'aide d'un microscope optique (Leica Microsystems Schweiz AG, CH-9435 Heerbrugg), et les images ont été photographiées à l'aide d'une caméra numérique (Leica ICC50 W).

II.7.8. Dosage des protéines

La concentration en protéines dans chaque organe a été réalisée en suivant le protocole de Bradford (1976). Le protocole qui suit décrit les étapes de sa mise en œuvre.

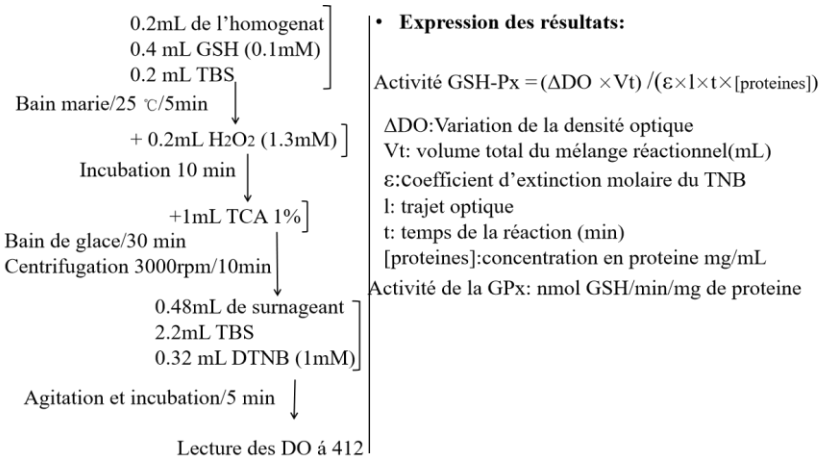
0.1 mL de l'homogénat
5 mL de la solution de Bradford
$\xrightarrow{\text{Incubation/5min}}$ Lecture des DO á 595.

- Courbe de calibration BSA

II.7.9. Biomarqueurs du stress oxydant

II.7.9.1. Glutathion peroxydase (GSH-Px)

Le dosage de la glutathion peroxydase selon Flohé et Günzler (1984) repose sur la réduction du peroxyde d'hydrogène (H_2O_2) en présence de glutathion réduit (GSH). La GSH-Px catalyse cette réaction, permettant la conversion du H_2O_2 en eau (H_2O), tout en oxydant le GSH en glutathion disulfure (GSSG).Le protocole qui suit décrit les étapes nécessaires à sa mise en œuvre.



II.7.9.2. Catalase (CAT)

Le dosage de l'activité de la catalase selon la méthode d'Aebi (1984) repose sur la mesure de la dégradation du peroxyde d'hydrogène (H_2O_2) en eau (H_2O) et en oxygène (O_2) par l'enzyme catalase.Le protocole qui suit décrit les étapes nécessaires à sa mise en œuvre.

20 µL de l'chantillon.
780 µL PBS; pH 7,5.
200 µL peroxyde d'hydrogène (500 mmol). → Lecture des DO á 240 nm chaque 15s pendant 1min.

Expression des résultats:

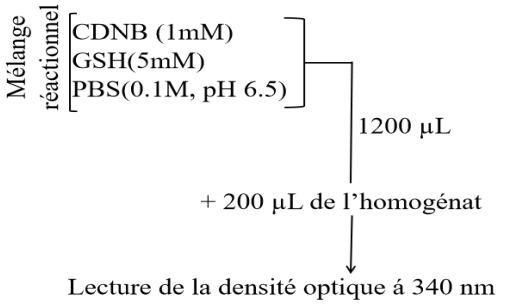
$$\text{Activité de la CAT (µmol } H_2O_2\text{/min/mg de proteines)} = \frac{\Delta DO}{\varepsilon \times L \times Fd \times [\text{proteines}]}$$

ΔDO: variation de la densité optique par minute.
ε: Coefficient d'extinction de H_2O_2 (0,043 $mM^{-1}cm^{-1}$).
L:Trajet optique.
Fd: Facteur de dilution.

II.7.9.3. Glutathion S-transférase (GST)

L'évaluation de l'activité de la GST selon Habig et al., (1974) repose sur la mesure de la réaction de conjugaison entre le GSH et le CDNB. Cette méthode est basée sur la formation d'un complexe glutathion-conjugué, qui peut être détecté

spectrophotométriquement grâce à l'augmentation de l'absorbance à 340 nm.Le protocole qui suit décrit les étapes nécessaires à sa mise en œuvre.



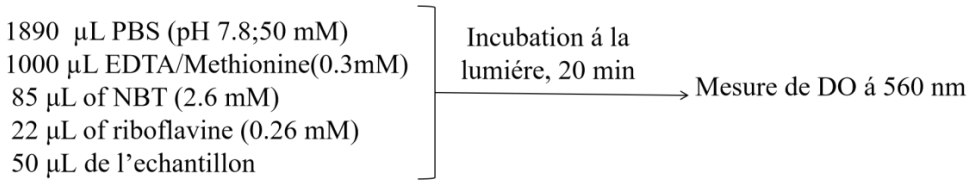
- **Expression des résutlats:**

Activité GST = ΔDO×Vt/ε×l×t×[proteines]

ΔDO:variation de l'absorbance
Vt:volume total
ε : coefficient d'extinction molaire du conjugué CDNB-GSH =9.6 $M^{-1}.Cm^{-1}$
l:trajet optique
[proteines]: concentration en proteines (mg/mL)
Activité GST :µmol CDNB/min/mg de proteines

II.7.9.4. Superoxyde dismutase (SOD)

La méthode de dosage de la superoxyde dismutase, selon Beyer et Fridovich (1987) repose sur l'inhibition de la réduction du NBT en formazan bleu par le radical superoxyde, généré par le couple riboflavine/méthionine sous exposition à la lumière. La SOD catalyse la dismutation du superoxyde, réduisant ainsi la formation de formazan. L'activité enzymatique est évaluée par la diminution de l'absorbance à 560 nm, proportionnelle à l'inhibition de la réduction du NBT. Une unité de SOD est définie comme la quantité d'enzyme nécessaire pour réduire de 50 % la formation de formazan. Le protocole qui suit décrit les étapes nécessaires à sa mise en œuvre.



- **Expression des resultats:**

%d'inhihbition = (Δ DO b - Δ DO e)/ Δ DO b × 100

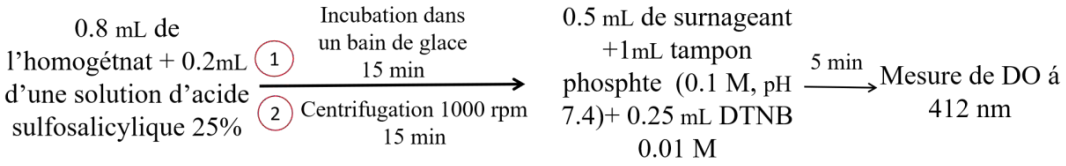
Δ DOb: variation de la densité optique du témoin
Δ DOe: variantion de la densité optique de l'echantillon

1 unité(U) correspond a la quantité de l'enzyme qui inhibe 50% de la réduction du NBT en formazan ⟷ Activité de la SOD (U)= %d'inhibition/50

Activité de la SOD = Activité SOD U/ [proteines]
[proteines]: concentration en proteine (mg/mL)

II.7.9.5. Glutathion réduit (GSH)

Le dosage du glutathion réduit selon la méthode d'Ellman (1959) repose sur une réaction entre le DTNB (5,5'-dithiobis (2-nitrobenzoïque acid)) et les groupements thiols libres du GSH. En présence de GSH, le DTNB est réduit pour former un produit jaune, la 5-thio-2-nitrobenzoïque (TNB), mesurable spectrophotométriquement à 412 nm. L'intensité de la coloration est proportionnelle à la concentration en GSH dans l'échantillon. Le protocole qui suit décrit les étapes nécessaires à sa mise en œuvre.



- **Expression des résultats:**

$$[GSH]\ (nmol/mg\ protein) = \frac{DO \times 1 \times 1.525}{13.1 \times 0.8 \times 0.5 \times mg\ protein}$$

- DO: Densité optique.
- 1 : Volume total de la solution utilisée pour la déprotéinisation (0,8 mL homogénat + 0,2 mL acide salicylique).
- 1,525 : Volume total du mélange réactionnel utilisé pour le dosage de GSH dans surnageant.
- 13,1 : Coefficient d'absorbance du groupement sulfhydryle (–SH) à 412 nm.

II.7.9.6. Malondialdéhyde (MDA)

L'estimation des niveaux de peroxydation lipidique selon Buege et Aust (1978) repose sur la mesure des produits de peroxydation lipidique, notamment le MDA, qui réagit avec l'acide thiobarbiturique pour former un complexe coloré. L'intensité de la coloration, mesurée par spectrophotométrie, est proportionnelle à la concentration en MDA, permettant d'évaluer le degré de peroxydation lipidique dans l'échantillon. Le protocole qui suit décrit les étapes nécessaires à sa mise en œuvre.

150 µL TBS (pH 7.4)
375 µL d'une solution BHT/TCA (1% p/v BHT dans 20% TCA)
375 µL de l'homogénat

Centrifugation 1000 rpm/10 min

400 µL de surnageant
80 µL de HCL
320 µL d' une solution de TBA

Incubation dans un bain-marie bouillant/10 min

Lecture des DO á 530 nm.

- **Expression des résutlats:**

$$[MDA]= \frac{DOe\text{-}DOt}{\varepsilon} \times \frac{1}{[proteines(mg/mL)]}$$

DOe:densité optique de l'échantillon.
DOt:Densité optique du témoin.
ε: coefficient d'extinction molaire du MDA=1.56×10^{5} $M^{-1}.cm^{-1}$.

[MDA]: nmol/mg de proteines.

II.7.9.7. Produit de l'oxydation avancé des protéinés (AOPP)

Le dosage des produits d'oxydation avancée des protéines selon Kayali *et al*., (2006) repose sur la formation d'un complexe coloré entre les AOPP et l'iodure dans un milieu acide. Ce complexe, mesurable à 340 nm, est proportionnel à la concentration en AOPP dans l'échantillon. Les AOPP, produits issus de l'oxydation des protéines par des espèces réactives de l'oxygène, servent de marqueurs du stress oxydatif. L'intensité de l'absorbance à 340 nm permet de quantifier les AOPP en comparant les résultats à une courbe de calibration utilisant des standards comme la chloramine-T. Le protocole qui suit décrit les étapes nécessaires à sa mise en œuvre.

200 µL de l'homogénat
800 µL PBS (0.1M; pH 7.4)

2 min

100 µL de iodure de potasium (1.16 M)
200 µL acide acétique glaciale

→ Lecture immédiate des DO á 340 nm.

- Courbe de calibration : Chloramine-T (0 -200 µL).
- Blanc: PBS + acide acétique+ iodure de potassium.

II.7.9.8. Oxyde nitrique (NO)

Le dosage du NO selon la méthode de Griess, en suivant le protocole d'Archer (1993), repose sur une réaction colorimétrique permettant de détecter les nitrites, principaux métabolites stables du NO. Cette technique implique une réaction de diazotation

suivie d'un couplage avec un composé aromatique pour former un complexe azoïque coloré. L'intensité de la coloration, mesurée à 570 nm, est directement proportionnelle à la concentration en nitrites.

100 µL de l'echantillon
50 µL du réactif de Griess A(une combinaison d'une solution de sulfanilamide 1% dans HCL 10 N)
50 µL du réactif de Griess B (N-(1naphthyl)éthylènediamine dihydrochloride (NED) préparé dans une solution de H_3PO_4 5%.

→ Incubation 2O min á l'obscurité → Lecture des DO á 570 nm

- Courbe de calibration: nitrite (0-50 µmol)

II.8. Activité de l'acétylcholine éstèrase dans le cerveau (AChE)

Le dosage de l'acétylcholinestérase selon Ellman *et al* 1961 repose sur la réaction entre l'acétylthiocholine (substrat) et l'enzyme AChE, qui produit de la thiocholine. Cette thiocholine réagit ensuite avec le DTNB, générant un composé jaune, le TNB, mesurable spectrophotométriquement à 412 nm. L'intensité de la coloration jaune, proportionnelle à la quantité de TNB formée, permet de calculer l'activité enzymatique de l'AChE dans l'échantillon.Le protocole qui suit décrit les étapes nécessaires à sa mise en œuvre.

1 mL PBS (0.1 M; pH7.4)
50 µL DTNB/NaHCO3 (150 %)
50 µLAcétylthiocholine (0.045 M)
5 µL de homogénat

→ Agitation → Lecture des DO á 412 nm chaque 3min pendant 25 min.

- **Expression des résultats:**

$$\text{Activité AChE(nmol/min/mg de proteines)} = \frac{\Delta DO \times V}{\varepsilon \times L \times [\text{proteines}]}$$

ΔDO: variation de l'absorbance par unité de temps.
V: Volume réactionnel.
ε: Coefficient d'absorption molaire du TNB (13.6 mM^{-1}. cm^{-1})
L: trajet optique.

II.9. Analyse statistique

Les résultats de l'étude 1 et 2 sont exprimés sous forme de moyenne ± erreur standard de la moyenne (SEM). Pour ces études, le test de Sidak a été utilisé pour les comparaisons multiples afin d'évaluer la signification statistique avec GraphPad

Prism version 7 (GraphPad Software, Inc., La Jolla, CA, USA). En ce qui concerne l'étude 3, une analyse de variance à un facteur (ANOVA unidirectionnelle) a été réalisée pour comparer les groupes expérimentaux, suivie du test post hoc de Tukey pour les comparaisons multiples, en utilisant IBM SPSS Statistics V26. Les différences ont été considérées comme significatives pour une valeur de $p < 0.05$.

CHAPITRE III. RESULTATS & DISCUSSION

Article 1

Evaluation of phytochemical and potential protective effects of antioxidants of *Moringa oleifera* leaves against abamectin-induced nephrotoxicity in *Wistar* female rats

K. Boudjema, K. Chouala, K. Yahia, S. Nani, K. Ouali, M. Boumendjel, A. Boumendjel and M. Messarah.

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July **24, 2024**

The article #7985 titled "Evaluation of phytochemical and potential protective effects of antioxidants of *Moringa oleifera* leaves against abamectin-induced nephrotoxicity in Wistar female rats " (authors: K. Boudjema, K. Chouala, K. Yahia, S. Nani, K. Ouali, M. Boumendjel, A. Boumendjel, M. Saoudi and M. Messarah) is accepted to publish in our journal Pharmaceutical Chemistry Journal with the Electronic ISSN:1573-9031 and Print ISSN: 0091-150X.

Sincerely,
FOLIUM Publishing House CEO
Olga Burmistrova

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Издательского дома «ФОЛИУМ» Бурмистрова О.П.

K. Boudjema [1], K. Chouala [1], K. Yahia[2], S. Nani [2], K. Ouali[3], M. Boumendjel[1], A. Boumendjel[1] and M. Messarah[1]

Evaluation of phytochemical and potential protective effects of antioxidants of *Moringa oleifera* leaves against abamectin-induced nephrotoxicity in wistar female rats

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Abstract: The purpose of this study is to explore the phytochemical composition and antioxidant activities of *Moringa oleifera* leaves aqueous extract (MO) and its potential nephroprotective effects on toxicity induced by abamectin (ABM). Due to its high antioxidant content, MO demonstrated a strong antioxidant activity against the 2,2-diphényl 1-picrylhydrazyle radical (DPPH), IC 50 = 0.125, and the acid 2,2'-azino-bis (ABTS) radical, IC 50 = 0.06. It also shown a significant ferric reducing capacity, EC 50 = 1.4. High performance liquid chromatography (HPLC) analysis revealed the presence of six phenolic compounds: gallic acid, chlorogenic acid, caffeic acid, vanillic acid, quercetin and nargining. Moreover, the fourier transform infrared (FTIR) analysis demonstrated several functional groups in MO. Female Wistar were divided into four equal groups: control received 0.5mL of water, MO group received *Moringa oleifera* leaves aqueous extract (200mg/kg BW), ABM group consumed abamectin in water (1mg/kg BW), ABM-MO received both treatments, during 21 days. Results showed that abamectin induced significant decrease in white blood cells and red blood cells counts and in haemoglobin level. Abamectin consumption resulted in renal failure, highlighted by significant elevation in kidney filtration indices. Moreover, it generated notable oxidative stress by reducing glutathione (GSH) concentrations and enzymatic activities of glutathione peroxidase (GPx), superoxide dismutase (SOD) and glutathione-S-transferase (GST). Nevertheless, MO supplementation reestablished oxidative imbalance, hematological parameters, and renal function. Indeed, rich and varied phytochemical composition of MO improved antioxidant status and reduced renal cellular damages and histological configuration induced by abamectin.

Keywords: abamectin; *Moringa oleifera*; oxidative stress; HPLC; FTIR.

Introduction

Environmental pollutants can have a range of adverse effects on humans and animals alikes, especially in developing countries [1]. According to the world health organization 3 million workers in these countries are subjected to serious pesticide poisoning every year [2]. In fact, these products are used to eradicate parasitic organisms and disease vectors [3]. However, the extensive use of pesticides has become a worldwide problem because of their non-specific mechanism, which makes it dangerous for living organisms and for the environment [4]. Abamectin, a combination of two groups of avermectin (Avermectin B1a 80%, Avermectin B1b 20%) belonging to the macrocyclic lactone family (Campbell. 2012), is a pesticide frequently used in animal therapy because of its effectiveness and pharmacological potential [6]. Furthermore, it is applied to agriculture to protect vegetables and fruit crops against insects and mites [7-8]. In invertebrates, avermectins acts by blocking the transmission of the nerve impulses by opening the glutamate gated chloride channels, thus causing hyperpolarization of nerve cells and immobilization of the parasite. In humans and animals, it stimulates GABA A receptor, although abamectin is less harmful to humans when used in therapeutic levels because of the presence of the blood-brain barrier [9]. That is why abamectin has been utilized not only for animal therapy but also as a safe drug against helminths in human use [10].

Despite these advantageous applications, several experiments reported the toxic effects of abamectin on various organs. Recent study, indicated that it impairs rat's liver and kidney functions, explained by a significant increase in hepatic biomarkers injury levels accompanied with a reduction in antioxidant enzymes activities [11]. In addition, the administration of this chemical was associated with significant generation of reactive oxygen species on cerebral rats' tissues [3]. ABM was also identified as an inducer of testis injuries, by impairing antioxidant defense system, causing inflammation and apoptosis [12].

In human, minor abamectin toxicity manifests as nausea, vomiting, diarrhea, and weakness, while, severe poisoning may result in coma, respiratory failure, acidosis, and uncommonly, death. Its toxic effects seem to be caused by GABA-A chloride channel potentiation [9]. Moreover, avermectins are able to induce genotoxic alterations in human cells, by chromatin condensation and DNA degradation [13].

Plants, in addition to preserving environmental harmony and supplying oxygen for life, play a crucial part in human diet and serve as an important source of contemporary pharmaceuticals [14]. *Moringa oleifera* or miraculous tree, one of the 13 species belonging to the *Moringacea* family, is largely farmed in the Middle-Est, Africa and Asia [15]. This plant is recognized as a beneficial dietary component due to its bioactive compounds [16-17], among which, polyphenols. The latter are present in abundance in all portions plant [18], and represent a large family of chemicals with antioxidant proprieties [19]. In general, the *Moringacea* are extremely oil-rich, with oleic acid serving as the primary fatty acid, much like olive oil [20]. *Moringa oleifera* leaves possess a large spectrum of therapeutic and medicinal properties, anti-inflammatory action, antibacterial effect, gastro-protective function, and an important capacity to reduce oxidative stress by scavenging free radicals [21-22]. An earlier experiment on diabetic rats showed that *Moringa* leaves reduced insulin resistance and increased insulin levels, preventing hyperglycemia [23]. Moreover, it has been claimed that its leaves and bark extracts exhibit cytotoxic activity against several cancer cells, including breast, colorectal and pancreatic cancers [24-25]

Being a major primary target for environmental toxins, the kidneys are extremely susceptible to their toxic effects [26], and knowing that kidney disease represent a major health problem [27]. The purpose of the present work is to investigate the nephroprotective effects of *Moringa oleifera* leaves aqueous extract on abamectin toxicity and to evaluate its capacity to reduce oxidative stress through the exploration of its chemical composition and antioxidant activity (*in vivo* and in *vitro study).*

Materials and Methods

Chemicals

All chemical products used in this study were purchased from Sigma Chemical Co. (St. 1 Louis, France).

The pesticide was a commercial formulation (Vertimec 1.8% E.C) containing abamectin (18g/l) as an active component (CAS 71751-41-2). This chemical (C94H14O28) is a mixture of two avermectins, avermectin B1a and avermectin B1b (fig 1). The product is formulated by INDUSTRIALS AFRASA firm, Spain, and distributed by FMP AGRI, Algeria.

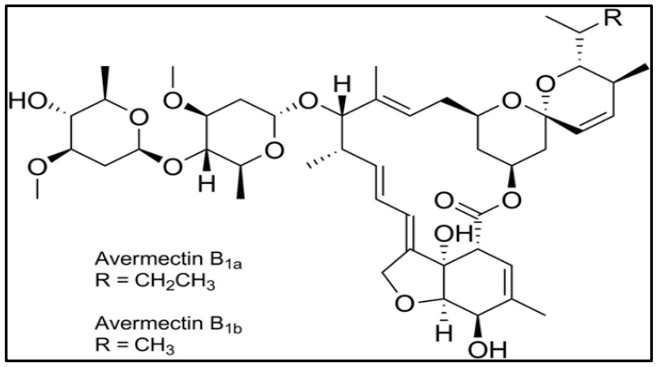

Fig. 1. Chemical structure of abamectin

Plant Material

Moringa Oleifera powdered leaves were obtained from BIOGATRANA (Gatrana Sidi Bouzid 9100, Tunisia), a company for Extracting Natural Products.

Plant extract preparation

Moringa oleifera aqueous extract preparation was performed by using the maceration method:10 grams of powder sample were extracted with 100 ml distilled water, during 24h, under stirring and at laboratory temperature (18-20°C). The solution was then filtered using Whatman filter paper No: 1.the filtrate was then dehydrated, using an oven at 37°C, and stored in the freezer until used.

Total polyphenol content: Total polyphenol content was assayed by the method of Wolfe, et al. [28] using Folin Ciocalteu reagent. The obtained results were calculated as gallic acid equivalent per gram plant extract (GAE/g E).

Total flavonoid content: Total flavonoid content was estimated spectrophotometrically according to the method reported by Zhichen, et al. [29]. Total flavonoid content was expressed as quercetin equivalent per gram plant extract. (QE/g E).

Total Tannins content: Tannin content was determined using the protocol described by Hagerman and Butler [30]. Total tannin content was expressed as catechin equivalent per gram plant extract (CE/gE)

Total antioxidant activity: Spectrophotometric evaluation of Total Antioxidant Activity was determined by the technique described by Prieto, et al. [31] based on the formation of a green colored complex after the reduction of molybdate ions to molybdenum in the presence of the sample. Absorbance was recorded at 695nm. Butylated hydroxytoluene (BHT) was used as standard.

DPPH free radical scavenging assay: The procedure described by Kirby and Shmidt [32] was adopted to evaluate the scavenging ability on DPPH radical. The result was represented as the necessary concentration (mg/ml) of extract to scavenge 50% of radicals (IC50).

ABTS reducing power assay: The capacity of MO aqueous extract to reduce ABTS radical was estimated following the protocol of Khan, et al. [33]. The sample's absorbance was determined at 734 nm. As previously explained for the DPPH test, the result was expressed as IC 50(mg/ml).

Ferric reducing antioxidant power assay (FRAP): The protocol described by Pan, et al. [34] was used to evaluate the ferric reducing antioxidant power. The sample's absorbance was determined at 700 nm The result was expressed as the half maximal effective concentration, EC 50 (mg/ml).

High-performance liquid chromatography analysis (HPLC): HPLC analysis was performed using a Shimadzu (LC 20 AL) equipped with a universal injector (Hamilton 25 µL), an analytical column a Shim-pack VP-ODS C18 (4.6 mm×250 mm, 5 µm), type (Shimadzu) and a UV-VIS detector SPD 20A (Shimadzu). The mobile phase, composed of Acetonitrile and Acetic acid 1%, was filtered using a 0.45 um membrane filter and derived to the column using a pump at a flow rate of 1ml/min. the result was obtained at 268 nm.

Fourier transform infrared analysis (FTIR): The Fourier transform infrared spectroscopy (FTIR Themo Fisher Scientific, Waltham, MA, USA) was used to characterize the different functional groups present in *Moringa oleifera* powdered leaves, FTIR spectra was recorded within the range of 4000 to 500 cm^{-1}.

Animals

Twenty mature female wistar rats, weighing 180-200g, were obtained from Pasteur Institute (Algiers, Algeria). They were acclimatized for two weeks prior in normal environmental conditions (25 ± 3°C, 12-hour light/dark cycle), housed in plastic cages given access to standard rodent food and water ad libitum.

After the adaptation period and during the three weeks experiment, animals were divided into four equal groups. The first group was the normal control group and received drinking water orally. The second group (MO-treated group) received *Moringa Oleifera* leave extract at a dose of 200 mg/kg BW orally [35]. The third group (ABM) was given abamectin dissolved in drinking water considering the daily water consumption of animals (24 ml/ 200 g BW) [36], at a dose of 1mg /kg BW [37]. The dose of ABM was adjusted weekly according to the average body weight of rats. The fourth group (ABM-MO) received a combination of abamectin and *Moringa Oleifera* with the same doses and in the same way as the second and third group. On the day following the final day of treatment, rats were sacrificed by cervical decapitation.

Blood sampling: In order to obtain serum for urea, creatinine and uric acid measurement, blood samples, collected without EDTA, were centrifuged at 3000 rpm for 15 min at 4°C. Serum samples were preserved at – 20°C. Blood used for hematological analysis (RBC, WBC, HTC, MCV, Hb, Platelets) was collected into EDTA tubes.

Hematological indices: Hematological indices were quantified using a full automated blood cells counter, ERMA PCE - 210N, Japan.

Biochemical Assay: The biochemical parameters were performed by using commercial kit from ERBA, Spain (refs: urea-XSYS0020-XSYS0075, Creatinine-XSYS0024-XSYS0076, uric acid-XSYS0042-XSYS0072)**.**

Tissue sampling: After the sacrifice, kidneys were promptly excised, rinsed with a 0.9 NACL solution and weighed. Relative kidney weight, as a percentage from body weight, was calculated. Kidney of each rat was divided into two sets. One gram was homogenized in 2 mL of tris-buffered saline (TBS: 50 mm Tris, 150 mm NaCl, pH 7.4) in cold ice conditions, followed by a centrifugation of the homogenates at 9000 rmp for 15 min at 4°C.supernatants were then collected and stored at -20°C for the assessment of GSH, MDA, AOPP levels and GPX, GST, SOD enzymatic activity. The second set was used to the histopathological study.

Histological examination: Histological examination was carried out following the protocol of Hould [38]. Kidney fragments were rinsed with physiological water, fixed in 10% formaldehyde, embedded in paraffin, sliced to sections of 5 um thickness, deparaffinized and stained with hematoxylin and eosin (H&E). The obtained sections were then air dried and scanned using light microscope (Leica Microsystems Schweiz AG, CH-9435 Heerbrugg) and photographed using a digital camera (Leica ICC50 W).

Lipid peroxidation assessment: The Malondialdehyde content as an indicator of lipid peroxidation was measured according to the technique of Buege and Aust [39], using thiobarbiturique acid. The absorbance was recorded at 532 nm after the formation of the red colored complex MDA-TBA, the latter was quantified with a molar extinction coefficient of 1.56×10^5 /M/cm. and expressed in nmol MDA/mg of protein.

Advanced oxidation protein products assessment: AOPP concentration was measured following the method of Kayali, et al. [40] The sample's absorbance was measured at 340 nm. The result was expressed in µmol/mg of protein.

Reduced Glutathione content: Reduced Glutathione level in kidney homogenate was determined using the technique of Ellman [41] modified by Jolow [42]. Based on the

reaction of DTNB (5,5-dithio-bis-2-nitrobenzoic acid) with compounds containing sulfhydryl groups, which result in the development of yellow color. Absorbance was recorded at 412 nm and result was expressed as nmol GSH/mg of protein.

Assessment of antioxidant enzymes activities: Glutathione Peroxidase (GPx) activity was estimated using the protocol defined by Flohe and Gunzler [43], based on the conversion of H2O2 to H2O by this enzyme in the presence of GSH. The absorbance was measured at 420 nm. The result was expressed as specific GPx activity in nmol GSH/mg of protein.

Superoxide dismutase (SOD) activity was assessed in line with the method reported by Beyer and Fridovich [44]. based on the competition between SOD and NBT for superoxide anion, generated by the photoreduction of riboflavin/methionine. In other words, the ability of SOD to inhibit the reduction of NBT by superoxide anion. After 20 minutes of incubation of the reactional mixture with tissue samples, absorbance was read at 560 nm. Units of SOD activity were expressed as the quantity of enzyme required to inhibit the reduction of NBT by 50%. The activity was expressed as units/mg of protein.

The spectrophotometric method of Habig, et al. [45] was employed to estimate the Glutathione-S-transferase activity using 1- chloro-2,4-dinitrobenzene (CDNB). The absorbance was recorded at 340 nm and GST activity was expressed as umol CDNB/GSH/min/mg of protein.

Protein content: Protein content was measured spectrophotometrically at 595 nm, depending on Bradford method [46]. Bovine serum albumin was used as standard.

Statistical analysis: All results were represented as mean ± standard error of the mean (S.E.M), one way ANOVA was used for the comparison between experimental groups. Sidak test for multiple comparisons was used to evaluate the significance with GraphPad prism version 7 (GraphPad Software, Inc., La Jolla, CA, USA). Differences were considered significant at a p-values <0.05.

Results

Antioxidants and in vitro Antioxidant activity: Results in Table 1 showed that Moringa oleifera extract contains phenolic acids (38.88±1.81 mg AGE /g extract), flavonoid (95.99± 4.71mg QE /g extract), and condensed tannins (0.201 ±0.01 mg RE /g extract). Data concerning the total antioxidant capacity, the potential antioxidant of

Moringa Oleifera aqueous extract on DPPH radical, ABTS radical and FRAP test are represented in Table 02.

Table 1: Antioxidant contents of Moringa oleifera leaf extract.

Studied Parameters	Values
Total phenolic acids (mg AGE/ g E)	38.59 ± 1.6
Total flavonoids (mg CE/g E)	95.99 ± 5.16
Condensed Tannins (mg CE/g E)	0.201 ± 0,01

n=3.Value are represented as (mean ± SEM).GAE (Gallic acid equivalent); CE (Catechin Equivalent)

Table 2: Antioxidant activity of Moringa oleifera leave extract.

Studied parameters	Values
50 % scavenging concentration (mg/ml) on DPPH radical	0.125± 0.009
50 % scavenging concentration (mg/ml) on $ABTS^{+}$ radical	0.06 ± 0.001
Total antioxidant Capacity (mg/ml)	0.201.46 ± 0.002
FRAP test (EC 50) (mg/ml)	1.04 ±0.01

n=3.Value are represented as (mean ± SEM).

HPLC analysis of antioxidant constituent present in Moringa oleifera leaves extract

Among the nine tested standard, represented in Table 3, six phenolic compounds including gallic acid (110.01ug/g), chlorogenic acid (13.320 ug/g), Vanillic acid (134.66 ug/g), caffeic acid (52.18 ug/g) and naringin (1405.35 ug/g) were revealed in the aqueous extract of *Moringa. oleifera* using the HPLC analysis. Chromatogram of the identified compounds is shown in (Fig.2).

Table 3: Standard mixture data.

Compound	Retention time	Equation	R^2
Gallic acid	5.29	Y=54681x	0.9956
Chlorogenic acid	13.392	Y=21665x	0.9853
Vanilic acid	15.531	Y=65077x	0.9921
Caffeic acid	16.277	Y=84066x	0.9974
Vanillin	21.46	Y=58930x	0.9966
p-Coumaric acid	23.817	Y=49495x	0.9961
Rutin	28.37	Y=28144x	0.9869
Nargining	34.788	Y=19379x	0.9968
Quercetin	45.047	Y=45378x	0.9962

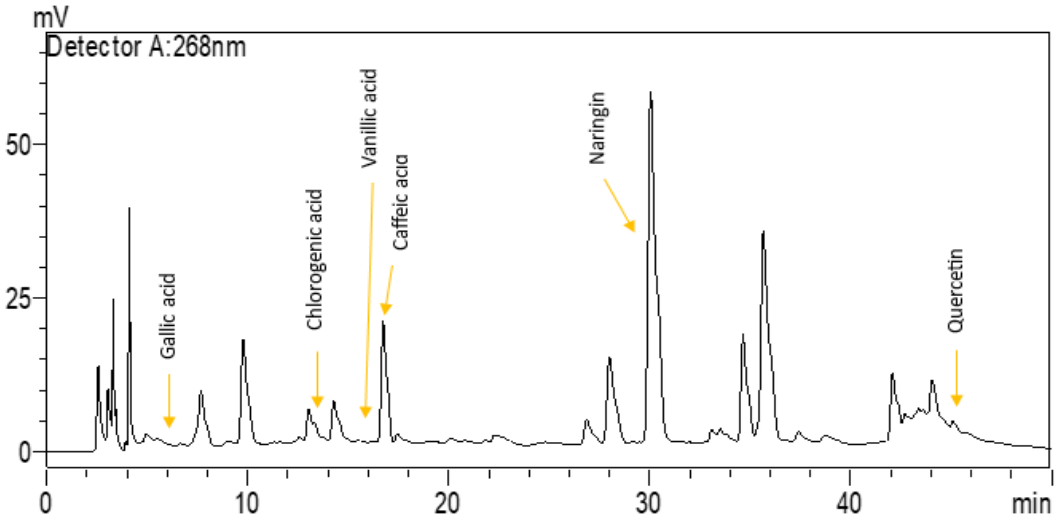

Fig.2. HPLC chromatogram of identified contents detected in aqueous extract of *Moringa Oleifera*.Retention Times: Gallic acid (5.468), Chologenic acid (13.320), Vanillic acid (15.472), Caffeic acid (16.183), Naringin (34 .640), Quercetin (45.080).

Fourier Transform Infrared (FTIR) Analysis: The Fourier transform infrared (FT-IR) was used to characterize the mains functional groups of active component present in Moringa Oleifera aqueous extract (Fig.3). The bands at 3265.86cm^{-1} would be due to O-H bending vibration. Whereas, the bands at 2915 cm^{-1} and 2846.9 cm^{-1} represent the asymmetric and the symmetric stretching of carbon (sp2, sp3) respectively in alkanes. In addition, the FT-IR spectrum showed the presence of carbonyl group (C=O) at 1621 cm^{-1}. The presence of harmonic bands between 1900 cm^{-1} and 2300 cm^{-1} confirmed the presence of aromatic cycle in M. oleifera leaves. The weak signal detected at 1300 cm^{-1} can be due to the C-N linkage. However, the bands at 1020 cm^{-1} is due to C-O functional group. These results confirmed the presence of phenolic compounds in *Moringa Oleifera* leaf extract.

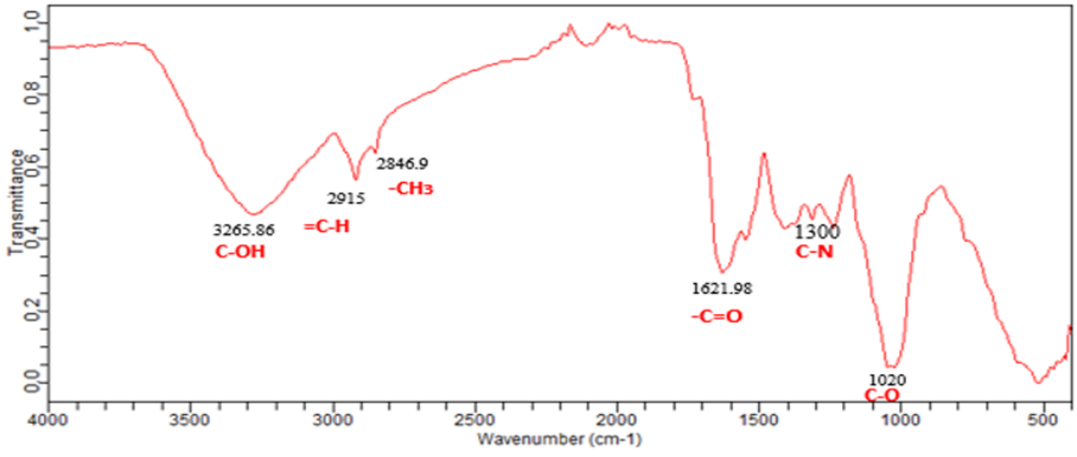

Fig.3. FTIR bands of biocompounds of *Moringa oleifera* leaf extract

Variations in hematological parameters: In comparison to control group, ABM administration caused a significant decrease in red blood cells ($p \leq 0.05$, -23.85%), white blood cells ($p \leq 0.001$, - 44%) and hemoglobin ($p \leq 0.001$). However, no significant changes were observed in platelets, and MCV levels. When treated with MO, intoxicated rats showed a significant increase in Hb level as well as a slight increase that is not statistically significant in WBC and RBC counts. No significant changes were found in MO treated group compared to control group. Table 04 summarizes the found results.

TABLE 4. Effect of treatment on Hematological parameters

Parameters	Control	MO	ABM	ABM-MO
WBC 10^3/ul	8.74± 0.45	8.4 ± 0.36	5 ± 0.51***	6.96 ± 0.31
RBC 10^6/ul	10.15 ± 0.69	9.22 ± 0.14	7.73 ±0.84*	8.65 ± 0.4
Platelet 10^3 /ul	295 ± 10.13	295.33± 22.08	252 ±17.44	258.70 ± 22.50
Hb g/dl	21.35 ± 0.35	18.22 ± 0.44	12.96 ± 2.7***	16.64 ± 0.6$^{\#}$
HTC %	46.58 ± 1.6	42.17 ± 0.7	36.98 ± 2.15**	39.38 ± 2.18
MCV (fL)	47.1 ± 0.32	46.06 ± 0.36	45.22 ± 0.40	45.330.71

Value are means ±S.E.M for groups of five rats each. Significant difference: MO, ABM groups are compared to control group ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$), ABM-MO group compared to ABM group (#p< 0.05).

Effect on body weight, absolute and relative kidney weights: As shown in Table 5, no significant changes in body weight were mentioned during the experimental period in all animal groups although a significant decrease in absolute kidney weight ($p \leq 0.001$) and relative kidney weight ($p \leq 0.05$) were noted in ABM-intoxicated rats compared to the control group. These renal damages were significantly restored ($p \leq 0.05$) when intoxicated rats were treated with MO. No significant variations were found in MO group compared to control group and no death was observed in any of the experimental groups.

TABLE 5. Effect of treatment on body weight, absolute and relative kidney weights

Studied parameters	Control	MO	ABM	ABM-MO
Initial body weights (g)	180.40 ±8.46	189.6± 4.29	197.33 ±7.77	190.4 ± 7,2
Final body weights (g)	215 ±7.16	207.8± 2.59	213 ± 2.90	200.6 ±2,4
Absolute kidney weights (g)	1.21±0.03	1.22 ± 0.04	1.03±0.04**	1.19 ± 0.03$^{\#}$
Relative weights (g/100 gr bw)	0.55±0.01	0.59 ± 0.02	0.50 ± 0.01*	0.59 ±0.01$^{\#}$

Values are means ± S.EM for five rats in each group. : MO, ABM groups are compared to control group (*p<0.05, **p<0.01), ABM-MO group compared to ABM group (#p< 0.05).

Biochemical parameters: Urea, creatinine and uric acid levels were measured to evaluate the kidney function. Our analysis indicates that administration of abamectin at dose of (1mg/kg bw) caused a significant elevation ($p \leq 0.05$) in serum concentrations of urea, creatinine and uric acid compared to control group. A significant decline ($p \leq 0.05$) in urea and uric acid levels were observed when ABM intoxicated rats were treated with MO (200 mg/kg bw). In addition, a significant increase in creatinine levels was noted in ABM-MO treated group compared to control group. No significant variations were observed in *Moringa oleifera* and MO-ABM groups compared to control group (TABLE.6).

TABLE 6. Changes in Serum urea, Creatinine and Uric Acid levels in different groups

Parameters	Control	MO	ABM	ABM-MO
Urea (mg/dl)	36.4 ± 3.88	35.2± 6.78	60.4± 4.64$^{*\#}$	37.2± 5.17
Creatinine (umol/l)	56.57 ± 2.16	63.64± 3.30	77.79 ± 6.49*	76.02 ± 3.53*
Uric acid (umol/l)	103.4 ± 7.52	120.4 ± 11.68	128.6 ±9 .12$^{*\#}$	103.2 ± 9.84

Values are means ± S.EM for five rats in each group. : MO, ABM groups are compared to control group (*p<0.05), ABM-MO group compared to ABM group (#p< 0.05).

Oxidative stress biomarkers: Data concerning the renal enzymatic and non-enzymatic antioxidants are represented in (Fig.4). Renal oxidative stress biomarkers are shown in (Fig.5). A significant (p<0.05) decrease in GPx, GST, SOD activities and GSH levels, as well as a significant increase in renal MDA and AOPPs concentrations were observed in ABM-intoxicated rats compared to control group. However, treatment with MO restored significantly the ABM induced damages in GPx, GST, SOD activities and GSH concentrations. No significant changes were detected in MO and ABM-MO groups compared to control group.

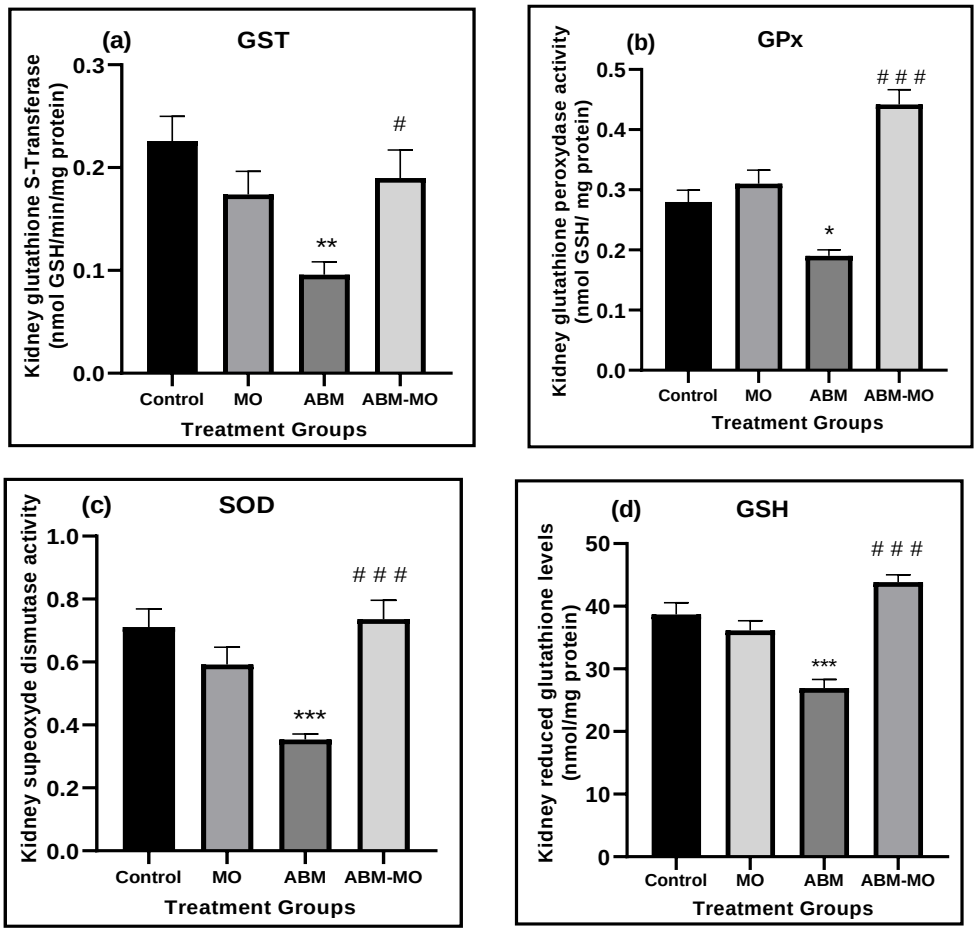

Fig.4. Renal enzymatic and non-enzymatic antioxidants; Value are means ±Sem. MO, ABM groups are compared to control group (*p<0.05, **p<0.01, ***p<0.001), ABM-MO group compared to ABM group. ($^{\#}$p<0.05, $^{\#\#\#}$p<0.001)

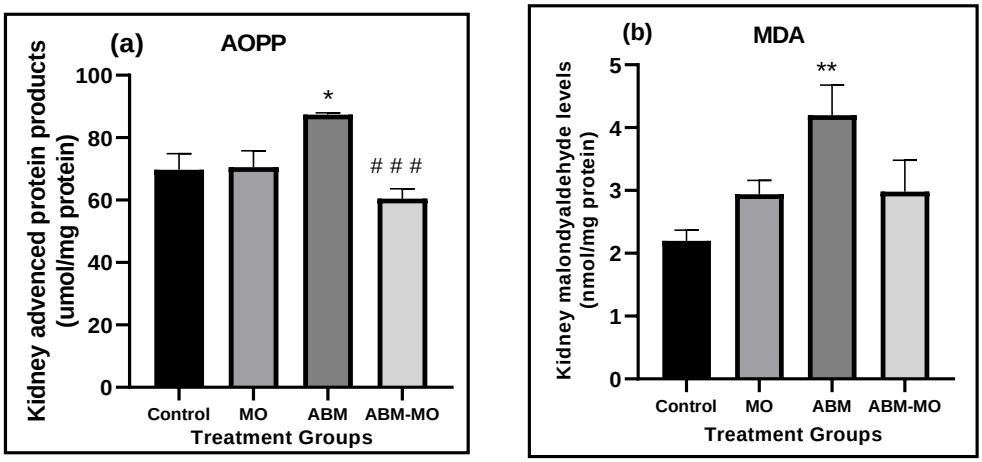

Fig.5. The renal tissue levels of oxidative stress biomarkers in different experimental groups. Value are means ±Sem. MO, ABM groups are compared to control group (*p<0.05, **p<0.01, ***p<0.001), ABM-MO group compared to ABM group. ($^{\#}$p<0.05, $^{\#\#\#}$p<0.001)

Histopathological findings: As shown in (Fig.6), kidney tissues sections from both control (Fig.6 A, B) and MO (Fig. 6 C, D) groups showed normal renal parenchyma

with normal glomerular and tubular architecture. However, in rats treated with ABM , the renal tissue shows severe degenerative alterations of glomerule, congestion with Bowman's space dilatation associated with glomerular atrophy, and tubular hydropic degeneration with presence of tubular necrosis(Fig. 6 E, F).Tissues sections from ABM-MO treated rats showed relatively normal kidney tissues with reduction in Bowman’s space and normal nephrocytes(Fig.6 G,H) compared to ABM trated rats.

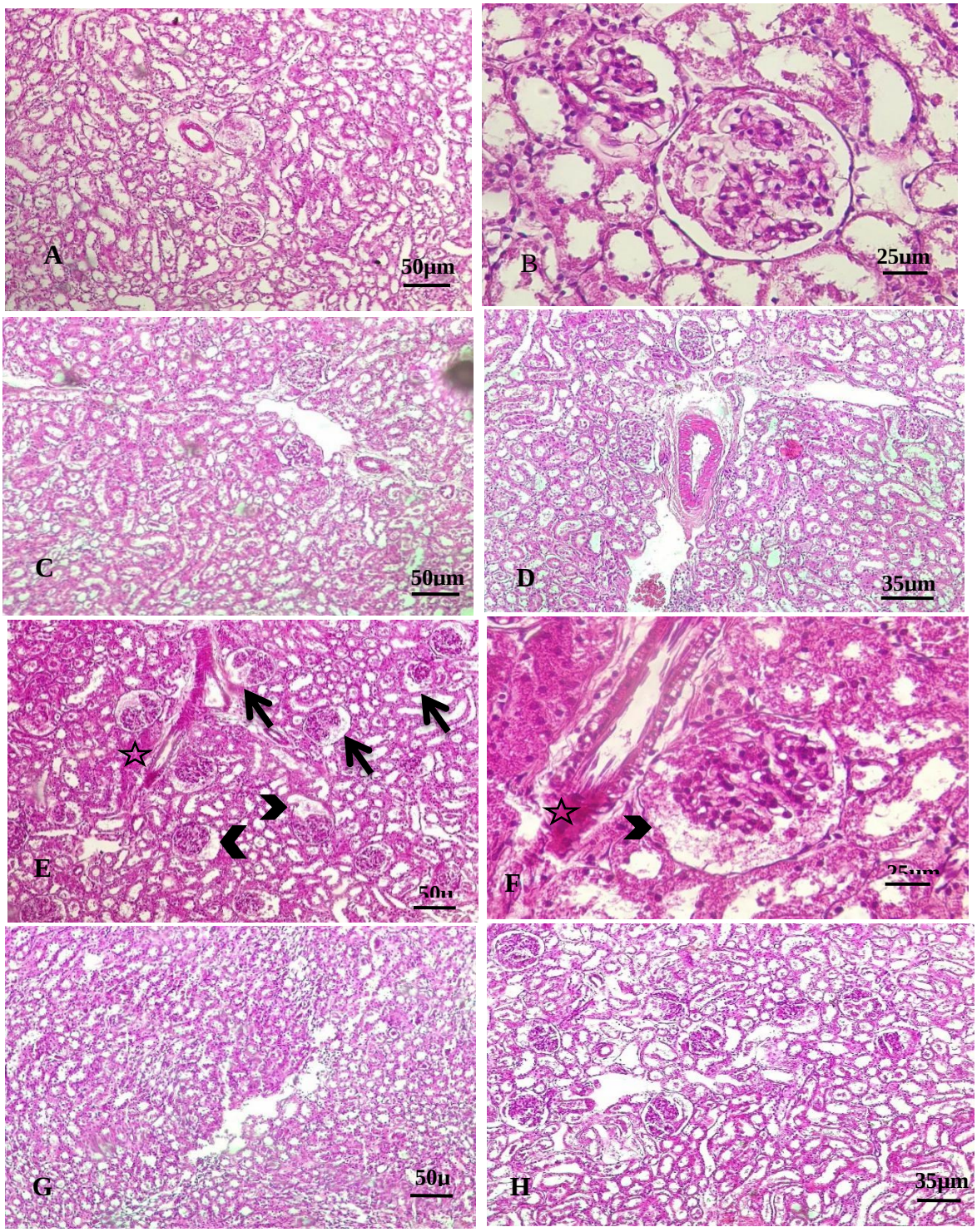

Fig.6. Photomicrographs of histological section of kidney tissue in control rats (A, B), after administration of MOLE (C, D), MOLE (E, F) and ABM-MOLE (G;H). In control and MOLE treated rats, the sections show normal renal parenchyma with normal glomerular and normal tubular structure (A,B,C,D). In rats treated with Pesticide, the renal tissue shows severe degenerative alterations of glomerule (arrow), congestion (arrowhead) (E,F) with Bowman's space dilatation associated with glomerular atrophy (arrow) and tubular hydropic degeneration with presence of tubular necrosis (asterix). In Pesticid-RAAE-treated rats, slices showed relatively normal renal tissue (G, H) (H&E staining, magnification 150X and 400X, scale bar = 25, 35 and, 50µm).

Discussion

In order to treat several diseases and toxicities, the use of medicinal herbs containing potent therapeutic compounds has taken precedence [47]. This study examined the antioxidant properties and phytochemical content of extract from the leaves of *Moringa oleifera* (MO) with the aim to determine how well it might protect the kidneys from the toxicity induced by abamectin. Phytochemical results showed that MO contains an appreciable quantity of total phenolic compounds, flavonoids and condensed tannins. These molecules are known to have a marked antioxidant activity [19-48], and regarded as strong protons donors [49]. The amount of total polyphenols in the studied extract of MO was 38.59 GAE/g E, which was similar to the findings of Moyo et al [50]. However, Bennour, et al. [49] reported a higher value of 101.81 GAE/g. Flavonoids are potent elements for nutraceuticals and medicines. Some of them have been used to develop new clinical treatments and dietary supplements [51]. Flavonoids concentration in this study was 95.99 mg CE/g extract. However, lower flavonoids concentration was obtained by Abdel Fattah, et al. [53]. Regarding the quantity of tannins, 0,201 mg of catechin equivalent per gram of extract was found in the current MO aqueous extract. While Adisakwattana and Chanathong [52] reported a higher content of 4.90 mg catechin equivalent/g extract.

According to investigations of its content in bioactive compounds, different environmental conditions have an impact on phytochemical composition of *Moringa Oleifera* plants grown in different countries [54], hence the differences in phenolic compounds counts might be referred to the variability of environmental and experimental factors [55]

HPLC analysis allowed the detection of gallic acid, chlorogenic acid, Vanillic acid, caffeic acid, quercetin and naringin in MO. This outcome is consistent with previous studies showing that MO has a wide range of bioactive molecules with strong antioxidant activities, including, gallic acid, protocatechuic acid, p-hydroxybenzoic acid, gentisic acid, catechin, chlorogenic acid, caffeic acid, Syringic acid, Vanillic acid, Ferulic acid, Sinapic acid, p-coumaric acid, Rutin, Apigenin-7-glucoside, rosmarinic acid, cinnamic acid, qurecetin, apigenin, kaempferol, rhamnetin and chrysin [53-56-57].

Flavonoid, polyphenols, amino acids, alkaloids and proteins present in MO include O-H, C=O, C-O, functional groups in their structures [18]. Phytochemical results, obtained in the current study, were confirmed by the FTIR spectrum. Moreover, all of these data

are supported by the observed ability of MO to scavenge DPPH and ABTS radicals, which is similar to the finding of Laoung et al who demonstrated that *Moringa oleifera* leaves tea was more powerful than gallic acid against ABTS radical [58]. In line with the results of Omodanisi, et al. [56], *Moringa oleifera* leaves showed a marked antioxidant activity that was observed in our study via the measurement of total antioxidant capacity and its ferric reducing power.

In toxicity studies, the decline in weight gain, body and relative weights are measured to evaluate pathophysiological state of experimental animals, which may be a sign of toxicity [59-60]. The present experiment shows that exposure to abamectin, at dose of 1mg/kg for 21 days, did not induce any significant changes in body weight when compared to control group, which is similar to the results found by Aggarwal [60] who studied the toxic effects of abamectin at dose of 2mg/kg in female rats. However, a significant decrease in absolute and relative renal weights was noticed in abamectin treated group as compared with control group, which may be a symptom of nephrotoxicity or chronic progressive renal diseases [61].Contrasting to our results, previous research on rabbits indicates that ABM cause an increase in the kidney relative weight after exposure to Voliam Tago, a mixture of abamectin and Chlorantraniliprole, they suggested that could be due to the decrease in body weight of intoxicated animals or a sign of pesticides toxicity [62]. In addition, Abdelrassoul [63] expressed that administration of ABM at dose of 0.5 mg /kg BW to male rats for 30 days caused a significant gradual decline in body weight, as well as a marked increase in relative kidney, liver and brain weights. Administered female rats with both abamectin and *Moringa* in this study showed nearly normalized absolute and relative kidney weight compered to control group and *Moringa oleifera* treated group. Such result may be related to the richness of MO in polyphenols including tannins, which are known to have vasodilatory effects, thus, glomerular filtration may be improved through renal vasodilatation [64].

The examination of hematological indices in the current study revealed that ABM induced reduction in RBC, hemoglobin and WBC counts when ABM-treated rats are compared to control group. The decrease in RBC and Hb level after ABM administration may be the result of hemolysis brought on by oxidative damage to cell membranes caused by ROS [11]. In agreement with our results, a study conducted by Manssour, et al. [65] on Nile tilapia fishes demonstrated that Abamectin causes severe anemia by reducing RBC and Hb level. In accordance with our results, Eissa and Zidan

[66], recorded that avermectins decreased white blood cells count, in this experiment the observed leukopenia may be the result of an altered immune system [67]. In contrast to our finding, Ahmed, et al. [68] reported that ivermectin and doramectin, belonging to avermectins family, increased leucocyte count, they explained this by the presence of different inflammatory focus after pesticides treatment. On the other hand, the co-administration of MO with ABM, in our study, positively affects hematological indices. These effects may be due to its rich micronutrient content, such as vitamin A, a key factor in reducing anemia [69], vitamin B, vitamin C, vitamin E, magnesium, and iron [70] which is involved in hemoglobin formation.

Regarding the renal biochemical analysis, a significant increment in urea, creatinine, and uric acid serum levels were observed following ABM administration, this result might confirm the nephrotoxicity of abamectin. The elevation in serum creatinine and urea concentrations in this study can be related to the decline in glomerular filtration and renal tubule dysfunction [71]. Indeed, high urea concentration is correlated with protein catabolism, as urea is the end-product of proteins degradation and a sign of kidney injury. The elevation in urea and creatinine reflects the kidney failure to eliminate these products [72-73]. This is corroborated by the observed histopathological changes in ABM treated group, including, glomerular atrophy and tubular hydropic degeneration with presence of tubular necrosis.Our results are in agreement with several studies confirming that ABM induced histopathological alterations in kidney, liver and brain tissues [3-11-71]. Interstingly, *Moringa oleifera* supplementation improved the elevation in urea and uric acid levels and reversed the negative histopathological effects induced by abamectin. This data is in coincidence with the findings of Elhamalawy [74] who mentioned that *Moringa oleifera* leaves attenuates the toxic effect of thiamethoxam in mouse. Moreover, Abou-Zeid, et al. [75] reported that *Moringa* leaves ethanolic extract reduced significantly the renal histological damages caused by tilmicosin, a macrolide antibiotic used in veterinary. In this study, the protective effects of *Moringa* might be attributed to the important bioactive molecules present in *Moringa oleifera* leaves extract, as demonstrated by the phytochemical analysis, such as gallic acid, Vanillic acid, chlorogenic acid, caffeic acid, naringin and quercetin. These components have been reported to have nephroprotective and antioxidant effects [76-81].

Free radicals are extremely reactive particles that are generated by physiological mechanisms in biological systems [82]. In the present work, ABM increased renal indicators of oxidative stress, malondialdehyde (MDA) and advanced oxidation

proteins products (AOPPs), and decreased kidney antioxidant status, including superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferase (GST) activities and reduced glutathione (GSH) levels. As proteins are the primary targets of free radicals and are prevalent in cells, plasma and the majority of tissues, AOPPs represent a crucial marker of oxidative stress [83]. Rats receiving abamectin in this study had significantly higher AOPPs levels compared to the control group. Elevated AOPPs levels positively correlate with acute and chronic kidney failure. Consequently, AOPPs are involved in the development of these renal damages through cellular and molecular processes that lead to the production of superoxide, podocyte death, and activation of monocytes [84]. MDA is a product of polyunsaturated fatty acids peroxidation. Having a strong cytotoxic power, MDA can easily bind to proteins or nucleic acids [85]. According to the present study the elevation in MDA levels reflect the triggering of oxidative stress, caused by ABM, via the generation of free radicals which are involved in the activation of lipid peroxidation pathway [86]. In agreement with our result, Scasselati, et al. [87] observed alteration in MDA liver levels when Oreochromis niloticus fishes were exposed to avermectins for 96 hours.

Due to their antioxidant activity and having the ability to protect cells against free radicals. SOD, GPx and GST are an essential enzyme involved in the cellular antioxidant defense system. SOD catalyzes the transformation of superoxide anion into H_2O_2 [88]. The latter is then cleaved into H_2O by GPx, which is in charge of preventing intracellular molecules from oxidation [11]. The significant decrease in SOD, GPx, GST activities after abamectin exposition may be due to the accumulation of free radicals and the failure of the redox system to scavenge these molecules, which would weaken cellular defense [89]. In addition, the reduction in GST activity may reflects insufficient ligation and detoxification of free radicals [72]. Knowing that GSH is implicated in the elimination of hydrogen peroxide and lipid peroxide through GPx action [90], the decline in its concentration causes oxidative stress [91]. Depletion in GSH concentration in this study may prove the overproduction of free radicals in rat's kidney following ABM treatment. In agreement with our results, Aioub, et al. [11] observed that ABM administration was associated with hepatorenal oxidative damage. Additionally, Radi, et al. [3] demonstrated that ABM could alter the antioxidant defense

system by promoting the generation of free radicals in brain and hepatic rats' tissues. Moreover, data obtained in the current study are in line with those of Liang, et al. [91] who studied the toxicity of abamectin on isolated mouse macrophage and found ABM causes a decrease in GSH levels as well as in GPx and SOD activities.

According to the results of this investigation, and in comparison, ABM-treated rats the co-administration of MO with ABM significantly restored the antioxidant machinery as evidenced by the significant improvement in GSH levels and antioxidant enzymes activities. Recently Turkish researchers have shown that pre-treatment with MO attenuates renal and hepatic methotrexate induced oxidative stress by increasing SOD and GSH levels [92]. Additionally, a study conducted by Akinrinde, et al. [93] showed that *Moringa oleifera* leaves at dose of 200 mg/Kg exhibit nephroprotective effects during oxidative damages caused by renal ischemia and reperfusion by reducing MDA and AOPP renal levels. The observed antioxidant effect of MO in the present study might be referred to its large polyphenolic compounds content. Taking the example of chlorogenic acid, a phenolic acid containing one carboxyl group and five active hydroxyl groups. The phenolic hydroxyl group structure interacts readily with free radicals and can produce hydrogen radicals that have an antioxidant effect on hydroxyl radical and superoxide anions [94]. Previous research indicated that chlorogenic acid might not only activate antioxidant enzymes but also reduce free radicals' generation and cell degradation caused by ROS [95-96]. Also, Bhattacharyya [97] highlighted that chlorogenic acid purified from green coffee bean attenuates the over production of lipid peroxide and ameliorates the antioxidant system activity by increasing SOD activity and GSH levels in renal tissues rats treated by chemical drugs. Moreover, flavonoids present in *Moringa* are recognized to serve as potent antioxidants [98]. *Moringa oleifera* is able to prevent or slow the oxidation of molecules generally by trapping free radicals and reducing development of inflammatory cytokines because of its high phenolic contents [56].

Conclusion

The present study revealed that abamectin administration resulted in serious perturbations including hemotoxicity, biochemical alterations, oxidative damages and histopathological changes in renal tissues. These results confirm the toxic effects of abamectin and its implication in the generation of free radicals. Treatment with MO showed a marked nephroprotective effects against abamectin induced renal toxicity and oxidative stress. According to FTIR and HPLC analyses, Moringa Oleifera leaves

contain a variety of bioactive and antioxidant chemicals that may be contributing to its positive effects, as well as its capacity to scavenge free radicals. Therefore, *Moringa Oleifera* leaves might be considered as a good source of natural antioxidants, which can be used in dietary and pharmaceutical industries. To the best of our knowledge the present study is the first to report the nephroprotective effect of *Moringa oleifera* on abamectin induced renal oxidative damages.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors contribution

B.K and C.K performed the experiments, analyzed the data, and wrote the article. KY carried out the HPLC analysis, N.S carried out the FTIR analysis, O.K carried out the histological study, B.M, B.A, and M.M conceived and designed the experiments.

Ethical Approval

All the experimental procedures were carried out in accordance with international guidelines for Care and use of laboratory animals (Ref: Council Instructions about the Protection of Living Animals Used in Scientific Investigations, Official Journal of the European Communities, 1986 (JO 86/609/CEE) L358, pp. 1-18)

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Article 2

Hepatoprotective potential of *Moringa oleifera* leaves against abamectin-induced toxicity in rats

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Abstract

The present work aims to evaluate the antioxidant abilities of *Moringa oleifera* leaves aqueous extract (MOLE) *in vitro*, as well as to clarify its hepatoprotective effects on toxicity and oxidative alterations induced by formulated abamectin (ABM) in rats. ABTS, DPPH and FRAP tests were used to assess its *in-vitro* antioxidant activity.

Rats were divided into four groups : group I served as controls which received standard diet, group II received MOLE by gavage at 200 mg/ kg BW Group III received orally ABM 1mg/kg BW and group IV received both ABM and MOLE for 21 days.The phytochemical investigation showed that MOLE exhibited potent antioxidant properties against the acid 2,2'-azino-bis (ABTS) radical (IC50 = 0.06) and the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) (IC50 = 0.125). Additionally, a notable ferric reducing capacity (EC50 = 1.4) was demonstrated. Administration of ABM by orally to rats caused hepatotoxicity as monitored by the increase in the levels of hepatic markers enzymes (transaminases "ASAT, ALAT"), alkaline phosphatase "ALP", gamma-glutamyl transferase "GGT"), free bilirubin (FBL) and total bilirubin (TB), cholesterol (CHL) and triglycerides (TG), as well as hepatic malondialdehyde (MDA) levels thus causing a drastic alteration in antioxidant defense system. Particularly, the activities of catalase (CAT), glutathione-S-transferase (GST), and glutathione peroxidase (GPx) and the level of reduced glutathione (GSH) increased by ABM. These biochemical alterations were accompanied by histological changes marked by slight and sinusoidal dilatation (moderate peliosis). Treatment with MOLE prevented the liver damage induced by ABM, as revealed by inhibition of hepatic lipid peroxidation accompanied by an improvement of liver histopathological changes, CAT, GPx and GST activities. It could be concluded that *Moringa oleifera* is promising a protective agent against hepatotoxicity during the exposure to abamectin.

Keywords: Abamectin. Rats. Oxidative stress. *Moringa oleifera*.Antioxidant activity.

Introduction

Avermectins are bio-pesticides produced by soil actinomycetes, specifically Streptomyces avermitilis, and belong to the macrocyclic lactones class with nematocidal, acaricidal, and insecticidal properties. Among the members of the avermectin family are moxidectin, milbemycin oxime, doramectin, selamectin, ivermectin, eprinomectin, and abamectin (Bai 2016). The latter is one of the most widely used avermectins in agriculture and veterinary medicine due to its broad spectrum of activity and simplicity of use (Reda *et al*.,2023). Abamectin is composed of avermectin B1a (80%) and avermectin B1b (20%). It acts by promoting cell membrane hyperpolarization by binding to glutamate or GABA (γ-amino butyric acid) receptors, which increases the influx of chloride ions. Consequently, neurotransmission is inhibited, causing targets to become paralyzed (Aioub *et al*., 2022). Thus, abamectin is highly toxic to insects and may also be highly toxic to mammals (Radi et al.,2020). Indeed, several studies have reported the toxic effects of avermectins on different living organisms. For example: In rats, Radi, et al.(2020) demonstrated that abamectin causes liver and brain toxicity. In rabbits, Bokreta, *et al*. (2021) showed that rabbits exposed to a mixture of abamectin and Chlorantraniliprole exhibited renal alterations. In fishes, Reda, *et al.* (2023) highlighted that abamectin induces immune response disruption and oxidative stress. In pigeons, Zhu, *et al*. (2013) demonstrated that avermectins disturb pigeon liver functions by inducing oxidative stress and apoptosis.

Worldwide, medicinal plants are utilized for a variety of medicinal purposes. Due to their abundant extracts and bioactive components, they are employed to treat, manage, and control various health disorders (Abdelazim *et al*., 2024). *Moringa oleifera* is a tropical tree native to the western and sub-Himalayan regions, including India, Pakistan, Africa, and Arabia, and belongs to the Moringacea family, which comprises 14 species. *Moringa oleifera* is the most extensively studied species in this family due to its intriguing profile (Elgandour *et al*.,2023). The plant is considered a rich source of nutrients such as vitamin C, calcium, potassium, iron, proteins, and vitamin A. For this reason, many tropical and subtropical nations include the tree's edible leaves, fruits, flowers, and immature pods in their traditional diets (Islam *et al*., 2021).

The liver is a vital organ that performs a variety of functions, including protein synthesis, energy metabolism, bile secretion, drug detoxification, vitamin and glycogen storage, and more. Owing to its crucial role in the body, any malfunction or failure of the liver often leads to serious health conditions and has emerged as a major global cause of morbidity and mortality (Slama *et al* .,2020). Containing a various phytochemical with therapeutic potential, *Moringa oleifera* possesses several medicinal properties including antioxidant, anti-inflammatory, anti-aging, antipyretic, antidiabetic, antihypertensive, immunomodulatory, hepatoprotective, and diuretic properties (Abdelazim *et al*.,2024) . Additionally, it exhibits neuroprotective (Azlan *et al*., 2023) and antiparasitic effects (Elgandour *et al*., 2023; El-Sayed and Fathy 2019; Korsor 2017).

Several papers have been interested on the chemical composition of *Moringa oleifera* extracts (Setiawan *et al.*,2022). while, the protective effect of *Moringa oleifera* on free radicals and on antioxidant enzyme activities in abamectin-induced oxydative stress and its complication has never been examined. Thus, the present investigation is carried out in order to study the possible hepatoprotective role and antioxidant capacity of *M. oleifera* leaf extract in order to attenuate abamectin -induced liver injury in rats.

Materiel and methods

Chemicals

The pesticide used in the present study was a commercial formulation (Vertimec 1.8% E.C) containing abamectin (18g/l) as an active component (CAS 71751-41-2) (Figure 1). The product is formulated by INDUSTRIALS AFRASA firm, Spain, and distributed by FMP AGRI, Algeria. *Moringa Oleifera* powdered leaves were obtained from BIOGATRANA (Gatrana Sidi Bouzid 9100, Tunisia), a company for Extracting Natural Products.

All reagents used for oxidative stress parameters, total polyphenolic contents and phenolic standards were purchased from Sigma–Aldrich (GmbH, Steinheim, Germany). Alcohol (HPLC grade) was purchased from Merck (Darmstadt, Germany). Ultrapure water was obtained from the Millipore system (Billerica, USA).

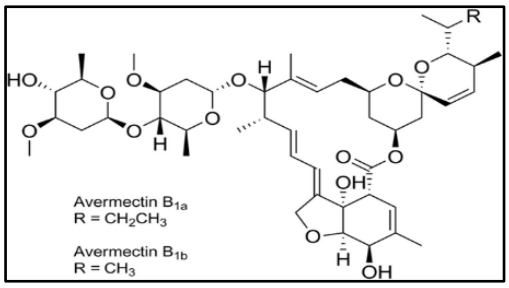

Fig.1. Chemical structure of abamectin

Total Antioxidant Activity

Spectrophotometric evaluation of Total Antioxidant Activity was determined by the technique described by Prieto *et al*. (1999) based on the formation of a green colored complex after the reduction of molybdate ions to molybdenum in the presence of the sample. Absorbance was recorded at 695nm. Butylated hydroxytoluene (BHT) was used as standard.

DPPH free radical scavenging assay

The procedure described by Kirby and Shmidt (1997) was adopted to evaluate the scavenging ability on DPPH radical. The result was represented as the necessary concentration (mg/ml) of extract to scavenge 50% of radicals (IC50).

ABTS reducing power assay

The capacity of MO aqueous extract to reduce ABTS radical was estimated following the protocol of Khan, *et al*. (2012) The sample's absorbance was determined at 734 nm. As previously explained for the DPPH test, the result was expressed as IC 50(mg/ml).

Ferric reducing antioxidant power assay (FRAP)

The protocol described by Pan, *et al*. (2008) was used to evaluate the ferric reducing antioxidant power.

Animals

The present work was carried out on twenty wistar rats, weighing approximately (180-200 g), obtained from Pasteur Institute (Algiers, Algeria). The animals were acclimatized for two weeks prior in normal environmental conditions (25 ± 3°C, 12-hour light/dark cycle), housed in plastic cages given access to standard rodent food and water ad libitum. The Ethical Committee of Directorate General approved all care procedures for Scientifique Research and Technological Development at Algerian Ministry PNR/SF 08/2012. Rats were divided into four equal groups and during 21 days: the healthy control group received drinking water by gavage. MOLE-treated group received *Moringa Oleifera* leave extract at a dose of 200 mg/kg bw orally by gavage (Albrahim and Binobead., 2018). ABM-treated group was given abamectin dissolved in drinking water considering the daily water consumption of animals 24 ml/ 200 g bw (Sahin et al., 2022), at a dose of 1mg /kg bw (Meligi and Hassan.,2017). The dose of ABM was adjusted weekly according to the average body weight of rats. ABM-MOLE group received a combination of abamectin and *Moringa Oleifera* with the same doses and in the same way as the second and third group. On the day following the final day of treatment, rats were sacrificed by cervical decapitation.

Blood collection

Blood samples were collected in tube without EDTA, centrifuged at 3000 rpm for 15 min at 4°C. The obtained serum samples were preserved at – 20°C, and served for the measurement of biochemical parameters aminotransferases (ASAT, ALAT), phosphatase alkaline (ALP), total and free bilirubin (TB, FBL), cholesterol and triglycerides.

Biochemical indices

The biochemical parameters were assayed using a biochemistry analyzer system (Erba Mannheim XL600) and the principle of each test was determined according to the ERBA XL-600 data sheet.

Liver homogenate preparation

Liver samples were quickly excised, rinsed with a 0.9 NACl solution and weighed. One gram of liver was homogenized in 2 mL of tris-buffered saline (TBS: 50 mm Tris, 150 mm NaCl, pH 7.4) in cold ice conditions, followed by a centrifugation of the cell suspension (9000×g for 15 min at 4 °C), the supernatants were divided into aliquots and then stored at -20 °C and served for the determination of MDA levels, GPx and GST activities.

Histological study

Following the approach of Hould (1984), liver fragments were washed with 0.9% NaCl, fixed in 10% formaldehyde, and then embedded in paraffin. The paraffin sections were cut into 5 µm slices and

stained with hematoxylin-eosin. Pictures were obtained using a light microscope (Leica Microsystems Schweiz AG, CH-9435 Heerbrugg) and photographed using a digital camera (Leica ICC50 W).

Lipid peroxidation assessment

Lipid peroxidation levels were estimated by measuring the formation of the substances reacting with thiobarbituric acid (TBARS) following the Buege and Aust (1978) protocol.

Measurement of reduced glutathione

GSH liver concentrations were measured by the method of Ellman (1959).

Assessment of antioxidant enzymes activities

Glutathione peroxidase (GPx) activity was measured according to the procedure of Flohe and Gunzler (1984). Glutathione-S-transferase activity was estimated following the method of Habig, *et al.*(1974) using 1- chloro-2,4-dinitrobenzene (CDNB).

Statistical analysis

Results are represented as mean ± standard error of the mean (S.E.M), one way ANOVA was used for the comparison between experimental groups. Sidak test for multiple comparisons was used to evaluate the significance with GraphPad prism version 7 (GraphPad Software, Inc., La Jolla, CA, USA). Differences were considered significant at a p-values <0.05.

Results

***In vitro* antioxidant activity**

Data concerning the total antioxidant capacity, the potential antioxidant of Moringa Oleifera aqueous extract on DPPH radical, ABTS radical and FRAP test are represented in **Table 1**.

Table 1 Antioxidant activity of *Moringa oleifera* leaves extract

Studied parameters	Values
50 % scavenging concentration (mg/ml) on DPPH radical	0.125± 0.009
50 % scavenging concentration (mg/ml) on $ABTS^{+}$ radical	0.06 ± 0.001
Total antioxidant Capacity (mg/ml)	0.201.46 ± 0.002
FRAP test (EC 50) (mg/ml)	1.04 ±0.01

n=3.Value are represented as (mean ± SEM)

Effect of treatment on body weight, absolute and relative weights

The obtained results show that there is no significant change in the body weight of rats in all groups, while a significant increase ($p < 0.05$) was recorded in the absolute and relative weights of the liver in rats treated with ABM compared to the control group. However, these two parameters were significantly restored when rats were treated by the combination MOLE+ABM (**Table 2**). No changes were recorded when MOLE group was compared to the control group.

Table 2. Effect of treatment on body weight, relative and absolute liver weights

Studied parameters	Control	MO	ABM	ABM-MO
Initial body weights (g)	180.40 ±8.46	189.6 ±4.29	197.33 ±7.77	190.4 ±7.2
Final body weights (g)	215 ±7.16	207.8 ±2.59	213 ±2.90	200.6 ±2.4
Absolute liver weights (g)	5.39 ±0.2	5.89± 0.3	6.63 ±0.2*	5.21 ±0.2$^{\#}$
Relative liver weights (g)	2.54 ± 0.09	2.71 ±0.08	3.14 ±0.1**	2.70 ±0.1$^{\#}$

Value are means ±S.E.M for groups of five rats each. Significant difference: MO, ABM groups are compared to control group (*p<0.05, **p<0.01), ABM-MO group compared to ABM group (#p< 0.05)

Biochemical indices

Data recorded after serum assays of biochemical parameters are represented in **Table 3**. The results indicate a major metabolic disturbance. The administration of ABM in rats resulted in a significant increase in TB and ASAT (p<0.001), in ALP, GGT and TG (p <0.01), as well as in CHL (p < 0.05) compared with control rats. However, the MOLE co-administration demonstrated a relative stability of these analyzed parameters with respect to, ASAT, ALP, triglyceride, cholesterol, unbound bilirubin.

Table 3. Changes in biochemical parameters levels in different groups

Studied parameters	Control	MO	ABM	ABM-MO
ALAT	21.6±1.02	19.8±1.4	52.8±2.2***	50.2±2.6
ASAT	86.4±3.5	94.6±3.3	171.4±16.2***	137.2±4.7$^{\#*}$
ALP	53± 8.8	67.2±3.2	126.6±6.6***	57±9.2$^{\#\# **}$
GGT	1.19±0.2	1.7±0.5	7±1.5**	4.2±0.6
Cholesterol	0.59±0.04	0.59±0.03	0.82±0.07*	0.58±0.05$^{\#}$
Triglycerides	0.57±0.05	0.62±0.1	1.07±0.07**	0.82±0.07$^{*\#}$
Total bilirubin	3.11±0.3	3.10±0.1	5.89±0.14***	5.21±0.4*
Free bilirubin	0.68±0.07	0.62±0.1	1.04±0.07*	0.63±0.05$^{\#}$

Value are means ±S.E.M for groups of five rats each. Significant difference: MO, ABM groups are compared to control group (*p<0.05, **p<0.01, ***p<0.001), ABM-MO group compared to ABM group ($^{\#}$p< 0.05, $^{\#\#}$p<0.01)

Effect of treatment on oxidative stress biomarkers

Oxidative stress assessment of the damaging effect of the free radicals generated by ABM was determined by the marker of lipid peroxidation (MDA), it was also evident by non-enzymatic antioxidants (GSH) and the enzymatic antioxidants (GPx and GST). Data are represented in **Fig.2**. ABM administration induced a significant decrease in the levels of the enzymatic (p < 0.01) and non-enzymatic antioxidants (p<0.001). While, the levels of MDA were seen to have increased significantly (p < 0.01)

compared to the control group. MOLE co-administration in ABM group significantly reduced the MDA ($p<0.01$) content in liver tissue homogenates. Also, the levels of GSH, GPx and GST significantly increased as compared to ABM-treated group ($p < 0.01$). The liver function parameters achieved by MOLE were equivalent to that control group.

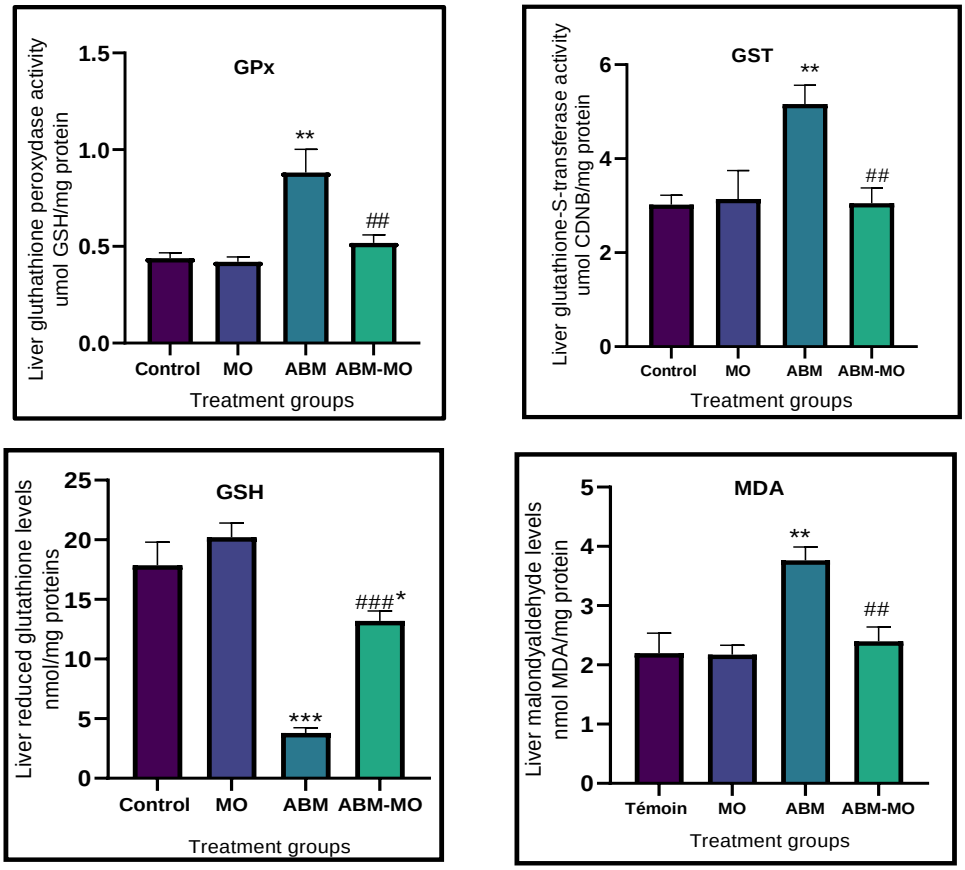

Fig.2. Effect of MOLE, ABM and their combination on liver oxidative stress biomarkers. Value are means ±Sem. MO, ABM groups are compared to control group ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$), ABM-MO group compared to ABM group. ($^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$)

Histopathological investigation

Results of histopathological examination are represented in **Fig.3**. Hepatic tissue sections of control rats (**Fig.3 A, B**) and MOLE- treated rats (**Fig.3 C**), showed normal hepatic architecture with uniformly stained hepatocyte and radiating central vein. Liver sections from ABM rats showed severe histological damage characterized by sinusoidal dilatation, degenerated hepatocyte, congested central vein and inflammatory process (**Fig.3 D, E**). In contrast, in the section of MOLE-ABM treated rat normal architecture of liver tissue was observed with a marked absence of inflammation compared to the control rats (**Fig.3 F, G**).

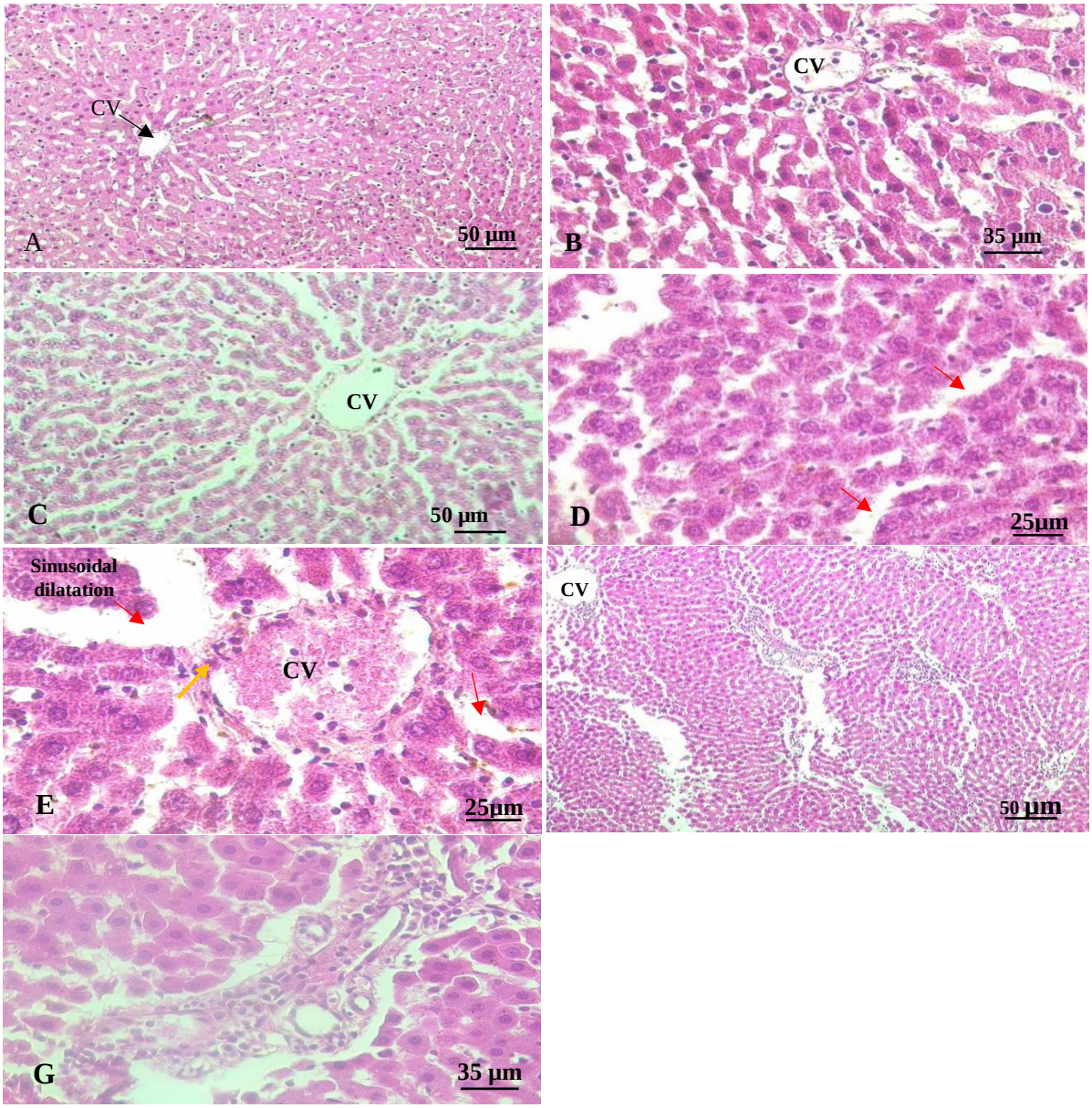

Fig. 3. Photomicrographs of histological section of liver tissues stained by hematoxilin and eosin (X 400). (A, B) the control rats showing normal hepatic architecture. (C) MOLE-group showed normal appearance of hepatic tissues. (D, E) ABM-treated group showing : sinusoidal dilatation (yellow arrow), congested central vein and inflamatory area (E, red arrow). CV: central vein.

Discussion

The current work research was carried out to examine the antioxidant proprieties *Moringa oleifera* leaves aqueous extract (MOLE) *in vitro* as well as, to evaluate its therapeutic effects on hepatic toxicity and oxidative damages induced by abamectin to wistar rats.

Results of the DPPH, ABTS and FRAP assays showed a notable antioxidant action of MOLE, which may be related to its various bioactive components characterized by their ability to scavenge free radicals through their proton or electron-donating properties (Reda et al., 2023).Moringa oleifera contain high levels of antioxidants such as , chlorogenic acid, gallic acid, quercetin, f p-coumaric acid, ferulic acid, and sinapic acid Khalid, et al. (2023)

Our results showed that oral administration of abamectin at a dose of 1mg/kg BW for 21 consecutive days did not induce any notable changes in rat body weight, which is in agreement with the findings of Kolianchuk *et al*. (2023), who reported that abamectin does not affect body weight in rats treated with different doses of abamectin (0.1, 1, and 2 mg/kg bw). However Madkour, *et al*. (2020) reported that emamectin benzoate, an avermectin insecticide, caused a significant decrease in rat body weight. They explained that this could be related to the reduction in food intake or to the toxic effect of the pesticide leading to the degradation of lipids and proteins.

Changes in the relative and absolute weights of an organ after drug or chemical administration reflect the toxic action of the ingested product (Hamed and Abdelrazik 2015). Our findings revealed a significant increase in both absolute and relative liver weights in ABM-treated rats compared to the healthy control rats, which may be due to the accumulation of abnormally high levels of fat, mostly triglycerides, in the parenchymal cells (Hamed and Abdelrazik 2015). On the other hand, MOLE supplementation significantly attenuates the toxic effect of ABM on liver absolute and relative weights, which is consistent with the results reported by Abdelrassoul *et al*. (2018). The observed effect of MOLE may be attributed to its contents of numerous antioxidative moieties (Setiawan et al., 2022).

Our results clearly demonstrate that abamectin induces a disturbance in lipid profile, as evidenced by a significant increase in serum total cholesterol and triglyceride concentrations. Consistent with our findings, Hamed, et al. (Hamed and Abdelrazik 2015) reported the ability of abamectin to affect liver metabolism in a way that increases lipid synthesis. They demonstrated that after sublethal oral administration of abamectin at 1/3 DL50 for 4, 24, and 336 hours, rats exhibited a significant elevation in cholesterol and triglyceride levels. In toxicology studies, mild to moderate increases or decreases in serum cholesterol and/or triglyceride concentrations are rather common, and the precise mechanisms underlying these changes are frequently unclear(Dong *et al*., 2020). Tests for liver function, including serum activities of AST and ALT, are crucial in assessing the effects of pesticide exposure on liver physiology. An increase in the activity and levels of these enzymes reflects dysfunction of liver tissues and hepatocyte injuries (Khaldoun-Oularbi et al., 2013; Berrouague *et al.*, 2019). In line with the

research of Radi *et al*. (2020), Khaldoun-Oularbi *et al*. (2013), and Abdel Daim, *et al*.(2018), the current results showed that ABM caused a significant elevation in AST, ALT, GGT, and alkaline phosphatase (ALP) levels compared to the control group. This may signify that ABM increased membrane permeability and led to blood leakage of intracellular enzymes from altered hepatocytes into the blood circulation (Radi et al., 2020).

Bilirubin is the final product of hemoglobin catabolism, and the liver's health status can be assessed by measuring bilirubin levels.

Our study revealed that ABM exposure caused a significant increase in total and free bilirubin concentrations, which may result from reduced liver uptake, conjugation, or increased bilirubin secretion (Bouasla *et al*., 2014). The rise in bilirubin levels may also be attributed to the accumulation of free radicals after exposure to abamectin. Indeed, a previous study reported the antioxidant effect of bilirubin and its capacity to scavenge free radicals. They noted that an elevation in bilirubin levels can be regarded as a natural antioxidant defense mechanism against reactive oxygen species (Hamed and Abdelrazik 2015). These findings were accompanied by histological alterations observed via sinusoidal dilatation, inflammatory infiltration, congested and central vein in ABM exposed rats which corroborate the study of Abdel-Daim and Abdellatief (2018). Also, Magdy *et al*.(2016) indicated that ABM is capable of inducing edema and dilatation of Disse's space. Pesticides may initiate inflammation in liver tissue, leading to the recruitment of mononuclear cells to the site of injury to clear damaged tissues (Essa *et al*., 2020). ABM cause apoptosis and increse TNFα levels (Radi et al., 2020). These affirmations may explain the observed inflammatory area and the altered hepatocytes in this study. Although several experiments have reported that ABM is rapidly excreted in mammals (Aioub *et al*., 2022), Howells et al. (2001) demonstrated the accumulation of ABM in bovine liver tissues. Additionally, a study conducted on female rats and their pups concluded that ABM accumulates in mothers' milk and can be transferred, via breastfeeding, to their male and female pups, causing biochemical and histopathological alterations accompanied with oxidative stress in the livers of both mothers and their suckling pups (Mossa *et al*., 2017). In our study, the recorded histopathological damages can be linkend to the accumulation of ABM in the liver leading to ROS generation (Khaldoun-Oularbi *et al.*, 2013).

Interestingly, treatment with MOLE ameliorated the histopathological changes caused by ABM and diminished the disruptive effect of ABM on liver function in the ABM-MOLE group by improving AST, GGT, ALP, FBL, and cholesterol levels. This might be the result of hepatocyte membrane stability and minimized enzymatic leakage, indicating the potential hepatoprotective effect of MOLE (Toppo *et al*., 2015).

Our results are consistent with those of Abdel Fattah *et al*.(2020) , who demonstrated that *Moringa oleifera* administration at a dose of 200 mg/kg for four weeks restored hepatic damage in rats treated with lead. Moreover, a study conducted by Reda, *et al.* (2020) on Oreochromis niloticus fishes highlighted the toxic effect of abamectin in the aquatic environment and demonstrated the protective effects of *Moringa oleifera* leaf ethanolic extract on hepatocytes by reducing serum concentrations of

AST, ALT, and albumin. Another report indicated that the hepatoprotective effect of *Moringa* could be related to its bioactive components, including alkaloids, quercetin, kaempferol, flavonoids, ascorbic acid, and benzyl glucosinolates (Pareek *et al*., 2023).

As the first line of defense against oxidative stress, antioxidant enzymes are responsible for preventing macromolecules from oxidative damage by scavenging and eliminating free radicals (Klibet *et al*.,2016). Our results showed that abamectin significantly altered the liver antioxidant system by reducing GSH levels, GPx and GST activities, and promoting lipid peroxidation through the increment in MDA levels. Indeed, depletion in antioxidant enzyme activities is linked to an aggravated oxidative stress situation (Rouag *et al*., 2020). Thus, the obtained results reflect the toxic effect of abamectin and its implication in triggering oxidative damages (Aioub *et al*., 2022).

The findings of the present study are in agreement with those reported by Radi, et *al*., (2020), who found that abamectin caused liver and brain oxidative stress by decreasing GSH levels, increasing MDA concentrations, and upregulating the expression of drug detoxifying genes in rats. In the same vein, Zhu, *et al*.(2013) stated the toxic effect of avermectin and affirmed that the mechanism of injury induced by avermectin is linked to apoptosis and oxidative stress. Moreover, a study conducted on isolated rat hepatocytes recorded that abamectin caused oxidative stress and changes in antioxidant enzyme activities (Al-Sarar *et al*., 2015). Hepatic GSH serves multiple functions as a redox partner, an electrophile, and a radical scavenger. Additionally, GSH may be utilized by a number of drug-metabolizing enzymes (such as GSTs) or act as a redox partner for antioxidant enzymes (such as GPx) (Amraoui *et al* ., 2018). It has been shown that ROS-mediated toxicity results from the oxidation of GSH to GSSG after pesticide exposure, which may explain the decline in GSH levels. In the same connection, Radi *et al*. (2020) reported that a decrease in GSH concentration resulted in H2O2 accumulation, cell damage, and lipid peroxidation. In fact, when toxic products are metabolized, more toxic substances are generated with an overproduction of (Danielson 2002), The latter triggers the Fenton's reaction through the degradation of the CYP hemeprotein and the liberation of iron, leading to lipid peroxidation (Caro and cederbaum 2004). These affirmations could explain the observed high levels of MDA in liver tissues in this study. GST represents an essential enzyme for tissue defense against oxidative stress, it catalyzes the conjugation of GSH to electrophilic xenobiotics, converting a reactive lipophilic compound to a non-reactive water-soluble molecule. Thus, the observed decline in GST activity may be linked to the decrease in the availability of GSH (Ahmed and Reda 2004)). The reduced activity of antioxidant enzymes GPx and GST following treatment with ABM may reflect an abnormal production of ROS and the incapacity of the antioxidant machinery to eliminate these molecules. Due to their ability to oxidize proteins, lipids, and even DNA, reactive oxygen species are considered to have the potential to cause tissue damage (Chouala *et al.*, 2024) . Such affirmation may support the histological alterations observed in liver tissue after ABM administration.

On the other hand, concomitant treatment with MOLE resulted in a significant amelioration in GPx and GST activities, as well as GSH levels. Moreover, MOLE has been proven to reduce MDA levels. This

result could be attributed to the antioxidant potential of *Moringa oleifera* leaves, known to contain a rich variety and high concentration of phenolic compounds (Cheng *et al*., 2017). In fact, Cheng *et a*l. (2017) reported that polyphenols are able to scavenge free radicals by donating a hydrogen atom or an electron to inhibit free radicals' generation or to deactivate their precursors. Additionally, polyphenols act as metal chelators, chelating metal transitions like Fe2+ and directly lowering the rate of the Fenton reaction, thus blocking oxidation brought on by extremely reactive hydroxyl radicals (•OH). Furthermore, these molecules have the ability to halt the amplification of lipid peroxidation (Chouala *et al*., 2024). Similar to our findings, previous studies have demonstrated the hepatoprotective effects of *Moringa oleifera* on several contaminants including lead (Abdel Fattah *et al*., 2020), chlorpyrifos (Ibrahim et al., 2019), cadmium (Toppo et al.,2015), and pendimethalin (Sharma and Paliwal 2012). In line with the obtained results of biochemical trials, here, the histological examination of liver sections proved the efficacy of MOLE when the observed hepatic lesions induced by abamectin were reduced after treatment with MOLE, Furthermore, these results correlate positively with the strong antioxidant capacity of MOLE observed *in vitro*.

Conclusion

The results of the current study revealed and confirmed the hepatotoxicity of abamectin in rats, leading to increased lipid peroxidation, as well as reduced antioxidant enzyme activity and GSH levels, thereby impairing the hepatic antioxidant system. These findings highlight the potential of *Moringa oleifera* as a natural detoxifying agent capable of alleviating the toxic effects of abamectin on the liver. Phytochemical analysis indicated the beneficial effects of MOLE and its ability to scavenge free radicals.

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Article 3

Antioxidant Effects of *Moringa oleifera* Against Abamectin-Induced Oxidative Stress in the Brain and Erythrocytes of Rats

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RESEARCH ARTICLE

Antioxidant Effects of *Moringa oleifera* Against Abamectin-Induced Oxidative Stress in the Brain and Erythrocytes of Rats

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Keywords: abamectin | antioxidants | brain | erythrocytes | HPLC | *M. oleifera*, oxidative stress | pharmacokinetics

ABSTRACT

The current study was conducted to explore the phytochemical composition and in vitro antioxidant activity of *Moringa oleifera* leaves aqueous extract (MOLE), as well as its in vivo modulatory effects on abamectin (ABM)-induced oxidative stress in rat erythrocytes and brain tissue. Following extraction, the total phenolic, flavonoid, condensed tannin and *ortho*-diphenolic contents of MOLE were determined. High-performance liquid chromatography (HPLC) analysis allowed the identification and the quantification of 12 bioactive compounds: gallic acid, chlorogenic acid, caffeic acid, vanillic acid, quercetin, ferulic acid, ascorbic acid, alizarin, hesperidin, neohesperidin, resveratrol, and naringin. In vitro study: the assessment of the antioxidant activity of MOLE on the 2,2-diphenyl-1-picrylhydrazyl radical DPPH and the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS), its ferric reducing power and its antioxidant effect on the β-carotene bleaching indicated that MOLE exhibited potent antioxidant activity, with an IC_{50} of 0.125 mg/mL against DPPH radical, and an IC_{50} of 0.06 mg/mL against ABTS radical. It also demonstrated notable ferric-reducing ability, with an EC_{50} of 1.4 mg/mL and a strong inhibition of β-carotene bleaching with an IC_{50} of 1.36 mg/mL. In vivo study: Twenty rats were equally divided into four groups. The first group served as a control and received distilled water by gavage. The second group (negative control) received ABM in drinking water at a dose of 1 mg/kg body weight. The third group received MOLE at a dose of 200 mg/kg of body weight by gavage. The fourth group received a combination of ABM and MOLE in the same manner and doses as described, for 3 weeks. Body weight, brain relative and absolute weights, and nitric oxide levels were not affected by ABM. However, ABM significantly inhibited acetylcholinesterase (AChE) activity ($p < 0.001$), decreased the activities of antioxidant enzymes, specifically superoxide dismutase (SOD) and glutathione S-transferase (GST) in cerebral tissue, and catalase (CAT) in erythrocytes ($p < 0.001$). ABM also decreased reduced glutathione (GSH) levels in both the brain ($p < 0.001$) and erythrocytes ($p < 0.05$). In addition, malondialdehyde (MDA) levels significantly increased in the brains of ABM-intoxicated rats ($p < 0.01$) compared to the control group. These results were accompanied by histopathological changes, notably the remarkable vacuolization of neuropil in brain tissue. Supplementation with MOLE in ABM-treated rats

significantly ameliorated brain AChE ($p < 0.05$) and GST activities, decreased MDA content, and improved GSH levels in both brain and erythrocyte homogenates ($p < 0.01$). MOLE also restored the histopathological alterations observed in the ABM group. Computational modeling revealed that some of the tested molecules, including some present in the studied extract, bound human peroxiredoxin 5, CAT, and glutathione peroxidase with acceptable affinities, which, together with the established molecular interactions and tight embedding satisfactory support the in vivo results. Thus, it may be concluded that ABM impairs brain and erythrocyte function through oxidative damage, and these effects could be prevented by MOLE, likely due to its antioxidant activity.

1 | Introduction

The use of pesticides in agriculture has contributed to boosting global food production. However, the growing dependence on pesticides to enhance agricultural output poses an increasing risk. Consequently, concerns have emerged about potential environmental contamination and the impact on nontarget organisms. Abamecin, a widely used insecticide around the world [1], is also one of the most prevalent pesticides in Algeria [2]. It consists of a blend of avermectins B1a (80%) and B1b (20%), both of which belong to the macrocyclic lactone family. Abamectin (ABM) is used in agriculture for its potent acaricidal, insecticidal, and nematicidal effects [3] and is commonly applied to treat parasitic diseases in animals [4]. As a neurotoxin, ABM acts by promoting cell membrane hyperpolarization through binding to glutamate or γ-amino butyric acid (GABA) receptors, increasing the influx of chloride ions. This inhibits neurotransmission, causing paralysis in target organisms [5]. Previous studies have reported the adverse effects of this pesticide on nontarget organisms. ABM may affect the respiratory system [6], the nervous system, and hepatic and renal functions. Moreover, ABM has been shown to induce testicular damage by provoking inflammation, oxidative stress, and apoptosis [7]. ABM also negatively impacts the health and vitality of honeybee colonies [8]. A study conducted by Chen et al. [9] on honeybees demonstrated the toxicity of ABM and its ability to significantly alter the expression of four genes (abaecin, cyp9e2, cyp302a1, and GstD1) linked to immune response and detoxification metabolism. Furthermore, Refes et al. [10] reported that acute inhalation exposure to ABM caused changes in body weight, altered hepatic function, and induced neurobehavioral complications in rats.

Around 80% of the global population relies entirely on plants for health and healing, and 25% of integrated pharmaceuticals are derived from medicinal and aromatic plants [11]. Native to India, *Moringa oleifera* is a plant from the Moringaceae family, renowned for its nutritional and medicinal properties. The effects *of M. oleifera*'s natural products have been extensively studied in various in vivo and in vitro models and have been the subject of substantial recent research [12]. *M. oleifera* has been reported to have antidiabetic, anti-inflammatory [13], and anticonstipation effects. Moreover, the plant's antiparasitic, neuroprotective, cerebroprotective [14], and antiproliferative properties against cancer have been recently confirmed [15]. Incorporating *M. oleifera* leaf powder into cookies or as an herbal drink has been shown to enhance the glycemic index, reduce blood sugar levels, improve appetite and digestive health, and lower diastolic and systolic blood pressure in models with high salt intake [12]. Different parts of the plant, including the seeds, roots, fruits, and leaves, contain bioactive molecules exhibiting a variety of beneficial properties, such as functioning as metabolic regulators, antioxidants, and antimicrobials [16]. Numerous phytochemicals, including phenolics, flavonoids, glycosides, alkaloids, sterols, carotenoids, vitamins, amino acids, and minerals, are believed to be present in *M. oleifera* leaves. Moreover, it has been demonstrated that flavonoids like kaempferol and quercetin are more potent antioxidants than conventional vitamins [17].

The goal of the present study is to explore the impact of ABM exposure on the antioxidant profile in rat brains and erythrocytes and to evaluate the protective capacity of *M. oleifera* leaves aqueous extract against ABM toxicity. Furthermore, binding affinities of some phytochemicals and their established molecular interactions with human peroxiredoxin 5 (PRDX5; 1H2D), catalase (CAT; 1QQW), and glutathione peroxidase (GPx; 1GP1) have been assessed by computational approach.

2 | Results

2.1 | Phytochemical Composition and Antioxidant Activities

The total phenols, total flavonoids, condensed tannins, and *ortho*-diphenol contents in MOLE are presented in Table 1. The results of its antioxidant activity (total antioxidant capacity, DPPH test, ABTS test, and ferric-reducing power [FRAP] test, β-carotene bleaching test) are presented in Table 2.

2.2 | High-Performance Liquid Chromatography Analysis of Antioxidant Constituent Present in *M. oleifera* Aqueous Extract

Twelve phenolic compounds, including gallic acid (110.01 µg/g), chlorogenic acid (13.320 µg/g), vanillic acid (134.66 µg/g), caffeic acid (52.18 µg/g), ferulic acid (1445.12 µg/g), alizarin (5416.8 µg/g),

TABLE 1 | Antioxidant content of *Moringa oleifera* leaves aqueous extract.

Studied parameters	Values
Total phenols (mg AGE/g E)	38.59 ± 1.6
Total flavonoids (mg CE/g E)	95.99 ± 5.16
Tannins (mg CE/g E)	0.201 ± 0.01
Ortho-diphenols (µg CAE/g E)	71.03 ± 0.5

Note: Values are presented as mean ± SEM ($n = 3$)

Abbreviations: CAE, cafeic acid equivalent; CE, catechin equivalent; GAE, gallic acid equivalent; gE gram extract; RE, rutin equivalents.

TABLE 2 | Antioxidant activity of *Moringa oleifera* leaves aqueous extract.

Antioxidant test	Parameter	Value (mg/mL)
DPPH test	IC50	0.125 ± 0.009
ABTS test	IC50	0.06 ± 0.001
Total antioxidant capacity	EC50	0.201 ± 0.002
FRAP test	EC50	1.04 ± 0.01
β-Carotene bleaching assay	IC50	1.36 ± 0.03

Note: $n = 3$. Values are represented as mean ± SEM.

TABLE 3 | Major phenolic and flavonoid compounds in *Moringa oleifera* leaf aqueous extract estimated by HPLC.

S/No.	Name of compound	Molecular weight (g/mol)	Molecular formula	Retention time (min)	Peak area (AU)	Concentration (µg/g extract)
1	Gallic acid	170.12	$C_7H_6O_5$	5.47	30 079	13.22
2	Ascorbibc acid	176.12	$C_6H_8O_6$	3.37	129 621	802.96
3	Chlorogenic acid	354.31	$C_{16}H_{18}O_9$	13.32	107 615	993.44
4	Vanillic acid	168.15	$C_8H_8O_4$	15.47	43 817	134.66
5	Caffeic acid	180.16	$C_9H_8O_4$	16.18	21 934	52.18
6	Ferulic acid	194.19	$C_0H_{10}O_4$	20.22	46 251	1445,12
7	Vanillin	152.15	$C_8H_8O_3$	21.46	19 900	—
8	*p*-Coumaric acid	164.16	$C_9H_8O_3$	23.817	—	—
9	Rutin	302.27	$C_{15}H_{14}O_6$	28.37	—	—
10	Neohesperidin	610.56	$C_{27}H_{34}O_{16}$	29.58	2898	1582.78
11	Resveratrol	228.24	$C_{14}H_{12}O_3$	32.4	44 763	0.048
12	Naringin	580.53	$C_{27}H_{30}O_{16}$	34.64	397 788	4105.35
13	Hesperidin	610.58	$C_{75}H_{60}O_{16}$	36.73	1227	0.050
14	Alizarin	240.21	$C_{14}H_8O_4$	38.66	52 834	5416.8
15	Flavone	252.26	$C_{16}H_{12}O_2$	40.76	—	—
16	Quercetin	302.24	$C_{15}H_{10}O_7$	45.08	119 336	45.05

hesperidin (0.05 µg/g), neohesperidin (1582.78 µg/g), resveratrol (0.048 µg/g), ascorbic acid (802.96 µg/g), naringin (1405.35 µg/g), and quercetin, were identified in the MOLE by high-performance liquid chromatography (HPLC) analysis out of the 16 tested standards (Table 3). The compounds' chromatogram is shown in (Figure 1).

2.3 | Effect of Treatment on Body Weight, Absolute and Relative Weights

The results show no significant change in the body weight of rats in absolute and relative brain weights in all groups (Table 4).

2.4 | Oxidative Stress Parameters

Figures 2 and 3 illustrate the impact of ABM administration on AChE levels and oxidative stress parameters in both the brain (Figure 2) and erythrocytes (Figure 3). ABM significantly inhibited AChE activity ($p < 0.001$) and decreased the activities of antioxidant enzymes, specifically superoxide dismutase (SOD) and glutathione S-transferase (GST) in cerebral tissue, and CAT in erythrocytes ($p < 0.001$), and reduced glutathione (GSH) levels in both the brain ($p < 0.001$) and erythrocytes ($p < 0.01$). In addition, the malondialdehyde (MDA) levels significantly increased in the brains of ABM-intoxicated rats ($p < 0.01$) compared to the control group. However, coadministration of MOLE with ABM significantly restored AChE and GST activities, GSH levels, and reduced MDA content in brain and erythrocytes homogenates ($p < 0.01$). Regarding NO levels, no significant changes were observed in any of the groups.

2.5 | Histopathological Examination of Brain Tissue

The results of histopathological examination are represented in Figure 4. Sections of brain tissue stained by hematoxylin and

TABLE 4 | Effect of treatment on body weight, absolute and relative brain weights.

Parameters studied	Control	MOLE	ABM	ABM-MOLE
Initial body weights (g)	180 ± 8	190 ± 4	197 ± 8	190 ± 7
Final body weights (g)	215 ± 7	208 ± 3	213 ± 3	201 ± 2
Brain absolute weights (g)	1.3 ± 0.1	1.5 ± 0.1	1.5 ± 0.04	1.3 ± 0.1
Brain relative weights (g/100 g bw)	0.6 ± 0.06	0.7 ± 0.07	0.7 ± 0.01	0.7 ± 0.1

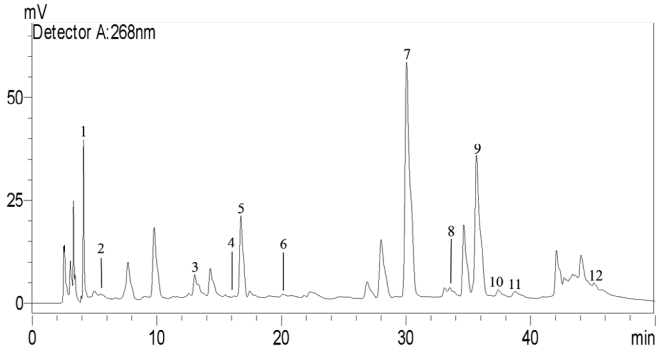

FIGURE 1 | HPLC chromatogram of identified contents detected in aqueous extract of *Moringa oleifera*. (1) Ascorbic acid; (2) gallic acid; (3) chlorogenic acid; (4) vanillic acid; (5) caffeic acid; (6) ferullic acid; (7) neohesperidin; (8) resveratrol; (9) naringin; (10) hesperidin; (11) alizarin; (12) quercetin.

eosin (H&E). (A) The control rats showed normal histological architecture. (B) The MOLE group showed a normal histological appearance. (C, D) The ABM-treated group showing a remarkable reduction in cell number with neuropil vacuolization (arrow). (E) The ABM-MOLE group showed nearly normalized histological structure with decreased neuropil vacuolization.

2.6 | In Silico Computational Findings

The binding affinity and root mean square deviation (RMSD) of nine antioxidant compounds with the three targeted receptors—PRDX5 (1H2D), CAT (1QQW), and GPx (1GP1)—are summarized in Table 4. All compounds exhibited negative binding affinities, with values of −8.5, −8.9, and −6.9 kcal/mol for 1H2D, 1QQW, and 1GP1, respectively. Among these, naringin displayed the lowest binding affinity compared to the other compounds (Table 5; Figure 5). It formed six or seven conventional hydrogen bonds, which were associated with carbon–hydrogen bonds, pi–cation interactions, pi–alkyl interactions, and pi–sigma networks, all contributing to the stability of the complexes (Figure 6).The chlorogenic acid–1HD2 complex exhibited the highest number of conventional hydrogen bonds (n = 9), with several closely interacting residues, including Glu16, Lys63, Val70, Arg86, Gly92, Val94, Arg95, Leu96, and Gln68. Notably, Gln68 was the closest interacting residue, with a distance of only 2.093 Å. The quercetin–glutathione peroxidase complex demonstrated the lowest embedding distance of 1.933 Å, indicating a strong interaction between the compound and the receptor.

3 | Discussion

Numerous environmental pollutants exert various types of stress on ecosystems, potentially harming organisms. Medicinal plants have recently gained considerable interest due to their rich chemical composition, including phenolic and flavonoid compounds, which exhibit antibacterial, anticancer, anti-inflammatory, antioxidant, and immunostimulant properties [18]. To the best of our knowledge, this study is the first to demonstrate the antioxidant effects of M. oleifera leaves against ABM-induced oxidative stress in the brain and erythrocytes of rats, supported by an in silico study revealing its underlying mechanism of action.

Phytochemical analysis revealed significant concentrations of phenols, flavonoids, tannins, and *ortho*-diphenols in the aqueous extract of M. oleifera leaves. HPLC analysis identified several bioactive compounds, including ascorbic acid, gallic acid, chlorogenic acid, vanillic acid, caffeic acid, ferulic acid, alizarin, hesperidin, neohesperidin, resveratrol, quercetin, and naringin in MOLE. These findings are consistent with previous studies reporting the richness of M. oleifera leaves in active constituents with antioxidant properties [11]. M. oleifera is known to contain antioxidants that scavenge free radicals [19]. In this study, the antioxidant activity of M. oleifera against DPPH and ABTS radicals, along with its potent ferric-reducing power, were demonstrated. Furthermore, M. oleifera exhibited strong anti-β-carotene bleaching activity (IC_{50} = 1.36 mg/mL), a value comparable to that of vitamin C (IC_{50} = 1.28 mg/mL). This suggests that M. oleifera may possess higher antioxidant activity than ascorbic acid and other common antioxidants [20]. Similarly, Laoung-On et al. [21] reported that tea made from M. oleifera leaves scavenged ABTS radicals more effectively than gallic acid. In addition, the present study revealed that M. oleifera leaves contain significant amounts of alizarin, a compound known for its potential to protect proteins from damage induced by hydroperoxyl radicals [22].

Body weight, as well as the absolute and relative weights of organs, are primary indicators of toxicity [23]. In this study, body weight and brain absolute and relative weights were measured in rats. No significant changes were observed in any of the experimental groups compared to the control. In line with our findings, Aggarwal et al. [24] reported that exposure to ABM does not affect rats' body weight. Conversely, Abdelrasoul [25] found that exposure to ABM at a dose of 0.5 mg/kg resulted in changes in body, liver, kidney, and brain weights in rats, suggesting that these alterations are linked to the toxicity of ABM.

Erythrocytes are primarily recognized for their role in transporting metabolic gases and nutrients to tissues. However, it

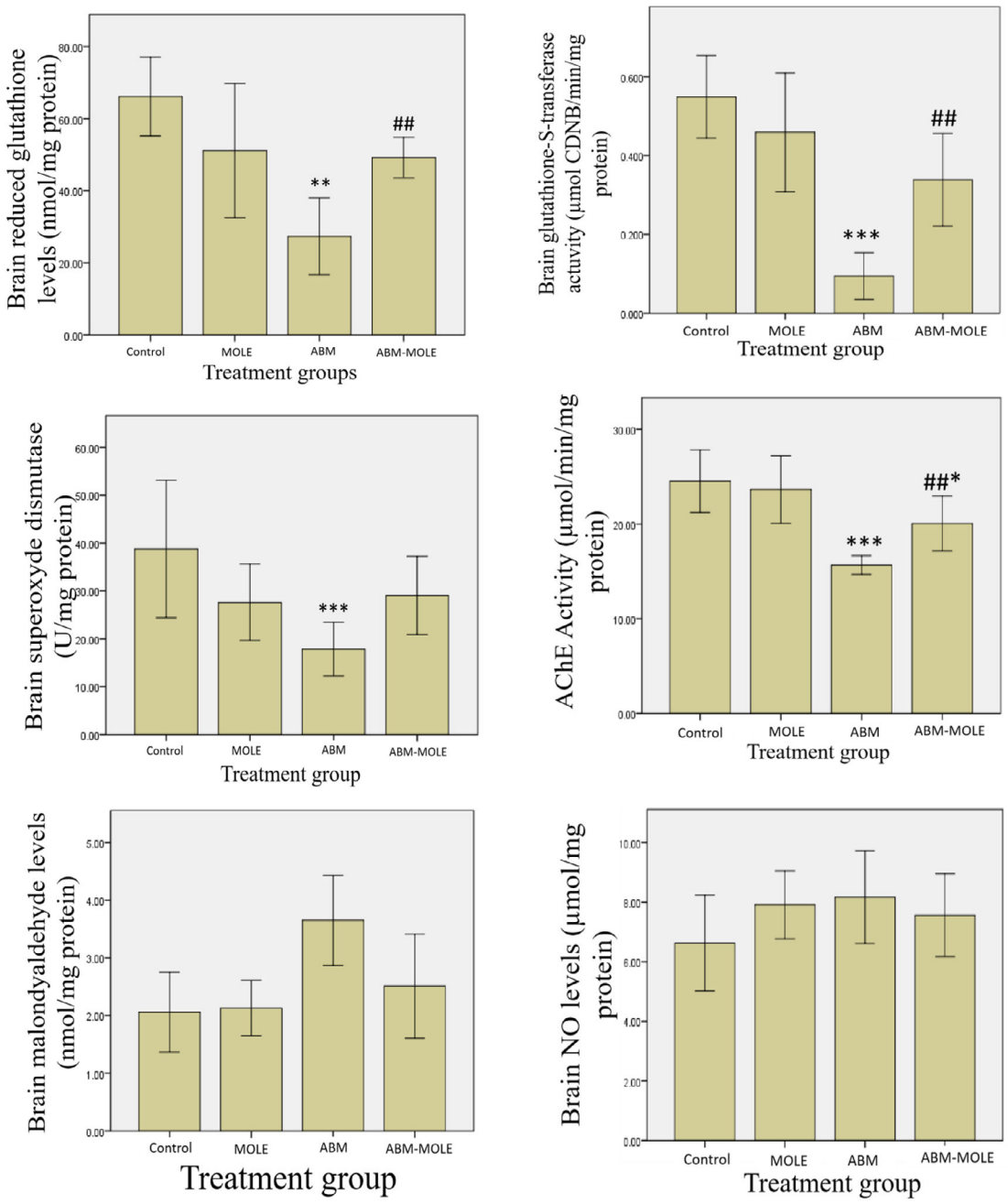

FIGURE 2 | Preventive effects of MOLE against abamectin on antioxidant enzyme activities, GSH, MDA, and NO levels in the brain of rats. Each bar represents mean ± SD (significance at $p < 0.05$). MOLE, ABM groups are compared to control groups ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$), ABM-MOLE group compared to ABM group ($^{\#\#}p < 0.01$).

has become clear over time that red blood cells also perform significant biological functions. They possess a highly specialized and organized cell membrane that interacts with inflammatory mediators and oxidative agents, leading to structural and functional alterations that quickly signal abnormal conditions [26]. Due to its high levels of polyunsaturated fatty acids and significant oxygen consumption, the nervous system is particularly vulnerable to oxidative stress [27]. Oxidative stress plays a crucial role in ABM-induced toxicity [28].

GSTs are key enzymes with multiple functions, playing a crucial role in the detoxification pathway by catalyzing the initial step in forming water-soluble end products, such as mercapturic acids. These enzymes metabolize various electrophilic compounds through conjugation with glutathione [29]. GSH is vital for detoxifying reactive oxygen species (ROS) in brain cells. Recent research has demonstrated that, in addition to its intracellular roles, GSH also performs significant extracellular functions within the brain [30].

In the current study, the reduction in GSH levels may be attributed to ABM's role in upregulating drug-detoxifying genes [28]. A compromised GSH system in the brain has been linked to oxidative stress observed in various neurological disorders,

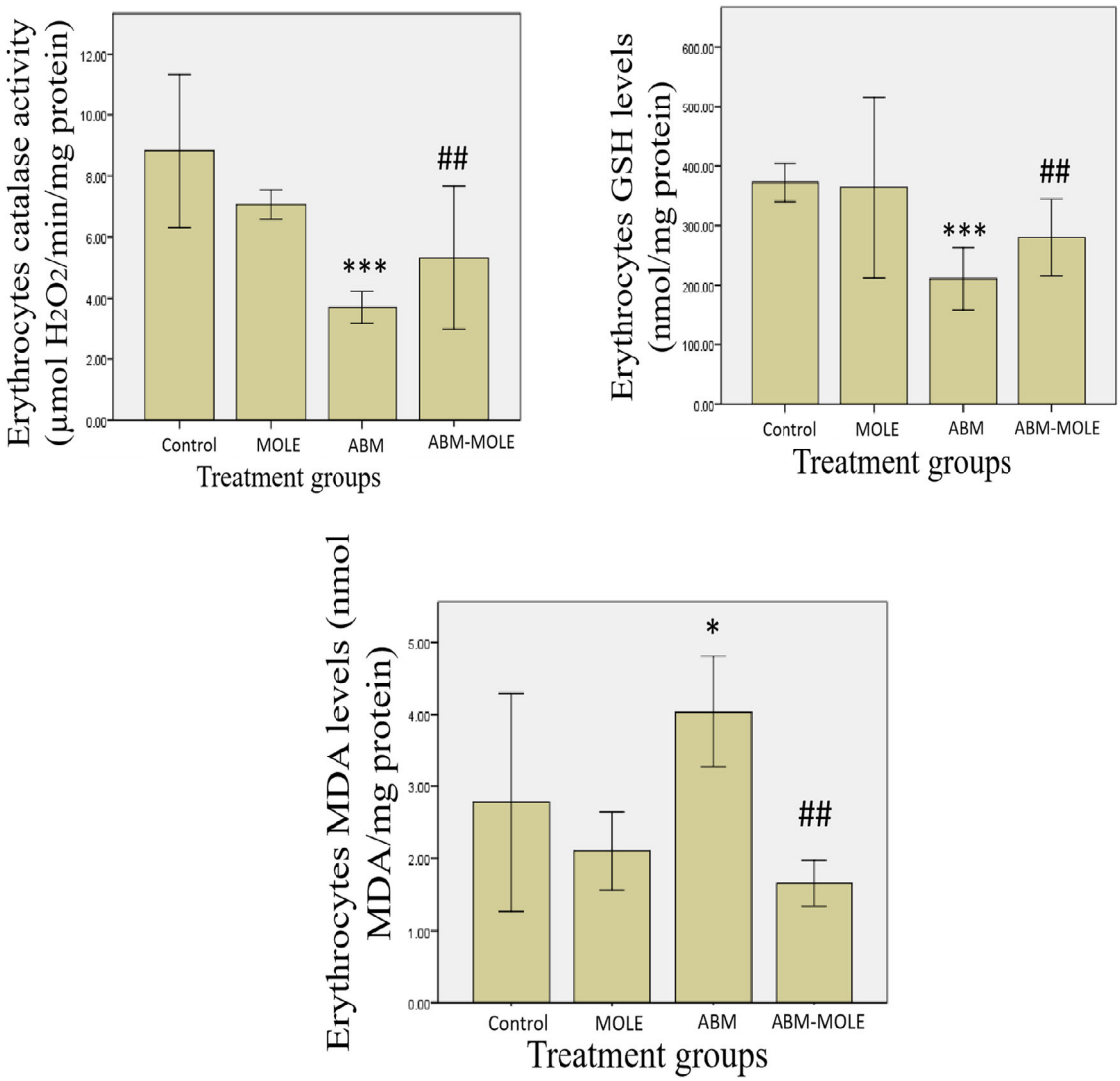

FIGURE 3 | ABM toxicity and the preventive effects of MOLE on CAT activity, GSH, and MDA levels in rat erythrocytes. Each bar represents mean ± SD (significance at $p < 0.05$). MOLE, ABM groups are compared to control groups (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$), ABM-MOLE group compared to ABM group ($^{\#\#}p < 0.01$).

including lipid peroxidation, DNA denaturation, and protein oxidation [30]. This could explain the increased MDA levels and the decreased GST activity in brain tissue.

ROS and reactive nitrogen species serve as an effective defense mechanism against stressors, yet their prolonged presence can damage host cells. To counteract this, protective antioxidant enzymes function to neutralize these harmful molecules. Consequently, the decline in antioxidant enzyme activities observed in this study may result from an overproduction of ROS and reactive nitrogen species, which is associated with the reduction in GSH levels and the disruption of the antioxidant system following ABM administration. In line with our findings, a study by Turkish researchers on the toxicological impact of five avermectins, including ABM, on GST enzymes isolated from human erythrocytes revealed that the tested compounds inhibited GST activity at millimolar concentrations [29]. Similarly, Radi et al. [28] reported that ABM induced oxidative stress by decreasing GSH and CAT levels while increasing MDA concentrations in both the brain and liver of rats.

Acetylcholinesterase is a crucial enzyme in the nervous system responsible for terminating nerve impulses by catalyzing the hydrolysis of the neurotransmitter acetylcholine. As a specific molecular target for organophosphate and carbamate pesticides, AChE activity and its inhibition are well-established early indicators of pesticide poisoning in humans [31]. In our study, the reduction in AChE activity following ABM exposure corresponds with the findings of Hashim, Abou-Beih, and Gbilily [32], who reported a decrease in serum acetylcholinesterase in rats exposed to ABM for 45 days. In addition, Zhang, Dong, and Liu [33] demonstrated that avermectins induce neurotoxicity in carp by reducing AChE activity.

The observed alteration in AChE activity in this study may be linked to the neurotoxic effects of ABM, confirming its ability to induce oxidative stress [28]. This oxidative stress leads to the damage of macromolecules, including enzymes [34]. Moreover, avermectins have been shown to alter cellular signaling pathways, triggering inflammation through NF-κB activation and promoting apoptosis by inhibiting the PI3K/Akt signaling pathway [33].

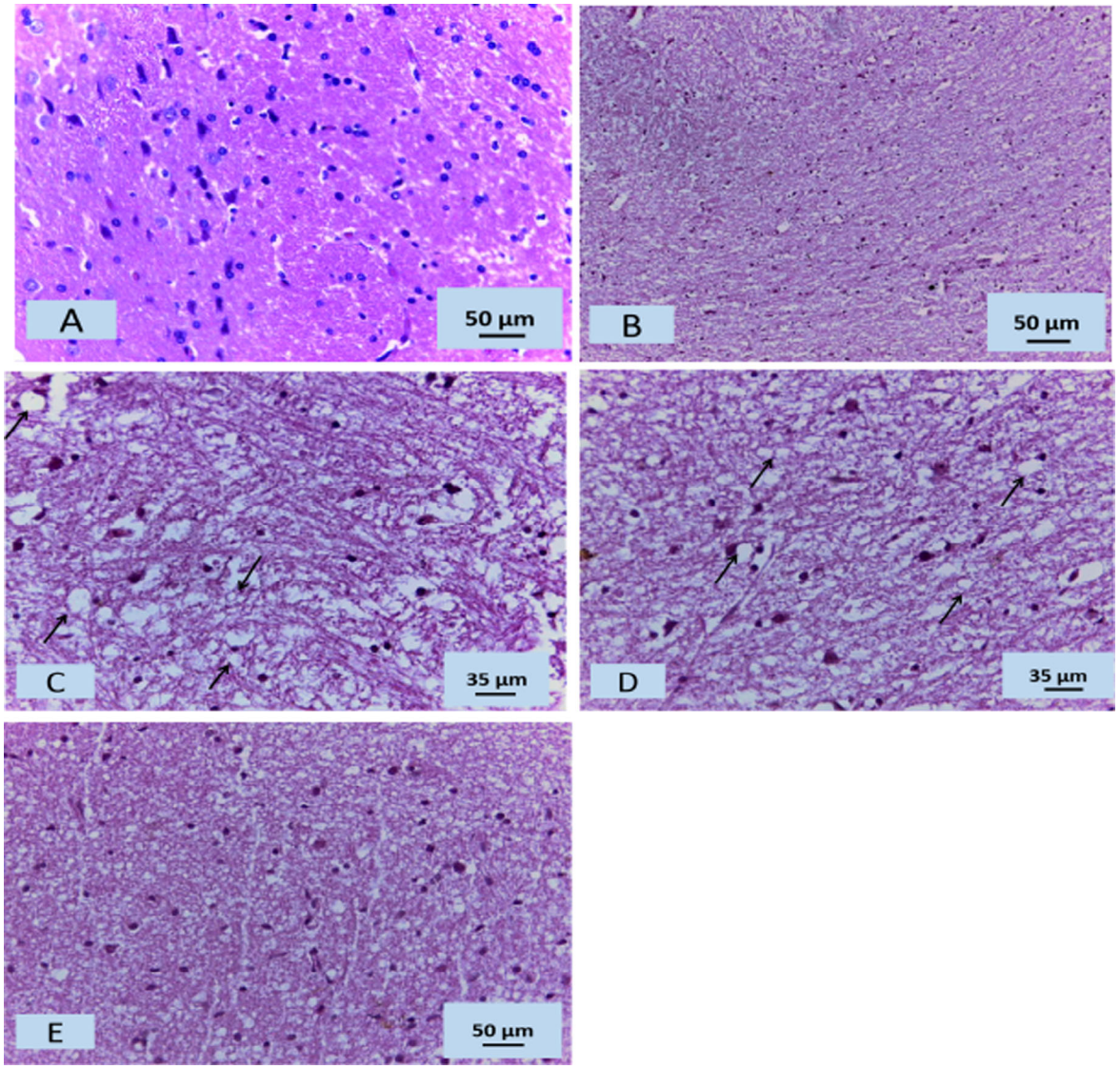

FIGURE 4 | Photomicrographs of histological section of brain tissues stained by hematoxylin and eosin. (A) The control rats showed normal histological architecture. (B) The MOLE group showed a normal histological appearance. (C, D) The ABM-treated group showed a remarkable reduction in cell number with neuropil vacuolization (arrow). (E) The ABM-MOLE group showed nearly normalized histological structure with reduced neuropil vacuolization.

In the current study, the observed biochemical changes were accompanied by histopathological alterations, notably vacuolation of the neuropil in the brain tissue of ABM-treated rats. These findings align with those of Radi et al. [28], who reported that exposure to ABM at a dose of 2 mg/kg for 5 days resulted in hemorrhage and neuropil vacuolation in the rat brain. Furthermore, an in vitro study by Chinese researchers on mouse neuroblastoma N2a cells showed that avermectins, including ABM and doramectin, significantly inhibited neurite growth in differentiating N2a cells in a dose-dependent manner. This was accompanied by a reduction in the expression of P-glycoprotein, an ATP-dependent efflux transporter that exports toxic drugs from cells and protects against drug-induced cell death [35].

M. oleifera is increasingly recognized as a potent chemoprotective plant [36]. In this study, MOLE demonstrated a notable ability to mitigate the toxic effects of ABM, particularly within the nervous system of rats, by restoring the activities of GST and acetylcholinesterase, as well as improving GSH levels in the brain and reducing MDA levels in both the brain and erythrocytes. These results are consistent with those of Al-Qahtani and Albasher [37], who found that *M. oleifera* acts as a neuroprotective agent by inhibiting MDA and nitric oxide production, while reducing inflammation and apoptosis in mice exposed to lead. Similarly, Soliman et al. [38] reported that pretreatment with *M. oleifera* reduced renal and hepatic oxidative stress caused by methotrexate by increasing levels of SOD and GSH. Moreover, our findings are supported by the study of Sakr, Rashad, and Abaza [39], which concluded that *M. oleifera* oil exerts protective effects against tributyltin-induced brain toxicity, primarily through its strong antioxidant and anti-apoptotic properties.

In the present study, neohesperidin emerged as one of the most abundant and notable compounds identified in the *M. oleifera* leaf extract. Neohesperidin, a flavonoid, is widely recognized for its diverse bioactive properties, which include alleviating cough

TABLE 5 | Binding affinity and root mean square deviation (RMSD) of some antioxidant compounds and the three targeted receptors: Human peroxiredoxin 5 (PRDX5; 1H2D), catalase (CAT; 1QQW), and glutathione peroxidase (GPx; 1GP1).

Receptor/ligand	Binding affinity (kcal/mol)			RMSD (lower–upper)		
	1H2D	1QQW	1GP1	1H2D	1QQW	1GP1
Caffeic acid	−5.5	−5.7	−5.3	0.0–27.18	0.0–43.55	0.0–27.43
Chlorogenic acid	−7.1	−7.0	−6.5	0.0–21.94	0.0–46.73	0.0–28.22
Gallic acid	−5.6	−5.5	−4.8	0.0–9.34	0.0–38.00	0.0–29.70
Naringin	−8.5	−8.9	−6.9	0.0–21.66	0.0–44.95	0.0–29.82
p-Coumaric acid	−5.2	−5.7	−5.0	0.0–26.96	0.0–35.58	0.0–33.48
Quercetin	−6.8	−7.8	−6.8	0.0–27.62	0.0–33.85	0.0–29.72
Rutin	−7.7	−7.5	−6.9	0.0–29.81	0.0–38.26	0.0–36.96
Vanillin	−5.0	−5.2	−4.6	0.0–4.32	0.0–44.89	0.0–30.86
Vanilic acid	−5.4	−5.2	−5.0	0.0–27.32	0.0–40.03	0.0–29.69

FIGURE 5 | 3D hydrophobic illustrations of naringin, as one of the best *Moringa oleifera* identified compounds, complexed with the three-targeted receptors: human peroxiredoxin 5 (A), catalase (B), and glutathione peroxidase (C).

and phlegm, reducing blood lipid levels, and exhibiting antioxidant, antiaging, anticancer, antiallergy, and antiatherosclerosis effects. Recent studies have demonstrated that neohesperidin also possesses significant neuroprotective effects. Specifically, it has been shown to mitigate neurodegeneration induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice, a model of Parkinson's disease by suppressing excessive activation of the NF-κB and MAPK signaling pathways [40].

Flavonoids and polyphenols in M. oleifera act as hydrogen donors, capable of neutralizing free radicals and protecting mammalian cells from damage induced by ROS [11]. The data from our study can be attributed to the presence of a wide array

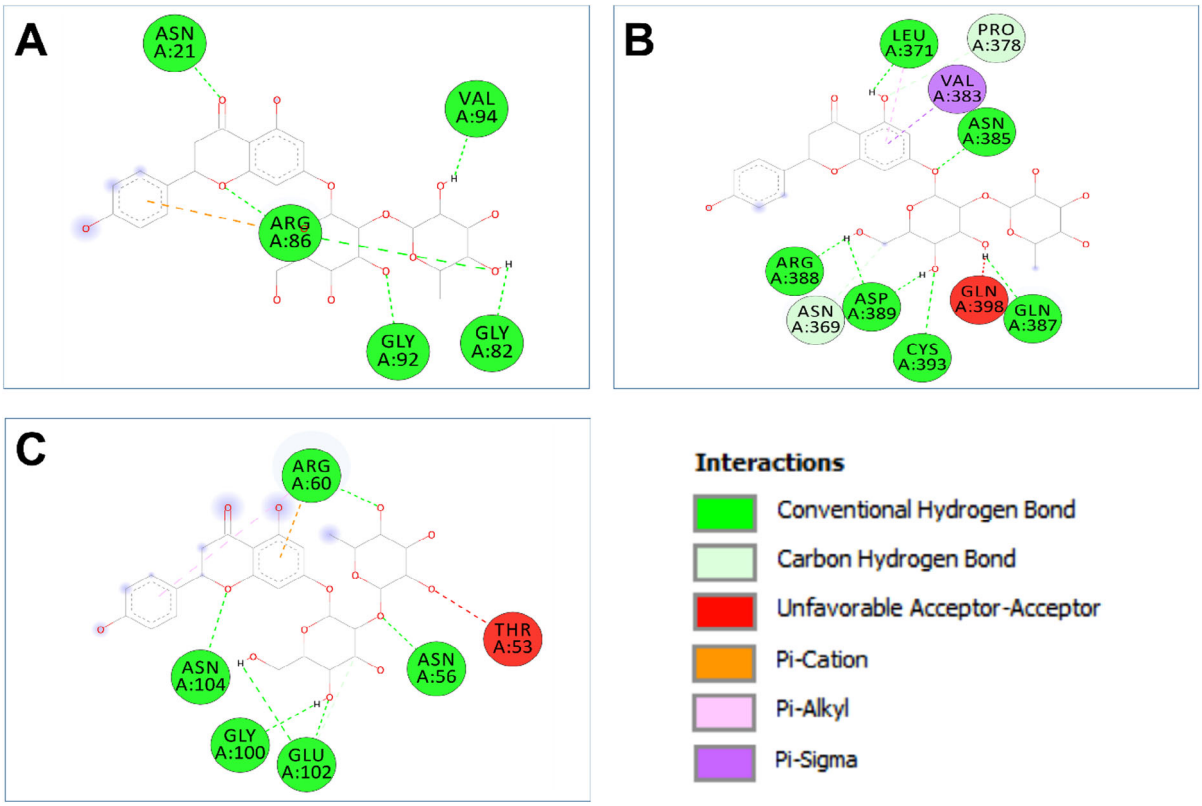

FIGURE 6 | Illustrations of the 2D diagrams of interactions for naringin, as one of the best *Moringa oleifera* identified compounds while complexed with the three targeted receptors: human peroxiredoxin 5 (A), catalase (B), and glutathione peroxidase (C).

of flavonoids and polyphenols in M. oleifera, as evidenced by the phytochemical analysis. The ability of M. oleifera to enhance GSH levels and modulate other protective enzymes is well established, likely due to bioactive compounds such as quercetin, gallic acid, kaempferol, and caffeic acid [41]. These findings are further corroborated by the HPLC analysis in this study. Previous studies have shown that quercetin mitigates oxidative damage in the brain caused by iron oxide nanoparticles in rats [42]. Similarly, hesperidin can alleviate oxidant-induced damage and cell death through mechanisms such as scavenging oxygen radicals, inhibiting lipid peroxidation, and chelating metal ions [43]. In addition, gallic acid is recognized as a potent antioxidant [44], and caffeic acid has been reported to reduce oxidative stress in the hippocampus [45]. These findings highlight M. oleifera as a valuable source of bioactive molecules with strong antioxidant properties.

Nanotechnology is advancing rapidly across several scientific and technological fields. Due to their unique properties, nanoparticles have diverse applications in medicine, agriculture, and other domains. However, many conventional methods for synthesizing nanoparticles have detrimental environmental effects [46]. In this context, nanoparticles synthesized using green techniques are highly regarded for their eco-friendliness, cost-effectiveness, safety, and biocompatibility, making them suitable for large-scale production [47]. Consequently, plant extracts have gained significant attention for their role in nanoparticle synthesis [48], with M. oleifera emerging as a promising candidate for producing nanoparticles with medicinal properties. Its rich content of bioactive molecules, such as antioxidants, free radical scavengers, and reducing agents, makes it an ideal candidate for the synthesis of various nanoparticles [49].

The results of the current study were further supported by histopathological examination of brain tissue, which demonstrated significant improvements in the structural integrity of brain tissue in ABM-MOLE-treated rats.

As shown in Table 4, all the tested compounds exhibited negative binding affinities, with values of −8.5, −8.9, and −6.9 kcal/mol for 1H2D, 1QQW, and 1GP1, respectively. Recent studies have indicated that binding affinity values are primarily determined by both the chemical structure and the geometry of ligand-receptor complexes [50–52]. Naringin was predicted to have the lowest binding affinity for all three studied compounds, followed by rutin (Table 6; Figure 5). Naringin formed either six or seven conventional hydrogen bonds with each of the targeted macromolecules. These bonds were associated with various interactions, including carbon–hydrogen bonds, pi–cation interactions, pi–alkyl interactions, and pi–sigma network bonds, which enhance the stability of the complexes (Figure 5) [50, 51, 53].

The chlorogenic acid–1HD2 complex exhibited the highest number of conventional hydrogen bonds (n = 9), involving several closely interacting residues, such as Glu16, Lys63, Val70, Arg86, Gly92, Val94, Arg95, Leu96, and Gln68. Notably, Gln68 was the closest interacting residue, with a distance of only 2.093 Å. All the analyzed compounds were deeply embedded (< 2.5 Å) in the targeted receptors (Table 6). The deepest embedding was observed for quercetin when complexed with GPx, which

TABLE 6 | Interactions, bond category, and closest interacting residues between the tested antioxidant compounds and the three-targeted receptors: Human peroxiredoxin 5 (PRDX5; 1H2D), catalase (CAT; 1QQW), and glutathione peroxidase (GPx; 1GP1).

Compound No.	No. H-bond	Closest interacting residues	
		Residue (letters and ID)	Distance to closest interacting residue (Å)
Human peroxiredoxin 5 (pdb id: 1HD2)			
Naringin	7	**Asn21, Arg86, Arg86, Arg86, Gly92, Gly82, Val94**, Arg86	Gly92:HN (1.847)
Rutin	8	**Asn21, Asn21, Asn21, Arg86, Arg86, Arg95, Leu96, Thr81,** Asn21, Glu16, Glu16, Gly92, Gly92:C,O;Lys93, Arg95, Arg95, Ala90, Leu96	Asn21:HD22 (2.172)
Chlorogenic acid	9	**Lys63, Arg86, Arg86, Gly92, Leu96, Val70, Gln68, Val94, Glu16**, Arg95, Gly92:C,O;Lys93:N, Arg95	Gln68:O (2.093)
Catalase (pdb id: 1QQW)			
Naringin	7	**Asn385, Cys393, Leu371, Arg388, Asp389, Gln387, Asp389**, Pro378, Asn369, Val383, Leu371	Asp389:O (2.106)
Quercetin	4	**Gln11, His14, Ala 270, Tyr274**, Gln11, Phe266, Phe266, Ala270, Ala270	Gln11:HE22 (2.316)
Rutin	6	**Asn338, Met339, Ile343, Ala418, Ser337, Met339**, Gly141, Asn338, Met339, Pro378, Asp140:C,O;Gly141:N, Ala381, Val383	Asn338:HD22 (1.969)
Glutathione peroxidase (pdb id: 1GP1)			
Naringin	6	**Asn56, Arg60, Glu102, Asn104, Glu102, Gly100**, Glu102, Arg60, Arg60, Arg60	Arg60:HH11 (1.997)
Rutin	4	**Gln59, Arg60, Gln59, Leu67**, Asn104, Asn104, Arg60, Arg60	Gln59:HE21 (2.220)
Quercetin	3	**Ala21, Pro103, Leu107**, Glu102, Leu89, Leu89, Ala21	Leu107:O (1.933)

Note: Bold residues interacting with conventional H-bonds.

showed a distance of just 1.933 Å. Several studies have reported that deep embedding is often linked to significant biological effects and health-promoting potentials, including antioxidant, anti-inflammatory, and anticancer activities [51–54].

Taken together, our computational modeling results confirm that the protective and antioxidant effects of M. oleifera compounds are thermodynamically feasible. These effects were also corroborated by the in vivo findings in rats, particularly in erythrocytes and brain tissues. Our results validate the health-promoting and phytotherapeutic potential of the plant's natural compounds [51, 54, 55]. In particular, several compounds from M. oleifera have previously been reported to exhibit acceptable lipophilicity, pharmacokinetics, and drug-likeness properties [40, 52], as evidenced by their interactions with the five major cytochrome P450 isoenzymes (CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4), which showed promising results [54, 56].

4 | Conclusion

The present study has shown that MOLE contains a variety of polyphenolic, flavonoid, tannins, and *ortho*-diphenolic components. On the other hand, this work revealed that ABM induces changes in oxidative stress indices in both rat's brain and erythrocytes, as well as alterations in brain AChE activity. *M. oleifera* leaf aqueous extract has demonstrated potent antioxidant activity both in vitro, by its strong free radical scavenging activity, and in vivo, by restoring the damage caused by ABM. The binding affinities of *M. oleifera* identified compounds might explain their protective effects, which can be the consequence of their deep embedding and the resulting molecular interactions of these compounds with some PRDX5 and GPx.

5 | Experimental Section

5.1 | Chemicals and Reagents

The pesticide used in the present study was a commercial formulation (Vertimec 1.8% E.C) containing ABM (18 g/L) as an active component (CAS 71751-41-2). This chemical ($C_{94}H_{14}O_{28}$) is a mixture of two avermectins, avermectin B1a and avermectin B1b (Figure 1). The product is formulated by INDUSTRIAS AFRASA firm, Spain, and distributed by FMP AGRI, Algeria. *M. oleifera* powdered leaves were obtained from BIOGATRANA (Gatrana Sidi Bouzid 9100, Tunisia), a company for Extracting Natural Products.

All reagents used for oxidative stress parameters, total polyphenolic contents, and phenolic standards were purchased from Sigma-Aldrich (GmbH, Steinheim, Germany). Alcohol (HPLC grade) was purchased from Merck (Darmstadt, Germany). Ultrapure water was obtained from the Millipore system (Billerica, USA).

5.2 | *M. oleifera* Leaf Aqueous Extract Preparation

M. oleifera powdered leaves were obtained from BIOGATRANA (Gatrana Sidi Bouzid 9100, Tunisia), a company for Extracting Natural Products (https://www.viveznature.com/28/bio-gatrana) and a voucher specimen (MO-0317) was deposited in the Herbarium of the Faculty of Sciences, University of Gafsa, Tunisia.

The preparation of *M. oleifera* aqueous extract was carried out using the maceration method. Ten grams of powdered sample were extracted with 100 mL of distilled water for 24 h, with continuous stirring at room temperature. The resulting solution was then filtered through Whatman filter paper No. 1. The filtrate was dried by evaporating the solvent in an oven at 37°C and stored at 4°C.

Total polyphenol content: Total polyphenol content was assayed by the method of Wolfe, Wu, and Liu [57] using Folin–Ciocalteu reagent. Briefly, a 200 mL volume of the extract was combined with 1 mL of Folin–Ciocalteu reagent diluted 10-fold. After 4 min, 800 µL of sodium carbonate solution (75 mg/mL in distilled water) was added. The mixture was left at room temperature for 2 h, and the optical density of the phenolic compounds was measured spectrophotometrically at 765 nm. The obtained results were calculated as gallic acid equivalent per gram plant extract (GAE/g E).

Total flavonoid content: Total flavonoid content was estimated spectrophotometrically according to the method reported by Zhishen, Mengcheng, and Wu [58]. After mixing 1 mL of the extract with 1 mL of aluminum chloride (2%) and letting the mixture sit at room temperature for 15 min, 430 nm optical density measurement. Total flavonoid content was expressed as quercetin equivalent per gram plant extract (QE/g E).

Condensed tannin content: Condensed tannin content was determined using the protocol described by Hagerman and Butler [59]. Briefly, an aliquot of 50 µL of the extract was mixed with 3 mL of a 4% (w/v) vanillin solution and gently stirred. To this mixture, 1.5 mL of concentrated hydrochloric acid was added, and the reaction was allowed to proceed at room temperature for 15 min. The absorbance was recorded at 550 nm, and the tannins concentration was determined using a catechin standard calibration curve. Condensed tannin content was expressed as catechin equivalent per gram plant extract (CE/g E)

Ortho-diphenols content: *Ortho*-diphenols content was measured following the method of Mateos et al. [60]. Briefly, 4 mL of the sample was mixed with 1 mL of a sodium molybdate solution. The mixture was vigorously stirred to ensure optimal homogeneity and then left to rest for 15 min. The absorbance of the mixture was measured at 370 nm, and the results were compared to a reagent blank. The concentration of *ortho*-diphenols was expressed in milligrams of caffeic acid equivalents (mg CAE) using a calibration curve prepared from caffeic acid standards (CAE/g E).

Total antioxidant capacity: The potency of a compound is typically measured using the half-maximal effective concentration (EC_{50}) for agonists or the half-maximal inhibitory concentration (IC_{50}) for antagonists or inhibitors. The EC_{50} represents the concentration of a substance (expressed in mg/mL) required to produce a response that is halfway between the baseline and the maximum effect. Similarly, the IC_{50} indicates the concentration needed to inhibit a biological or biochemical function by 50%. These metrics are key indicators of a compound's efficiency

and potency in pharmacological studies [61]. Spectrophotometric evaluation of total antioxidant capacity was determined by the technique described by Prieto, Pineda, and Aguilar [62] based on the formation of a green-colored complex after the reduction of molybdate ions to molybdenum in the presence of the sample. A 0.1 mL aliquot of the sample was mixed with 1 mL of a reagent solution (comprising 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) in an Eppendorf tube. The mixture was sealed and heated at 95°C for 90 min. After cooling to room temperature, the absorbance was recorded at 695 nm against a blank prepared under the same conditions. After calculating the percentage of reduction using the formula: [Reduction % = ((Ac − Ae)/Ac) × 100] where Ac is the control absorbance and Ae is the sample absorbance, the curve is the percentage of inhibition as a function of the extract concentration was plotted. The result was represented as the necessary concentration (mg/mL) of extract to reduce 50% of molybdate ions (EC_{50}).

DPPH free radical scavenging assay: The protocol described by Kirby and Shmidt [63] was adopted to evaluate the scavenging ability of DPPH radical. The intensity of the DPPH solution's color decreases inversely with the antioxidant activity in the medium, reflecting the ability of the extract to inhibit the DPPH radical. Nine hundred fifty microliters of DPPH solution (0.1 mM) was combined with 50 µL of the extract solution at varying concentrations. After incubation for 30 min in the dark at room temperature, the optical density was measured at 515 nm. After calculating the percentage of inhibition using the formula [Inhibition (%) = ((Ac − Ae)/Ac) × 100] where Ac is the control absorbance and Ae is the sample absorbance, the curve [Inhibition % = *f* (Concentration)] was plotted. The result was represented as the necessary concentration (mg/mL) of extract to scavenge 50% of radicals (IC_{50}).

5.3 | ABTS Reducing Power Assay

The capacity of *M. oleifera* aqueous extract to reduce ABTS radical was estimated according to the procedure of Re et al. [64]. ABTS (7 mM), used as the free-radical provider, was treated with potassium persulfate (2.45 mM) to generate free radicals by mixing 88 µL of potassium persulfate with 5 mL of ABTS solution. The mixture was incubated in the dark for 12 h. The solution was then diluted to achieve an absorbance of 0.7 ± 0.02 at 734 nm. Finally, 2.9 mL of the diluted ABTS solution was added to 100 µL of the sample, and the absorbance was recorded at 734 nm. As previously explained for the DPPH test. The result was represented as the necessary concentration (mg/mL) of extract to scavenge 50% of radicals (IC_{50}).

5.4 | Ferric-reducing antioxidant power assay (FRAP)

The method of Pan, Wang, and Huang [65] is based on the ability of the extract to reduce Fe^{3+} to Fe^{2+}. Briefly, 1 mL of the extract at various concentrations is combined with 0.25 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide solution (10 mg/mL). The mixture is incubated in a water bath at 50°C for 25 min. Afterward, 2.5 mL of 10% trichloroacetic acid (TCA) is added, followed by centrifugation at 3000 × *g* for 10 min. A 2.5 mL volume of the resulting supernatant is mixed with 2.5 mL of distilled water and 0.5 mL of a 1% aqueous $FeCl_3$ solution. The absorbance is measured at 700 nm, using a blank as the reference. In this experiment, ascorbic acid served as a positive control under identical conditions and concentrations. The result was expressed as the half maximal effective concentration, EC_{50} (mg/mL).

5.5 | *β*-Carotene Bleaching Test

The in vitro antioxidant activity of MOLE was assessed by the *β*-carotene discoloration test following the protocol of Ismail, Marjan, and Foong [66]. After dissolving 2 mg of *β*-carotene in 10 mL of chloroform, 1 mL was extracted and added to a flask that already contained 0.02 mL of linoleic acid and 0.2 mL of Tween 20. A rotary evaporator was used to evaporate the mixture for 10 min at 40°C. Following that, 100 mL of distilled water was gradually added to the flask, and the mixture that resulted was then vigorously shaken. Under the same experimental conditions, 5 mL aliquots of the emulsion were transferred to separate test tubes with 0.2 mL of the extract solution at varying concentrations. After that, the tubes were carefully shaken and incubated for two hours at 45°C in a water bath. The extract's reference absorbance was measured at 470 nm at an initial time (*t*¼ 0) against a blank that was an emulsion without any *β*-carotene. Every 15 min for 120 min. The following formula was used to calculate the absorbance reading, which was repeated every 15 min against a blank: [Inhibition % = (A0 − At)/(Ao0 − Aot) × 100], where At and Aot are the absorbance values measured in the samples or standards and control at t 1 = 4 120 min, respectively, A0 and Ao0 are the absorbance values measured at the initial incubation time for samples and control. The result was represented as the necessary concentration (mg/mL) of extract to scavenge 50% of radicals (IC_{50}).

5.6 | HPLC Analysis

A Shimadzu (LC 20 AL) fitted with a UV-VIS detector SPD 20A (Shimadzu), an analytical column of the Shim-pack VP-ODS C18 (4.6 mm × 250 mm, 5 µm) type, and a universal injector (Hamilton 25 µL) were used for the HPLC analysis. The 1% acetic acid and acetonitrile mobile phase was supplied to the column at a flow rate of 1 milliliter per minute via a pump after being filtered through a 0.45 µm membrane filter. At 268 nm, the result was obtained.

5.7 | Animals and Experimental Design

This study was conducted on twenty Wistar rats, each weighing approximately 180–200 g, obtained from the Pasteur Institute (Algiers, Algeria). The rats were acclimated for 2 weeks under standard environmental conditions (25 ± 3°C, 12-h light/dark cycle), housed in plastic cages with free access to standard rodent food and water. All care procedures were approved by the Ethical Committee of the Directorate General for Scientific Research and Technological Development at the Algerian Ministry (PNR/SF

08/2012). The rats were divided into four equal groups. The first group served as control and received distilled water by gavage. The second group received ABM dissolved in drinking water, considering the daily water consumption of animals 24 mL/200 g bw [67] at a dose of 1 mg/kg bw [68]. The dose of ABM was adjusted weekly according to the average body weight of rats. The third group received MOLE at a dose of 200 mg/kg bw [69] by gavage, the fourth group (ABM-MOLE) received a combination of ABM and MOLE administered in the same manner and at the same doses as before, for 3 weeks. On the day after the final treatment, the rats were sacrificed via cervical decapitation.

5.8 | Histological Examination

Histological examination was carried out following the protocol of Hould [70]. Brain fragments were rinsed with physiological water, fixed in 10% formaldehyde, embedded in paraffin, sliced to sections of 5 µm thickness, deparaffinized, and stained with H&E. The obtained sections were then air dried and scanned using a light microscope (Leica Microsystems Schweiz AG, CH-9435 Heerbrugg) and photographed using a digital camera (Leica ICC50 W).

5.9 | Reduced Glutathione Levels, Antioxidant Enzymes Activities, and Lipid Peroxidation Assessment

Reduced glutathione: GSH levels were measured using Ellman's [71] method. A volume of 200 µL of 0.25% sulfosalicylic acid was added to 800 µL of the organ supernatant, and the mixture was centrifuged at 1000 rpm for 15 min. Then, 500 µL of the resulting supernatant was combined with 1000 µL of phosphate buffer (0.1 M, pH 7.4) and 25 µL of DTNB (10 mM). The absorbance at 412 nm was measured to determine the glutathione content.

SOD (EC 1.15.1.1): The activity of SOD was measured using the protocol described by Beyer and Fridovich [72]. A total of 1890 µL of phosphate buffer pH 7.8 (50 mM), 1 mL of EDTA/methionine (0.3 mM), 85 µL of NBT (2.6 mM), 22 µL of riboflavin (0.26 mM), and 50 µL of the sample were combined. After 20 min of exposure to light, the mixture's absorbance at 560 nm was measured.

Catalase (EC 1.11.1.6): CAT activity was determined following the Aebi [73] method. The reaction mixture consisted of 20 µL of the sample, 780 µL of phosphate buffer (pH 7.5), and 200 µL of hydrogen peroxide (500 mM) in a total volume of 1 mL. The change in absorbance at 240 nm was monitored every 15 s for one minute to measure enzyme activity.

GST (EC 2.5.1): GST activity was assessed according to the method of Habig, Pabst, and Jakoby [74] by measuring the conjugation reaction between glutathione and 1-chloro-2,4-dinitrobenzene (CDNB). A volume of 200 µL of the sample was mixed with 1200 µL of a reaction mixture containing 1 mM CDNB and 5 mM GSH, both dissolved in 0.1 M phosphate buffer (pH 6.5). The absorbance at 340 nm was recorded at 1-min intervals for a total duration of 5 min to monitor the reaction.

MDA levels: lipid peroxidation levels were estimated following the Buege and Aust [75] protocol. The method relies on the formation of a yellow color upon the reaction of 5,5-dithio-bis-2-nitrobenzoic acid with compounds containing sulfhydryl groups. A volume of 375 µL of the sample was combined with 150 µL of TBS (pH 7.4) and 375 µL of butylated hydroxytoluene-trichloroacetic acid solution (1% w/v BHT in 20% TCA). The mixture was then centrifuged at 1000 rpm for 10 min. Following centrifugation, 400 µL of the supernatant was mixed with 80 µL of HCl and 320 µL of thiobarbituric acid reagent. The resulting solution was incubated in a boiling water bath for 10 min, after which absorbance was measured at 530 nm.

Nitric oxide levels: Nitric oxide levels were measured according to Griess's [76] method. The colorimetric detection of NO_2^- using Griess reagents is one of the simplest and, therefore, most widely used methods. This technique relies on a diazotization reaction that occurs in two steps. First, the acidification of NO_2^- produces N_2O_3, a derivative of NO· with strong nitrosating potential. This compound reacts with sulfanilamide to form a diazonium ion, which then couples with *N*-(1-naphthyl)ethylenediamine dihydrochloride. One hundred microliters of the sample are mixed with 50 µL of Griess Reagent A and 50 µL of Griess Reagent B. After incubating in the dark for 20 min at room temperature, the absorbance of the nitrite-containing samples relative to the reference sample is measured at 570 nm. Nitrite concentration was compared to a standard curve for sodium nitrate.

5.10 | Acetylcholine-Esterase Activity

Acetylchline-esterase activity was estimated according to the protocol of Ellman et al. [77]. A 1 mL aliquot of phosphate buffer (pH 7.4) was added to 50 µL of a DTNB/$NaHCO_3$ solution and 50 µL of acetylthiocholine. To this mixture, 5 µL of the sample was added, followed by agitation. The absorbance at 412 nm was recorded at 3-min intervals over a 25-min period.

5.11 | Computational Modeling and Interactions Assay

The studied biological activities of some antioxidants were further assessed by computational modeling and interaction assays. In this context, the PRDX5 (1H2D), CAT (1QQW), and GPx (1GP1) have been targeted. The crystal structures of these macromolecules have been retrieved from RCSB databases, then prepared following the deletion of the crystalized water and the addition of polar hydrogen and Kollman charges. Later, the targeted macromolecules have been processed for the CHARMm force field as previously reported [50, 55, 78]. The predicted bond categories, molecular interactions and tight embedding have also been assessed as previously described [54, 79, 80].

5.12 | Statistical Analysis

Results are represented as mean ± standard error of the mean (SEM), one-way ANOVA was used for the comparison between experimental groups. Tukey's post hoc test for multiple comparisons was used to evaluate the significance with IBM

SPSS Statistics V26. Differences were considered significant at a $p < 0.05$.

Author Contributions

Boudjema Kahina, Chouala Khadidja, and Chenna Houssem performed experiments, analyzed the data, and wrote the article. Badraoui Riadh Carried out the in sillico study. Khelef Yahia carried out the HPLC analysis. Boumendjel Mahieddine, Boumendjel Amel, and Messarah Mahfoud conceived and designed the experiments.

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Conflicts of Interest

The authors declare no conflict of interest.

Data Availability Statement

Research data are not shared.

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DISCUSSION GENERALE

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Les résultats de l''analyse phytochimique ont révélé des concentrations significatives en phénols, flavonoïdes, tanins et *ortho*-diphénols dans l'extrait aqueux des feuilles de *M.oleifera*.

L'HPLC a permis d'identifier plusieurs composés bioactifs, notamment l'acide ascorbique, l'acide gallique, l'acide chlorogénique, l'acide vanillique, l'acide caféique, l'acide férulique, l'alizarine, l'hespéridine, la néohespéridine, le resvératrol, la quercétine et la naringine dans l'extrait aqueux de *M. oleifera*. Ces résultats sont en accord avec ceux d'une études antérieures rapportant la richesse des feuilles de MO en constituants actifs aux propriétés antioxydantes (Dhakad *et al*., 2019)

Les flavonoïdes, polyphénols, acides aminés, alcaloïdes et protéines présents dans *M.oleifera* incluent dans leur structure des groupes fonctionnels tels que O-H, C=O et C-O (Kurniawan, 2021). Ceci a été confirmé dans cette étude par le spectre FTIR, qui a permis d'identifier différents groupements fonctionnels des composés bioactifs de l'extrait.

De plus, ces données sont corroborées par la capacité de *M.oleifera* à neutraliser les radicaux DPPH et ABTS, ce qui est en accord avec les travaux de Laoung *et al*., (2021), qui ont démontré que l'infusion de feuilles de *M.oleifera* était plus efficace que l'acide gallique contre le radical ABTS.

En accord avec les résultats d'Omodanisi *et al*., (2017), les feuilles de MO ont montré une activité antioxydante marquée, observée dans notre étude à travers la mesure de la capacité antioxydante totale, de son pouvoir réducteur du fer et de sa capacité à inhiber le blanchiment du β-carotène.

Concernant l'étude *in vivo*, les résultats obtenus ont montré que l'administration de l'ABM n'a provoqué aucun changement dans le poids corporel, ce qui est similaire aux résultats obtenus par Aggarwal *et al*., (2022), qui ont étudié les effets toxiques de l'ABM à une dose de 2 mg/kg chez les rates *Wistar*.

Cependant, une modification notable du poids total et relatif des reins a été notée, se traduisant par une atrophie rénale. Ce phénomène peut être un symptôme de néphrotoxicité ou de maladies rénales chroniques progressives (Hamed et Abdelrazik, 2015).

Contrairement à nos résultats, des études précédentes menées sur des lapins ont montré que l'exposition au Voliam Targo, un mélange d'ABM et de chlorantraniliprole, entraînait une augmentation du poids relatif des reins. Selon les auteurs, cette augmentation pourrait être due à la diminution du poids corporel des animaux intoxiqués ou constituer un signe de toxicité des pesticides (Bokreta *et al*., 2022).

Nos résultats ont révélé une augmentation significative des poids absolu et relatif du foie chez les rats traités par l'ABM par rapport aux rats témoins sains, ce qui pourrait être dû à l'accumulation anormalement élevée de lipides, principalement des triglycérides, dans les cellules parenchymateuses (Hamed et Abdelrazik, 2015). Ceci peut expliqué l'élévation du cholestérol et des triglycérides après exposition à l'ABM.

Par ailleurs, Abdelrassoul (2018) a rapporté qu'une administration de l'ABM à une dose de 0,5 mg/kg de poids corporel chez des rats mâles pendant 30 jours entraînait une diminution progressive et significative du poids corporel, ainsi qu'une augmentation marquée des poids relatifs des reins, du foie et du cerveau.

Dans la présente étude, la coadministration de MO avec l'ABM a permis de rétablir presque à la normale le poids absolu et relatif des reins et du foie, comparé au groupe témoin et au groupe traité uniquement avec MO. Ce résultat pourrait être attribué à la richesse de *M.oleifera* en molécules bioactives, y compris les alcaloïdes, la quercétine, le kaempférol, les flavonoïdes, l'acide ascorbique et les benzyl glucosinolates (Pareek *et al*., 2023), en plus des tanins, qui sont connus pour leurs effets vasodilatateurs. Par conséquent, la filtration glomérulaire pourrait être améliorée grâce à une vasodilatation rénale (Slama *et al*., 2021).

Les résultats de l'analyse hématologique ont révélé que l'ABM a induit une réduction du nombre de globules rouges (GR), de l'hémoglobine (Hb) et des globules blancs (GB) chez les rates traitées par l'ABM, par rapport au groupe témoin. La diminution des GR et des taux d'Hb après l'administration de l'ABM pourrait résulter d'une hémolyse provoquée par des dommages oxydatifs aux membranes cellulaires, causés par les espèces réactives de l'oxygène (Aioub *et al*., 2022).

En accord avec nos résultats, une étude menée par Manssour *et al*., (2017) sur des poissons *Tilapia du Nil* a démontré que l'ABM entraîne une anémie sévère en réduisant les concentrations de GR et d'Hb.

Aussi, Eissa et Zidan (2010) ont rapporté que les avermectines réduisaient le nombre de GB, dans cette expérience, la leucopénie observée pourrait être le résultat d'une altération du système immunitaire (Picolli *et al*., 2019).

En revanche, Ahmed *et al*., (2020) ont rapporté que l'ivermectine et la doramectine, appartenant à la famille des avermectines, augmentaient le nombre de GB. Ils ont expliqué cela par la présence de foyers inflammatoires après le traitement aux pesticides.

D'un autre côté, la coadministration de MO avec l'ABM dans notre étude a eu un effet positif sur les indices hématologiques. Ces effets pourraient être dus à la richesse de MO en micronutriments, notamment en vitamine A, un facteur clé dans la réduction de l'anémie (Shija *et al*., 2019), ainsi qu'en vitamines B, C et E, en magnésium et en fer (Patil et al., 2022), ce dernier jouant un rôle essentiel dans la formation de l'hémoglobine.

En accord avec les résultats de Radi *et al*., (2020) et Abdel Daim *et al*., (2018), les résultats actuels ont montré que l'exposition à l'ABM a entraîné une augmentation significative des niveaux d'ASAT, ALAT, GGT et de phosphatase alcaline (PAL) par rapport au groupe témoin.

Cela pourrait indiquer que l'ABM a accru la perméabilité membranaire, provoquant ainsi la fuite des enzymes intracellulaires altérées des hépatocytes dans la circulation sanguine (Radi *et al*., 2020).

La bilirubine est le produit final du catabolisme de l'hémoglobine, et l'état de santé du foie peut être évalué en mesurant ses niveaux. Notre étude a révélé que l'exposition à l'ABM a entraîné une augmentation significative des concentrations de bilirubine totale et libre, ce qui pourrait résulter d'une diminution de la captation hépatique, d'un défaut de conjugaison ou d'une augmentation de la sécrétion de bilirubine (Bouasla *et al*., 2014).

L'élévation des niveaux de bilirubine peut également être attribuée à l'accumulation de radicaux libres après une exposition à l'ABM. En effet, une étude antérieure a mis en évidence l'effet antioxydant de la bilirubine et sa capacité à éliminer les radicaux libres. Les auteurs ont noté qu'une augmentation des niveaux de bilirubine peut être considérée comme un mécanisme naturel de défense antioxydante contre les espèces réactives de l'oxygène (Hamed et Abdelrazik, 2015).

Concernant les indices biochimiques rénaux, l'élévation des concentrations sériques de créatinine et d'urée observée dans cette étude peut être liée à une diminution de la filtration glomérulaire et à un dysfonctionnement des tubules rénaux (Abdel Daim et Abdellatief, 2018).

En effet, une concentration élevée d'urée est corrélée au catabolisme des protéines, l'urée étant le produit final de leur dégradation et un indicateur de lésions rénales.

L'augmentation des niveaux d'urée et de créatinine reflète l'incapacité des reins à éliminer ces déchets (Nasr *et al*., 2016 ; Djaber *et al*., 2020).

L'effet toxique de l'ABM a été significativement atténué dans le groupe ABM-MO, comme en témoigne l'amélioration des niveaux d'ASAT, GGT, PAL, BL et de cholestérol. Ces résultats corroborent ceux d'Abdel Fattah *et al*., (2020), qui ont

rapporté que l'administration de *M.oleifera* à une dose de 200 mg/kg pendant quatre semaines permettait de restaurer les altérations hépatiques induites par l'exposition au plomb chez le rat. De plus, une amélioration significative des taux d'urée et d'acide urique a été enregistrée après la coadministration de MO avec l'ABM.

Dans cette étude, les effets protecteurs de *M.oleifera* pourraient être attribués aux molécules bioactives présentes dans l'extrait de ses feuilles, comme l'a démontré l'analyse phytochimique.

La protection hépatique pourrait être liée à la stabilisation de l'intégrité membranaire des hépatocytes et à la réduction de la perméabilité cellulaire, limitant ainsi la fuite des enzymes hépatiques dans la circulation sanguine (Toppo *et al*., 2015).

L'acétylcholinestérase (AChE) est une enzyme essentielle du système nerveux, responsable de l'arrêt des influx nerveux en catalysant l'hydrolyse du neurotransmetteur acétylcholine.En tant que cible moléculaire spécifique des pesticides organophosphorés et carbamates, l'activité de l'AChE et son inhibition sont des indicateurs précoces bien établis de l'intoxication aux pesticides chez l'homme (Kotb *et al*., 2021).

Dans la présente étude, la diminution de l'activité de l'AChE après une exposition à l'ABM est en accord avec les résultats de Hashim *et al*., (2018), qui ont rapporté une réduction de l'AChE sérique chez des rats exposés à l'ABM pendant 45 jours. De plus, Zhang *et al*., (2022) ont démontré que les avermectines induisent une neurotoxicité chez la carpe en réduisant l'activité de l'AChE.

L'altération observée de l'activité de l'AChE dans cette étude pourrait être liée aux effets neurotoxiques de l'ABM, confirmant son aptitude à induire un stress oxydatif (Radi *et al*., 2020). Ce stress oxydatif entraîne des dommages aux macromolécules, y compris aux enzymes (Radi, 2018).

Les radicaux libres sont des particules extrêmement réactives générées par des mécanismes physiologiques dans les systèmes biologiques (Zaric *et al*., 2023). Dans la présente étude, l'ABM a entraîné une diminution significative des niveaux de GSH dans tous les organes étudiés ainsi que dans les érythrocytes, parallèlement à une baisse de l'activité des enzymes antioxydantes, notamment une réduction de l'activité de la GSH-Px dans le foie et les reins, de la GST dans tous les organes, de la SOD dans le cerveau et de la CAT dans les érythrocytes. En outre, les niveaux de MDA étaient significativement augmentés dans l'ensemble des organes, accompagnés d'une élévation des niveaux d'AOPP dans les reins. Concernant le cerveau, aucune variation significative des niveaux de NO n'a été relevée.

En raison de leur activité antioxydante et de leur capacité à protéger les cellules contre les radicaux libres, la SOD, la GSH-Px, la GST et la CAT sont des enzymes essentielles impliquées dans le système de défense antioxydant cellulaire, la SOD catalyse la transformation de l'anion superoxyde en H_2O_2 (Seif *et al*., 2021), ce dernier est ensuite clivé en H_2O par la GSH-Px, qui est responsable de la prévention de l'oxydation des molécules intracellulaires (Aioub *et al*., 2022). La diminution significative des activités de la SOD, GSH-Px et GST après l'exposition à l'ABM pourrait être due à l'accumulation des radicaux libres et à l'échec du système redox à éliminer ces molécules, ce qui affaiblirait la défense cellulaire (Ahmed *et al*., 2016). Une étude menée par des chercheurs turcs sur l'impact toxicologique de cinq avermectines, y compris l'ABM, sur les enzymes GST isolées à partir d'érythrocytes humains a révélé que les composés testés inhibaient l'activité de la GST à des concentrations millimolaires, la réduction de l'activité de la GST pourrait ainsi refléter une insuffisance dans la conjugaison et la détoxification des radicaux libres (Nasr *et al*., 2016).

Sachant que le GSH est impliqué dans l'élimination du peroxyde d'hydrogène et du peroxyde lipidique via l'action de la GSH-Px (Liang *et al*., 2019), la diminution de sa concentration entraîne un stress oxydatif (Soliman *et al*., 2020), l'épuisement de la

concentration en GSH observé dans cette étude pourrait témoigner de la surproduction de radicaux libres chez les rates traités par l'ABM, en accord avec nos résultats, Aioub *et al*., (2022) ont observé que l'administration de l'ABM était associée à des dommages oxydatifs hépatorénaux, de plus, Radi *et al*., (2020) ont démontré que l'ABM pouvait altérer le système de défense antioxydant en favorisant la production de radicaux libres dans les tissus cérébraux et hépatiques des rats, par ailleurs, les données obtenues dans la présente étude sont cohérentes avec celles de Liang *et al*., (2019), qui ont étudié la toxicité de l'ABM sur des macrophages isolés de souris et ont constaté que l'ABM provoquait une diminution des niveaux de GSH ainsi que des activités de la GSH-Px et de la SOD.

Le MDA est un produit de la peroxydation des acides gras polyinsaturés, doté d'un puissant pouvoir cytotoxique, il peut facilement se lier aux protéines ou aux acides nucléiques (Ferrante *et al*., 2021), selon la présente étude, l'élévation des niveaux de MDA reflète le déclenchement du stress oxydatif induit par l'ABM via la génération de radicaux libres impliqués dans l'activation de la voie de peroxydation lipidique (Scassellati *et al*., 2020).

Une augmentation des AOPPs est positivement corrélée aux insuffisances rénales aiguës et chroniques, par conséquent, ces produits jouent un rôle dans le développement des lésions rénales via des processus cellulaires et moléculaires conduisant à la production de superoxyde, à la mort des podocytes et à l'activation des monocytes (Gyurászová, 2019).

Tous les dommages biochimiques ayant résulté de l'administration de l'ABM ont été accompagnés de dommages tissulaires observés dans le rein via des lésions dégénératives sévères des glomérules, une congestion associée à une dilatation de l'espace de Bowman menant à une atrophie glomérulaire ainsi qu'une dégénérescence hydropique tubulaire accompagnée de foyers de nécrose tubulaire, dans le foie, une légère dilatation sinusoïdale a été observée tandis qu'une vacuolisation marquée du neuropile a été mise en évidence dans le tissu cérébral.

Selon les résultats de cette étude, la coadministration de MO avec l'ABM, en comparaison avec les rats traités uniquement par l'ABM, restaure de manière significative la machinerie antioxydante, comme en témoigne l'amélioration notable des niveaux de GSH et des activités des enzymes antioxydantes, une étude récente a montré que le prétraitement des rats avec *M.oleifera* atténuait le stress oxydatif rénal et hépatique induit par le méthotrexate en augmentant les niveaux de SOD et de GSH (Soliman *et al*., 2020), de plus, une étude menée par Akinrinde *et al*., (2020) a révélé que les feuilles de MO, à une dose de 200 mg/kg, exerçaient des effets néphroprotecteurs lors de dommages oxydatifs causés par l'ischémie et la reperfusion rénale en réduisant les niveaux rénaux de MDA et d'AOPP, ces résultats sont en accord avec ceux d'Al-Qahtani et Albasher (2021), qui ont démontré que *M.oleifera* agit comme un agent neuroprotecteur en inhibant la production de MDA et d'oxyde nitrique tout en réduisant l'inflammation et l'apoptose chez des souris exposées au plomb.

Les flavonoïdes et les polyphénols présents dans *M.oleifera* agissent comme des donneurs d'hydrogène capables de neutraliser les radicaux libres et de protéger les cellules des mammifères contre les dommages induits par les espèces réactives de l'oxygène (Dhakad *et al*., 2019), les résultats de notre étude peuvent être attribués à la présence d'une large gamme de flavonoïdes et de polyphénols dans les feuilles de *M.oleifera*, comme l'a démontré l'analyse phytochimique, la capacité de *M.oleifera* à augmenter les niveaux de GSH et à moduler d'autres enzymes protectrices est bien établie, probablement en raison de la présence de composés bioactifs tels que la quercétine, l'acide gallique, le kaempférol et l'acide caféique (Albasher *et al*., 2020), ces résultats sont également confirmés par l'analyse HPLC réalisée dans cette étude.

L'alizarine, présente en quantité remarquable dans l'extrait étudié, est un composé connu pour son potentiel à protéger les protéines contre les dommages induits par les radicaux hydroperoxyles (Marković *et al*., 2023), des recherches antérieures ont montré que la quercétine atténue les dommages oxydatifs dans le cerveau causés par

les nanoparticules d'oxyde de fer chez les rats (Dora *et al*., 2021), de même, l'hespéridine peut réduire les dommages oxydatifs et la mort cellulaire par divers mécanismes, notamment l'élimination des radicaux oxygénés, l'inhibition de la peroxydation lipidique et la chélation des ions métalliques (Desmiaty *et al*., 2024), par ailleurs, l'acide gallique est reconnu comme un puissant antioxydant (Bouasla *et al*., 2021), et l'acide caféique a été rapporté pour son effet protecteur contre le stress oxydatif dans l'hippocampe (Koga *et al*., 2019). Les résultats de la présente étude ont été corroborés par l'examen histopathologique du tissu rénal, hépatique et cérébral, révélant des améliorations significatives de l'intégrité structurelle des organes étudiés chez les rats traités avec ABM-MO.

D'après les résultats de l'étude *in silico*, tous les composés testés (Acide gallique, Acide chlorogénic, acide vanillic, acide caféique, naringine et quercétine) ont présenté des affinités de liaison négatives, avec des valeurs de -8,5, -8,9 et -6,9 kcal/mol pour 1H2D, 1QQW et 1GP1, respectivement, des études récentes indiquent que les valeurs d'affinité de liaison sont principalement déterminées par la structure chimique et la géométrie des complexes ligand-récepteur (Badraoui *et al*., 2023; Jedli *et al*., 2024), la naringine a été prédit comme ayant la plus faible affinité de liaison pour les trois composés étudiés, suivi de la rutine, la naringine a formé six à sept liaisons hydrogène conventionnelles avec chacune des macromolécules ciblées, ces liaisons étaient associées à diverses interactions, notamment des liaisons carbone-hydrogène, des interactions pi-cation, pi-alcane et des réseaux de liaisons pi-sigma, contribuant à la stabilité des complexes (Badraoui *et al*., 2023; Bensaad et al., 2023; Mhadbi *et al*., 2023).

Le complexe acide chlorogénique–1H2D a présenté le plus grand nombre de liaisons hydrogène conventionnelles (n = 9), impliquant plusieurs résidus interagissant étroitement, tels que Glu16, Lys63, Val70, Arg86, Gly92, Val94, Arg95, Leu96 et Gln68. Notamment, Gln68 était le résidu interagissant le plus étroitement, avec une distance de seulement 2,093 Å.

Tous les composés analysés étaient profondément enfouis (< 2,5 Å) dans les récepteurs ciblés. L'enfouissement le plus profond a été observé pour la quercétine lorsqu'elle était complexée avec la GSH-Px, qui a présenté une distance d'interaction de seulement 1,933 Å. Plusieurs études ont rapporté que l'enfouissement profond d'un ligand dans un récepteur est souvent associé à des effets biologiques significatifs et à un potentiel bénéfique pour la santé, incluant des activités antioxydantes, anti-inflammatoires et anticancéreuses (Bensaad *et al*., 2023; Rahmouni *et al*., 2024).

Pris dans leur ensemble, nos résultats de modélisation computationnelle confirment que les effets protecteurs et antioxydants des composés de *M.oleifera* sont thermodynamiquement plausibles. Ces effets ont également été corroborés par les résultats in vivo obtenus chez les rats, notamment au niveau des érythrocytes et des tissus cérébraux. Nos résultats valident ainsi le potentiel phytothérapeutique et les bienfaits pour la santé des composés naturels de cette plante (Bensaad *et al*., 2023; Rahmouni *et al*., 2024; Chira *et al*., 2025).

En particulier, plusieurs composés de *M.oleifera* ont déjà été rapportés comme présentant une lipophilicité acceptable, des paramètres pharmacocinétiques favorables et des propriétés correspondant aux critères d'un candidat-médicament (Jedli *et al*., 2024). Ces propriétés ont été mises en évidence par leurs interactions avec les cinq principales isoenzymes du cytochrome P450 (CYP1A2, CYP2C19, CYP2C9, CYP2D6 et CYP3A4), qui ont montré des résultats prometteurs (Rahmouni *et al*., 2024; Bédoui *et al*., 2025).

CONCLUSION & PERSPECTIVES

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Dans la présente étude, nous avons exploré la composition phytochimique et l'activité antioxydante de *M.oleifera*, tout en évaluant son potentiel protecteur contre la toxicité induite par l'abamectine. L'approche adoptée a combiné des analyses *in vitro*, visant à caractériser les métabolites bioactifs et leur capacité antioxydante, ainsi qu'une étude *in vivo* permettant d'examiner les effets toxiques de l'abamectine sur divers organes (foie, reins, cerveau et érythrocytes) et l'impact protecteur de *M. oleifera*. Afin d'approfondir la compréhension des mécanismes d'action de cette plante, une étude *in silico* a été réalisée.

Les résultats obtenus à partir de cette investigation ont fait l'objet de trois publications scientifiques. Les principaux résultats obtenus dans chaque étude étaient présentés comme suit :

La première publication a porté sur l'effet néphroprotecteur de cet extrait. Une analyse HPLC a permis d'identifier six molécules bioactives dans l'extrait aqueux de MO, tandis qu'une analyse FTIR a révélé la présence de plusieurs groupes fonctionnels impliqués dans son activité biologique. Les résultats obtenus ont confirmé la néphrotoxicite de l'ABM à une dose correspondant à 1/10 de la dose létale. Cette toxicité s'est manifestée par une altération de la fonction rénale, mise en évidence par un déséquilibre biochimique et un stress oxydatif marqué. L'administration de l'extrait de M.oleifera a permis de restaurer la fonction rénale en réduisant de manière significative les niveaux des biomarqueurs de la fonction rénale, notamment l'urée, l'acide urique. De plus, le traitement a favorisé une amélioration du statut antioxydant, traduite par une augmentation quasi-normale des niveaux de GSH et de l'activité des enzymes antioxydantes, la GSH-Px, la SOD et la GST. Une diminution significative des dommages oxydatifs, notamment l'oxydation lipidique et protéique, a également été observée.

Afin de compléter les résultats de la première publication, **la deuxième publication** s'est intéressée à l'étude de l'effet hépatoprotecteur de l'extrait aqueux de *M.oleifera*. Les résultats ont mis en évidence un déséquilibre de la fonction hépatique ainsi qu'une altération du statut antioxydant hépatique suite à l'administration de l'abamectine. De manière similaire à son effet néphroprotecteur, le traitement des rats avec l'extrait a permis d'atténuer ces altérations hépatiques en réduisant de manière significative les niveaux élevés des marqueurs de cytolyse hépatique, notamment les transaminases, la phosphatase alcaline, la gamma-glutamyl-transférase, ainsi que la bilirubine libre et conjuguée. Par ailleurs, une diminution des niveaux de malondialdéhyde a été observée dans le foie des rats exposés à l'abamectine. En parallèle, le traitement par l'extrait de MO a induit une augmentation significative des niveaux de GSH ainsi que de l'activité des enzymes antioxydantes, notamment la GSH-Px et la GST, témoignant d'une restauration du système de défense antioxydant hépatique.

La troisième publication a concerné l'étude de l'effet protecteur de *M.oleifera* contre la toxicité de l'abamectine dans le cerveau et les érythrocytes. La coadministration de MO avec l'ABM a confirmé la capacité de la plante à réduire le stress oxydatif induit par l'ABM, comme en témoigne l'amélioration du statut antioxydant dans le cerveau et les érythrocytes, ainsi que la restauration de l'activité de l'acétylcholinestérase. Cette amélioration a été accompagnée par une prévention de l'altération histologique du tissu cérébral, indiquant l'effet neuroprotecteur du traitement.

De plus, une étude complémentaire a permis l'identification de six nouveaux composés phénoliques, en plus de ceux identifiés dans les premières études par HPLC. Aussi, une modélisation *in silico* a été réalisée, mettant en évidence la capacité de certains composés antioxydants, dont certains présents dans l'extrait étudié, à se lier à la peroxyrédoxine 5 humaine, à la catalase et à la glutathion peroxydase, avec des affinités considérées comme acceptables.

Ces trois études complémentaires ont permis d'évaluer de manière approfondie la toxicité de l'ABM sur plusieurs organes essentiels, tout en mettant en évidence le

potentiel protecteur de *M.oleifera*. Les résultats obtenus confirment que l'exposition à l'abamectine induit un stress oxydatif marqué, contribuant à des altérations biochimiques, fonctionnelles et histologiques des reins, du foie, du cerveau et des érythrocytes. En parallèle, l'administration de l'extrait aqueux de *M.oleifera* s'est révélée efficace pour atténuer ces effets délétères, grâce à ses propriétés antioxydantes et protectrices.

De plus, cette étude s'inscrit dans une démarche globale visant à alerter sur les dangers liés à l'utilisation abusive et anarchique des pesticides, notamment en Algérie. Elle rejoint ainsi d'autres recherches ayant mis en évidence les risques environnementaux et sanitaires de ces substances chimiques, soulignant la nécessité de stratégies alternatives pour minimiser leurs effets toxiques.

Cependant, cette étude présente certaines limites et nécessite des investigations supplémentaires pour approfondir ces observations. Ainsi, nous envisageons une future étude comparative entre l'extrait aqueux et l'extrait éthanolique de *M.oleifera*, en termes de composition en molécules bioactives, à l'aide de l'analyse LC-MS. Par ailleurs, une évaluation de l'effet de ces deux extraits sur trois types de lignées cellulaires cancéreuses le cancer du sein, le cancer du côlon et le carcinome hépatique sera également menée afin d'explorer leur potentiel anticancéreux. Ces perspectives permettront d'élargir les connaissances sur les applications thérapeutiques de *M.oleifera* et d'optimiser son utilisation dans le domaine de la phytothérapie et de la prévention des effets toxiques des pesticides.

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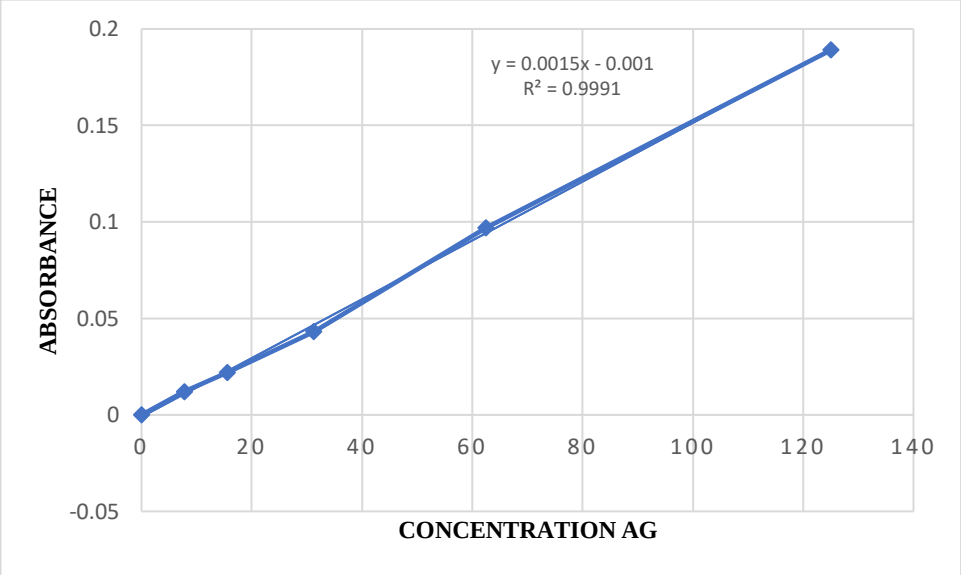
ANNEXES

Annexe 1 : Dosage des polyphénols totaux

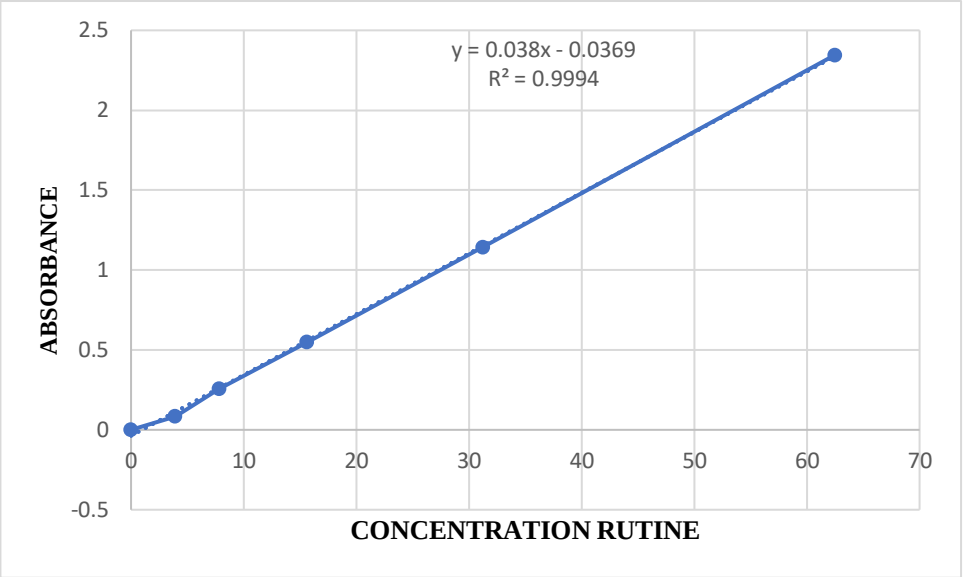
Réactif	Blanc	Échantillon
Extraits	0	0,25ml
Eau / Ethanol	0,25ml	0
Folin–Ciocalteu (1/10)	1ml	1ml
Na_2CO_3 (1%)	0,75ml	0,75ml
Agitation au vortex, incubation 2h et lecture à 760nm		



Annexe 1. Gamme d'étalonnage de l'acide gallique (suite)

Annexe 3 : Dosage des flavonoïdes totaux

Réactif	Blanc	Échantillon
Extraits	0	1ml
Eau / Ethanol	1ml	0
$AlCl_3$ (2%)	1ml	1ml
Agitation au vortex, incubation 10min et lecture à 415nm		



Annexe 2. Gamme d'étalonnage de la rutine (suite)

Dosage des tanins condensés

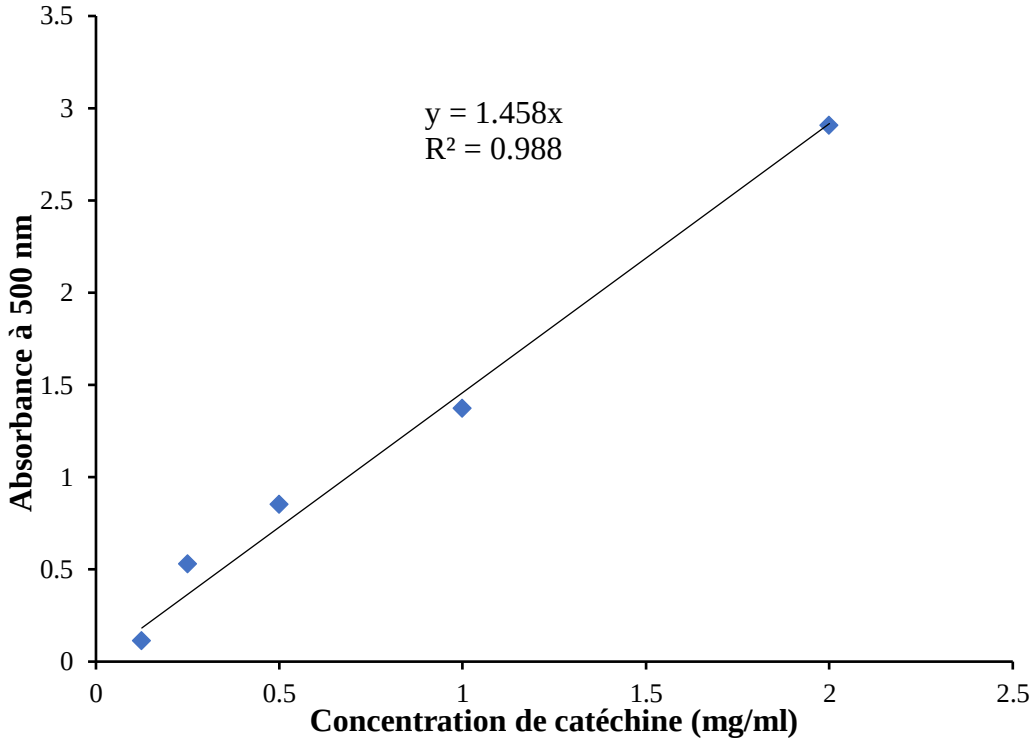


Figure 3 : Courbe de variation de la densité optique à λ= 500 nm en fonction de la concentration de catéchine.

Dosage des *ortho*-diphénols

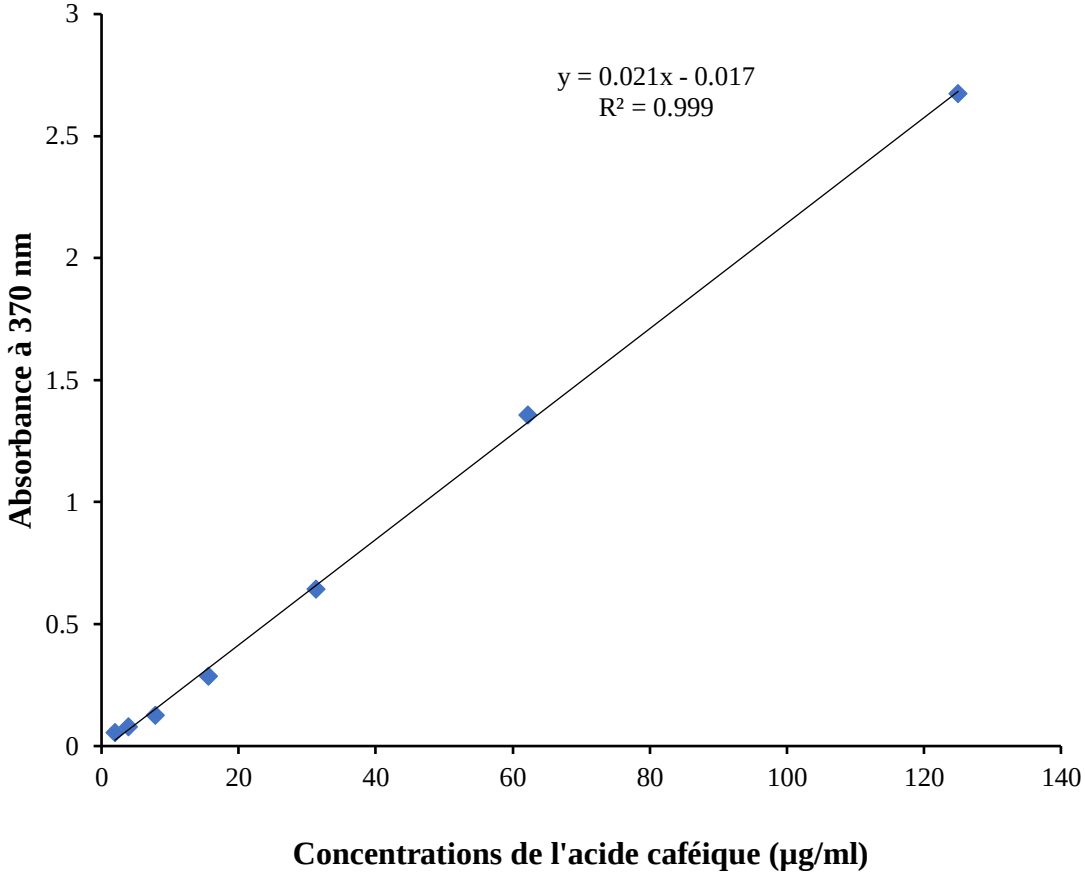
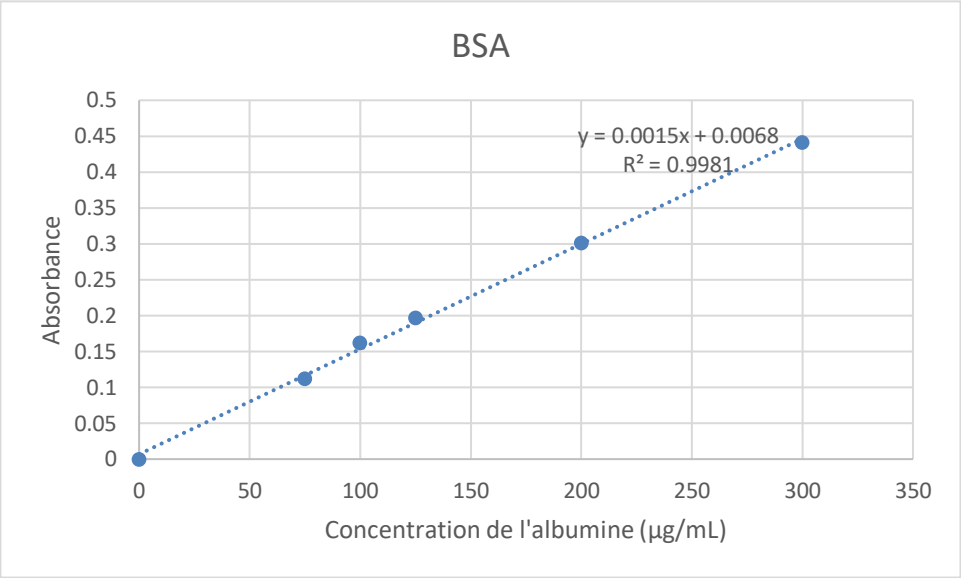


Figure 5 : Courbe de variation de la densité optique à λ= 370 nm en fonction de la concentration de l'acide caféique

Annexe 3 : Dosage des protéines par la méthode de Bradford

Réactif	Blanc	Échantillon
Surnageant	0	50µl
TBS	50µl	0
Réactif de Bradford	2.5ml	2.5ml
Agitation au vortex, incubation 5min et lecture à 595nm		



Annexe 4 : Gamme d'étalonnage BSA (1mg/ml)

Annexe 5 : Dosage du MDA

Réactif	Blanc	Échantillon
Surnageant	0	375µl
TBS	200µl	150
TCA-BHT	375µl	375µl
Agiter au vortex puis centrifuger a 1000 trs/min pendant 10 minutes		
Surnageant	400µl	400µl
HCl (0,6M)	80µl	80µl
Tris-TBA	320µl	320µl
Agitation au vortex et incubation à 80°c pendant 10 minutes et lecture à 530nm		

Annexe 6 : Dosage des taux des AOPP

Réactif	Blanc	Échantillon
Surnageant	0	200µl
PBS (0,1M, pH 7,4)	700µl	500µl
Incubation 2min		
KI (1,16M)	100µl	100µl
Acide glaciale	200µl	200µl
Agitation au vortex et lecture à 340nm		

Annexe 7 : Dosage du glutathion

Réactif	Blanc	Échantillon
Surnageant	0	800µl
TBS	1000µl	200µl
Acide Sulfosalicylique	200µl	200µl
Incubation 15min dans un bain de glace et centrifugation 1500 trs/min pendant 5minutes		
Surnageant	500µl	500µl
Tris-EDTA (pH 9,6)	1ml	1ml
DTNB (0,01M)	25µl	25µl
Agitation au vortex et incubation 5 minutes et lecture à 412nm		

Annexe 8 : Dosage de l'activité enzymatique de la glutathion peroxydase (GSH-Px)

Réactif	Blanc	Échantillon
Surnageant	0	200µl
TBS	400µl	200µl
GSH (0,1mM)	400µl	400µl
Incubation au bain marie à 25°c (5 minutes)		
H_2O_2 (1,3mM)	200µl	200µl
LAISSER 10 MINUTES AU REPOS		
TCA (1%)	1ml	1ml
Mettre le mélange 30 minutes dans la glace centrifugation a 3000 trs/min (10 minutes)		
Surnageant	480µl	480µl
Na_2HPO_4 (0,32M)	2,2ml	2,2ml
DTNB (1mM)	320µl	320µl
Agitation au vortex et incubation *5* minutes et lecture a *412*nm		

Annexe 9 : Dosage de l'activité enzymatique de la Glutathion-S-Transférase (GST)

Réactif	Blanc	Échantillon
Surnageant	0	200µl
Mélange (GSH 5mM+CDNB 1mM)	1,4ml	1,2ml
Lecture à 340 nm toutes les 1 minute durant 5minutes		

Annexe 10 : Dosage de l'activité enzymatique de la superoxyde dismutase (SOD)

Réactif	Blanc	Etalon	Échantillon
EDTA-Met (0.3mM)	1ml	1ml	1ml
PBS (50mM, pH :7,8)	892,2µl	892,2µl	892,2µl
Surnageant	0	0	50µl
PBS (50mM, pH :7,8)	1ml	1ml	950µl
NBT	85,2µl	85,2µl	85,2µl
Riboflavine	22,6µl	22,6µl	22,6µl
Apres incubation a la lumière pendant 20 min et lecture à 580nm			

Annexe 11 : Dosage de l'activité enzymatique de la catalase

Réactif	Blanc	Échantillon
Surnageant	0	20µl
PBS (0,1M, pH :7,4)	800µl	780µl
H_2O_2 (500mM)	200µl	200µl
Lecture à 240 nm toutes les 15sec durant 60 secondes		

Préparation des solutions

1. Dosage des protéines par la méthode de Bradford

❖ BSA (1 mg/ml) :

Dissoudre 5 mg BSA dans 5 ml d'eau distillée pour réaliser la gamme d'étalonnage.

❖ Réactif de Bradford :

Dissoudre 100 mg de bleu de Coomassie (G 250) dans 50 ml d'éthanol (95%). Agiter pendant 2 heures, puis ajouter 100 ml d'acide orthophosphorique (85%) et 850 ml d'eau distillée (pour obtenir 1 L de solution). Ce réactif doit être filtré puis conserver pendant 1 mois au maximum à une température de 4°C et à l'abri de la lumière.

2. Dosage du MDA

❖ La solution du tampon TBS (pH 7,4)

0,6057g de Tris (50 mM) et 0.877g Na Cl (150 mM) dans 100 ml d'eau distillé et ajuster le pH par NaOH ou HCl pour avoir un pH de 7,4.

❖ La solution d'H Cl (0,6 M) :

5.1569 ml de H Cl 36% dans 100ml d'eau distillé.

❖ La solution de Tris - TBA :

1.73g de TBA (120 mM) dans 100ml d'une solution Tris (26 mM).

❖ La solution de TCA – BHT :

1g de BHT (Butylehydroxytoluène) dans 100 ml de TCA (acide trichloracétique) à 20%.

3. Dosage des taux des AOPP

❖ Tampon phosphate (pH=7.4) :

Na2HPO4 (0.1M) : 3,4 g dans 240 ml d'eau distillée

NaH2PO4 (0.1M): 4,8 g dans 240 ml d'eau distillée

❖ Iodure de potassium (KI, 1.16 M) :

4.46 g dans 23,16 ml de H2O

4. Dosage du glutathion réduit

❖ L'acide salicylique (0,25%) :

Dissoudre 250 mg d'acide salicylique dans 100 ml d'eau distillée

❖ Solution Tris (0,4 M), EDTA (0,02 M) pH 9,6 :

❖ Dissoudre 12,114 g Tris et 1,871 g EDTA dans 250 ml d'eau distillée et ajuster le pH à 9,6. DTNB (0,01 M) :

Dissoudre 20 mg DTNB dans 5 ml de méthanol absolu (préparation le jours meme).

5. Dosage de l'activité enzymatique de la glutathion peroxydase (GSH-Px)

- GSH (0.1 mM) :

Dissoudre 3,073 mg GSH dans 100 ml d'eau distillée.

- TCA (1%) :

Dissoudre 1 g TCA dans 100 ml d'eau distillée.

- DTNB (1,0 mM) :

Dissoudre 20 mg DTNB dans 50 ml de méthanol absolu.

6. Dosage de l'activité enzymatique de la Glutathion-S-Transférase (GST)

- Tampon phosphate (0.1 M, pH 6) :

Solution A, dissoudre 3.58g Na_2HPO4 (12 H2O) dans 100 ml d'eau distillée.

Solution B, dissoudre 1.38 g NaH_2PO4 (2 H2O) dans 100 ml d'eau distillée.

Mélanger 12.3 ml de la solution A avec 87.7 ml de la solution B

- Mélange CDNB (1mM) GSH (5mM) :

Dissoudre 20,26 mg CDNB et 153.65 mg GSH dans 1 ml d'éthanol absolu puis dans 100 ml de tampon phosphate.

7. Dosage de l'activité enzymatique de la superoxyde dismutase (SOD)

- Le tampon phosphate (50 mM), PH = 7, 8 :

Na2HPO4 : 4,476g, NaH2PO4 : 1,95g : dans 250ml d'eau distillée

- La solution de Na2 – EDTA : 50mg dans 50 ml de tampon phosphate. La solution EDTA – Méthionine (0,1 mM, 13 mM) :

11,16 ml (Na2 – EDTA) + 580 mg Méthionine et compléter à 100 ml par le tampon phosphate.

- La solution de NBT (75 µM) :

10, 79 mg dans 5ml de tampon phosphate.

- La solution de Riboflavine (2 µM) :

10 mg dans 100 ml de tampon phosphate.

8. Dosage de l'activité enzymatique de la catalase

- Tampon phosphate (0.1 M, pH 7.4) :

Solution A, dissoudre 6,24 g NaH2PO4 (2 H2O) dans 200 ml d'eau distillée.

Solution B, dissoudre 14.32 g Na2HPO4 (12 H2O) dans 200 ml d'eau distillée.

Mélanger 16 ml de la solution A avec 84 ml de la solution B.

COMMUNICATIONS SCIENTIFIQUES

People's Democratic Republic of Algeria, Ministry of High Education and Scientific Research

Badji Mokhtar University-Annaba. Faculty of Sciences - Department of Biochemistry

Laboratory of Biochemistry and Environmental Toxicology

CERTIFICATE OF ATTENDENCE

Boudjema Kahina

For attending : **Applied Research in Food Sciences, Health and Environment (1st IS-ARFSHE 23)**
December, 17-18th 2023, Annaba

And Presenting an **Poster Communication** entitled :

The ameliorative effect of Moringa *Oleifera leaves* against abamectin-induced brain oxidative damages in Wistar rats

Authors : Boudjema Kahina, Chouala Khadidja, Boumendjel Amel, Messarah Mahfoud.

President of the Scientific Committee

Pr Boudjema SAMRAOUI
President of Scientific Committee
1st ISARFSHE 2023

Head of Biochemistry Department

الأستاذ الدكتور:
برانس زيدان

CERTIFICATE Of PARTICIPATION

Boudjema Kahina

has participated in 1st International Conference on Scientific and Academic Research on 10-13 December in 2022 at Konya/Turkey.

PAPER TITLE	*Antioxidant Potential and Phytochemical Analysis of Moringa Oleifera*
PRESENTATION TYPE	*Oral*

ICSAR

ICSAR 2022 CONFERENCE CHAIRMAN

Asst. Prof. Dr. Umut ÖZKAYA

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Département des Sciences Naturelles

ATTESTATION
DE PARTICIPATION

N°: D. CO. 4

Le comité scientifique du 1er Séminaire National sur Plantes, Molécules Bioactives et Valorisation (SN-PMBV-2022), organisé les 22 et 23 Novembre 2022, atteste que:

Mme. BOUDJEMA Kahina

a présenté une **communication orale.**

Titre: ÉTUDE DE L'EFFET NEPHROPROTECTEUR DU MORINGA OLEIFERA SUR LE STRESS OXYDANT INDUIT PAR L'ABAMECTINE.

Co-auteur: CHOUALA Khadidja, BOUMENDJEL Amel et MESSARAH Mahfoud.

Le directeur adjoint
Dr. OUMELAZ Fayça

La présidente du séminaire
Dr. HENI Sonia

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LABORATOIRE DE TOXICOLOGIE MOLECULAIRE

5èmes Journées Scientifiques Annuelles du Laboratoire de Toxicologie Moléculaire
23-24 février 2022, Université de Jijel, Algérie

ATTESTATION DE PARTICIPATION

Le Comité Scientifique ***des 5èmes Journées Scientifiques Annuelles du Laboratoire de Toxicologie Moléculaire*** *atteste que*

Mme. Kahina BOUDJEMA

a présenté une communication ***affichée*** *intitulée :*

«Effet protecteur d'un régime alimentaire enrichi en acide gallique sur la toxicité induite par l'abamectine chez des rattes femelles Wistar»

Co-auteurs : *CHOUALA K., BOUMENDJEL A., & MESSARAH M.*

Présidente des 5èmes Journées du Laboratoire de Toxicologie Moléculaire

Dr. Lamia BENGUEDOUAR

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