الجمـهورية الجـزائرية الديمقـراطية الشعبيـة

وزارة التعليـم العالـي والبحـث العلمـي

Ministry of Higher Education and Scientific Research

University of Ferhat Abbas – Sétif 1
Faculty of Natural and Life Sciences
Department of Biochemistry

جامعـة فرحـات عبـاس - سطيـف1
كليـــة علوم الطبيعة و الحياة
قســـــم البيوكيمياء

Master's Dissertation.

Submitted in partial fulfillment of the requirements for the degree of:

Master of Science .

Major : Biology

Option : Applied Biochemistry

Theme:

Sub-Acute Toxicity of Alkaloids Extract from *Drimia maritima* in Pregnant & Non-Pregnant Females of Wistar Rats.

Presented by:

FADLI Hazar, DEROUICHE Fatiha & DJABOU Chaima

Jury :

President : Dr. MAHDEB Nadia	**MCA**	University of Ferhat Abbas – Sétif 1
Supervisor : Pr. BOUZIDI Abdelouahab	**Professor**	University of Ferhat Abbas – Sétif 1
Examinator : Dr. GHADJATI Nadra	**MCB**	University of Ferhat Abbas – Sétif 1

Academic year : 2024-2025

Acknowledgment:

First and foremost, our deepest gratitude goes to the Almighty Allah, whose endless mercy, guidance, and blessings made this journey possible. We are truly thankful for the good health, strength, clarity of mind, and patience that He granted us throughout every stage of this research. Without His divine will and support, none of this work could have been accomplished.

We would like to begin these acknowledgments with hearts full of gratitude and deep emotion for someone who was not only our supervisor, but truly like a father throughout this journey **Professor Abdelouahab Bouzidi**. His presence, his guidance, and his remarkable human qualities shaped both this work and the people we became while completing it. He did not simply supervise us he cared, he listened, he motivated, and he believed in us. His kindness, wisdom, and patience accompanied us during the most delicate phases of this research. We will always carry with us the sincerity of his words, the strength of his support, and the immense impact he had on our academic and personal lives. For all of this and more, we say thank you from the deepest part of our hearts.

We would also like to extend our sincere thanks to **Dr. Mahdeb Nadia**, his wife, for her gentle presence and the support she offered during this work. Her advice, encouragement, and quiet kindness added warmth and reassurance at important moments. We are truly grateful for her thoughtful contributions.

Our most heartfelt and special thanks go to **Rahma Hassaine**, a name we will never forget. She was, without a doubt, the reason the histopathological study came to life. More than a collaborator, she was a guide, a generous helping hand, and a source of genuine support when it was needed the most. She welcomed us with openness, offered her time and effort with sincerity, and did everything she could to ensure that this critical part of the research was accomplished. Her trust in the value of our work and her dedication to helping us complete it went far beyond what was expected. We owe her more than simple thanks **we owe her a deep and lasting respect**, for both her scientific mind and her generous heart.

We also warmly thank **Dr. Ben Daoued Soumia**, whose openness and welcoming spirit made it possible for us to carry out the hematological and biochemical analyses. She received us with kindness and made the laboratory available in a way that was both professional and human. Her collaboration and support helped push this research forward and were deeply appreciated.

A respectful thanks goes to **Dr. Merzouki**, who authorized us to carry out the hormonal analyses in her laboratory. Even though this service was conducted on a paid basis, her willingness to make the facility accessible contributed to completing this phase of the study.

We also extend our gratitude to **Dr. Allouni Rima**, whose presence and advice during the practical sessions were meaningful and motivating. Her insights helped refine our work and encouraged us during challenging moments in the lab.

Dedication

To **myself**,
For holding on through the hard days, for choosing perseverance over giving up, and for growing through every challenge—you made it, and I'm proud of you.

To **my mother**,
Your love is my greatest strength. Your prayers, your patience, and your unwavering belief in me carried me when I couldn't carry myself. This is yours as much as it is mine.

To **my father**,
Thank you for your quiet support and constant presence. Your trust gave me comfort, and your pride gave me courage.

To **my brothers Ahmed & Abderrahmane**,
My brothers, my joy. Thank you for your laughter and your encouragement. You reminded me to smile when things felt heavy.

To **my sweet grandmother**,
Your prayers protected me, your love gave me peace. You are a blessing in every sense.

To **my aunts & uncles**,
Thank you for your warmth, your kindness, and your constant support. Your love has always lifted me.

To **Fatiha & Chaima**,
Thank you for walking this journey with me. Your effort, energy, and friendship made all the difference.

To **my teachers & professors**,
Seventeen years of learning shaped by your dedication. Thank you for every lesson and every push forward.

And to **my loved ones**,
Thank you for listening, for staying close, and for being my safe space. Your presence gave me the strength to keep going.

Hazar

To myself,

for every sleepless night, silent struggle, and moment of doubt—for choosing to keep going when it would have been easier to give up. This accomplishment stands as a reminder of my strength, growth, and unwavering determination.

To my beloved father, Ahmed

your presence in my life has been my greatest blessing. You are my foundation, my role model, and the silent force behind every success I've achieved. Your sacrifices, though quiet, were endless. Your love, though unspoken, was always felt. Every step of this journey was guided by your strength and your unwavering belief in me. This work is for you, Baba. May God bless you with peace, health, and long life.

To my dear mother,

thank you for your limitless love, your comforting words, and your constant prayers. You are the heart of my strength and the light that guided me through every challenge.

To my sister Samia, & to my brothers Nabil, Ali, Lyes, Hichem, & Aymen

thank you for your love, support, and pride. A warm thank you to Saida and Chaïma for your kindness and encouragement throughout this journey.

To my wonderful thesis companions, Hazar &Chaïma

your support made the difficult moments easier, and your presence meant more than words can say.

To my dear friends Imen, Asma, Manar, & Kaouther,

thank you for bringing joy, strength, and friendship when I needed it most.

Fatiha.

To myself
For every effort I made throughout my academic years.
For every step I took that cost a piece of my soul so I could learn something new.
Thank you for staying strong, for pushing forward, and for believing in your dreams.

To my mother
The great woman who gave me life,
Who embraced me with unconditional love,
Who has always been my safe place, full of warmth that never runs out.Thank you for everything.

To my father
My support, my security,
The hand that held me tightly in this life.
Thank you for always being there, with care and strength.

To my brothers :
Abdelhakim,my role model ,the one who helped shape my character.Thank you for being such a strong and inspiring presence in my life.To Abdelnaser and Mouhamed Abdelmoez, thank you for surrounding me with your love and constant support. I'm truly grateful for your presence.

To my sisters :
To Abla, my second mother, the source of tenderness.Thank you for raising me and caring for me with so much love. To Asma,the one who has always been one step ahead since the day we met,The hand that never let go. Thank you for walking beside me all thecaring.To Nourhene, may you always remain my support,
A beautiful heart that fills my life with kindness and light.

To my companions on this journey Fatiha and Hazar thank you for being with me through it all.

To the friends life gifted me: Kawthar, Lilia, Hedia, and Fatma – the one with the kindest heart.
Thank you for the laughter, for the support, and for the memories.

To everyone who taught me a letter, a word, or a sentence,
To all my teachers throughout my academic journey –
With all my love and appreciation.
You have helped shape the person I am today.

Chaima

الملخص

تُعدّ نبتة بصل العنصل (*Drimia maritima*) من النباتات البصلية التي يعود أصلها إلى حوض البحر الأبيض المتوسط، بما في ذلك الجزائر. لطالما كانت هذه النبتة مكوناً أساسياً في الطب التقليدي، حيث استُخدمت لخصائصها القلبية المدعمة، والمدرّة للبول، والمضادة للالتهابات. وعلى الرغم من استعمالها لعلاج حالات مثل الربو واليرقان، إلا أنها تُعرف بسمّيتها العالية، التي تُعزى إلى احتوائها على مركبات قوية وفعالة، أبرزها البوفادينوليدات، الهدف من هذه الدراسة هو إجراء تحليل كيميائي نباتي لمستخلص القلويدات الموجود في وتقييم سُميته بشكل شامل. وقد ركّزت الدراسة بشكل خاص على تأثير المستخلص على المؤشرات البيوكيميائية والدموية، بالإضافة إلى دراسة تأثيره على تطور الأجنة لدى إناث فئران ويستار.

تم جمع البصلات من منطقة سطيف الواقعة شمال شرق الجزائر. وتم استخراج القلويدات الكلية من المادة النباتية الطازجة عبر تقنية الاستخلاص السائل-السائل، ما أسفر عن مردود يُقدّر بحوالي 0.036% من المادة الخام. وأُكّد وجود القلويدات في المستخلص النهائي من خلال تحليل نوعي باستخدام كروماتوغرافيا الطبقة الرقيقة (TLC)، مع كاشف دراغندورف كمادة كاشفة.

شملت الدراسة استعمال إناث فئران ويستار بالغة وسليمة لإجراء اختبار السمية شبه الحادة، حيث تلقّت مجموعة المعالجة جرعة فموية يومية قدرها 5.1761 ملغ/كغ لمدة خمسة أيام، وهي تعادل 100/1 من الجرعة المميتة المتوسطة (LD50)، المبلّغ عنها. وشمل التقييم مراقبة تطور الوزن الجسماني ووزن الأعضاء، بالإضافة إلى إجراء تحاليل دموية وكيميائية حيوية لتقييم وظائف الكبد والكلى، وتحليل مستويات الهرمونات التناسلية.

لم تُسجَّل أي وفيات خلال اختبار السمية، لكن ظهرت علامات سريرية خفيفة وعابرة، مثل تسارع في ضربات القلب وتشنجات طفيفة بعد الجرعة. فيما يخص الفئران غير الحوامل، لوحظ انخفاض كبير في الوزن الجسماني في نهاية التجربة، مقارنةً بالمجموعة الضابطة. لم تُسجَّل تغيّرات ظاهرية في الأعضاء، إلا أنه لوحظ ارتفاع معنوي في الوزن النسبي للطحال.

كشفت التحاليل البيوكيميائية عن انخفاض معنوي ومفاجئ في مستويات اليوريا والكرياتينين، إلى جانب انخفاض في إنزيمات الكبد AST و ALT ، أما التحاليل الدموية، فأظهرت انخفاضًا معنويًا في عدد الصفائح الدموية (PLT) ومؤشر كريات الصفيحات (PCT) والهيموغلوبين (HGB)

بالنسبة للفئران الحوامل، لم يُسجَّل تغيّر في وزن الأمهات خلال فترة الحمل، إلا أن المستخلص أظهر مؤشرات واضحة على وجود سمية نمائية. إذ سُجّل انخفاض كبير جدًا بنسبة تُقدَّر بحوالي 81.93% في متوسط وزن الأجنة في مجموعة المعالجة مقارنةً بالمجموعة الضابطة، مما يدل على تقييد شديد للنمو داخل الرحم.، كما لوحظ انخفاض في عدد الأجنة، غير أن هذا التغيير لم يكن معنويًا. أما التحاليل الهرمونية، فقد أظهرت ارتفاعًا معنويًا في مستوى هرمون البروجسترون لدى الإناث المعالَجة، تُشير نتائج هذه الدراسة إلى أن مستخلص قلويدات (*Drimia maritima*) ، حتى بجرعة منخفضة ولفترة قصيرة، يُمكن أن يُسبب سمية معتدلة قابلة للعكس، مع تأثير واضح على وظائف الأعضاء والتكاثر. يُبرز الانخفاض الحاد في وزن الأجنة خطورة هذا المستخلص على تطور الأجنة، ما يُعزّز فرضية عبور المركبات السامة للمشيمة. كما أن الانخفاض غير المتوقع في مؤشرات الكبد والكلى يستدعي مزيدًا من البحث لفهم آلية تأثير هذه القلويدات..

ورغم أهمية النتائج التي توصلنا إليها، لا تزال المركبات الفعالة المسؤولة عن هذه التأثيرات غير معروفة. من الضروري مستقبلاً عزل هذه القلويدات وتحديد تركيبها البنيوي، إلى جانب دراسة آليات تأثيرها بدقة، لتحقيق فهم أعمق للتوازن بين الإمكانات العلاجية لهذا النبات والمخاطر السمية المرتبطة به.

الكلمات المفتاحية:نبات العنصل، السمية الجنينية، السمية شبه الحادة، جرذان ويستار، فئران ويستر، المؤشرات البيوكيميائية، التحليل الدموي، السمية التطورية، البروجستيرون،فقر الدم.

Abstract:

Drimia maritima, commonly known as sea squill, is a bulbous plant native to the Mediterranean basin, including Algeria. It has been used for centuries in traditional medicine for its cardiotonic, diuretic, and anti-inflammatory properties. Despite its therapeutic uses for conditions like asthma and jaundice, the plant is also known for its high toxicity, mainly due to potent compounds such as bufadienolides. Its toxicity is so notable that it has historically been used as a rodenticide.

The primary aim of this study was to perform a phytochemical analysis of the alkaloids present in *Drimia maritima* bulbs and to carry out a comprehensive toxicity assessment. Specifically, the study sought to evaluate its effects on biochemical and hematological markers and embryonic development in female Wistar rats.

Bulbs of *Drimia maritima* were collected from the Sétif region in northeastern Algeria. Total alkaloids were extracted from fresh plant material using a liquid-liquid extraction method, with a yield of approximately 0.036% from the raw plant material. The presence of alkaloids was confirmed in the final extract using thin-layer chromatography (TLC) with Dragendorff's reagent as a detection agent.

For toxicity evaluation, healthy adult female Wistar rats were used. A sub-acute toxicity study was conducted, where the treated group received a daily oral dose of 5.1761 mg/kg for five days. This dose corresponds to 1/100 of the reported LD_{50}. Assessments included body and organ weight monitoring, complete hematological and biochemical analysis (to evaluate liver and kidney function), and reproductive hormone measurements.

To assess embryotoxicity, pregnant females were treated during gestation. On gestation day 20 (GD20), they were sacrificed, and the number and weight of fetuses were carefully examined.

No mortality was observed during the sub-acute test. However, transient mild clinical signs such as tachycardia and slight convulsions were recorded shortly after administration.

In non-pregnant rats, significant body weight loss was observed in the treated group. While no gross anatomical changes in organs were noted, a statistically significant increase in the relative spleen weight was recorded. Biochemical analysis revealed a notable and significant decrease in serum urea and creatinine levels (renal markers) and in liver enzymes AST and ALT. Hematological evaluation showed a significant reduction in platelet count (PLT) and plateletcrit (PCT).

In pregnant rats, maternal body weight remained stable throughout gestation. However, the extract showed strong signs of developmental toxicity. The average pup weight in the treated group was reduced by approximately 81.93%, indicating severe intrauterine growth restriction. The number of pups also decreased, although this change was not statistically significant. Hormonal analysis revealed a significant increase in progesterone levels in treated females.

These findings confirm that the alkaloid extract of *Drimia maritima*, even at a low sub-acute dose, induces moderate and potentially reversible toxicity. It clearly affects organ function and reproductive outcomes. The sharp decline in pup weight points to a major embryotoxic risk, likely due to placental transfer of toxic compounds.

The atypical decrease in liver and kidney markers is unusual for a toxic substance and warrants further investigation into the specific mechanism of action of this extract. While this research provides crucial preliminary data, the specific alkaloids responsible for these effects remain unidentified. Future work should focus on isolating and structurally characterizing these active compounds and exploring their mechanisms to better understand the balance between the plant's therapeutic potential and its toxicological risks.

Keywords: *Drimia maritima*, alkaloid extract, embryotoxicity, sub-acute toxicity, Wistar rats, biochemical markers, hematological analysis, developmental toxicity, progesterone, anemia.

Résumé:

La plante *Drimia maritima*, connue sous le nom d'oignon marin, est une espèce bulbeuse originaire du bassin méditerranéen, y compris l'Algérie. Elle est utilisée depuis des siècles dans la médecine traditionnelle pour ses propriétés cardiotoniques, diurétiques et anti-inflammatoires. Malgré ses usages thérapeutiques contre l'asthme ou l'ictère, elle est aussi connue pour sa toxicité élevée, en raison de la présence de composés puissants, notamment les bufadiénolides. Cette toxicité est telle que la plante a été historiquement utilisée comme raticide.

Cette étude a pour objectif d'analyser le profil phytochimique des alcaloïdes extraits des bulbes de *Drimia maritima* et d'évaluer leur toxicité. Elle s'intéresse particulièrement à leurs effets sur les paramètres biochimiques, hématologiques, et sur le développement embryonnaire chez les femelles de rats Wistar.

Les bulbes ont été récoltés dans la région de Sétif, au nord-est de l'Algérie. Les alcaloïdes totaux ont été extraits à partir de matériel végétal frais par extraction liquide-liquide, donnant un rendement d'environ 0,036 %. La présence des alcaloïdes a été confirmée par chromatographie sur couche mince (CCM) avec le réactif de Dragendorff.

Pour l'étude de la toxicité, des femelles adultes et saines de rats Wistar ont été utilisées. Une étude de toxicité subaiguë a été menée avec une dose quotidienne par voie orale de 5,1761 mg/kg pendant cinq jours, correspondant à 1/100 de la DL_{50} rapportée. L'évaluation a inclus le suivi du poids corporel et des organes, des analyses sanguines (hématologiques et biochimiques concernant le foie et les reins) et l'analyse des hormones de reproduction.

Pour la toxicité embryonnaire, les femelles ont été traitées pendant la gestation. Le 20e jour de gestation (GD20), elles ont été sacrifiées et le nombre ainsi que le poids des fœtus ont été examinés.

Aucun décès n'a été enregistré pendant l'étude subaiguë, bien que des signes cliniques transitoires de toxicité légère aient été observés (tachycardie et spasmes mineurs après l'administration).

Chez les femelles non gravides, une perte significative de poids corporel a été observée dans le groupe traité. Aucune anomalie visible n'a été détectée dans les organes, mais une augmentation significative du poids relatif de la rate a été notée. Les analyses biochimiques ont montré une baisse marquée de l'urée et de la créatinine sériques (fonctions rénales), ainsi qu'une diminution des enzymes hépatiques AST et ALT.

L'analyse hématologique a révélé une baisse significative du nombre de plaquettes (PLT) et du pourcentage plaquettaire (PCT).Chez les femelles gestantes, le poids corporel est resté stable pendant toute la période. Toutefois, le traitement a induit une toxicité développementale marquée : le poids moyen des fœtus du groupe traité a diminué d'environ 81,93 % par rapport au groupe témoin, traduisant un retard sévère de croissance intra-utérine. Le nombre de fœtus a également diminué, mais sans signification statistique. Sur le plan hormonal, une augmentation significative du taux de progestérone a été observée chez les femelles traitées.

Les résultats suggèrent que l'extrait alcaloïdique de *Drimia maritima*, même à faible dose et sur une courte durée, peut induire une toxicité modérée potentiellement réversible, affectant les fonctions organiques et reproductives. La baisse importante du poids fœtal indique un effet embryotoxique notable, probablement lié au passage placentaire des composés toxiques.

La baisse inhabituelle des marqueurs hépatiques et rénaux nécessite des recherches supplémentaires pour élucider les mécanismes d'action de ces alcaloïdes. Bien que cette étude fournisse des données importantes, les composés actifs spécifiques restent à identifier. Des travaux futurs sont nécessaires pour isoler, caractériser et comprendre les mécanismes moléculaires de ces substances, afin d'évaluer le potentiel thérapeutique de cette plante face à ses risques toxiques.

Mots clés : *Drimia maritima*, extrait alcaloïdique, embryotoxicité, toxicité subaiguë, rats Wistar, paramètres biochimiques, analyse hématologique, toxicité du développement, progestérone, anémie**.**

- ***** : Significant difference, $P < 0.05$
- **ALAT** : Alanine Aminotransferase
- **ASAT** : Aspartate Aminotransferase
- **BCE** : Before Common Era
- **CBC** : Complete Blood Count
- **D. altissima** : *Drimia altissima*
- **D. maritima** : *Drimia maritima*
- **D. robusta** : *Drimia robusta*
- **D. sanguinea** : *Drimia sanguinea*
- **EDTA** : Ethylenediaminetetraacetic Acid
- **ERα** : Estrogen Receptor subtype alpha
- **Erβ** : Estrogen Receptor subtype beta
- **FSH** : Follicle Stimulating Hormone
- **GD** : Gestational Day.
- **GnRH** : Gonadotropin Releasing Hormone
- **H&E** : Hematoxylin and Eosin
- **HPLC-ESI** : High Performance Liquid Chromatography – Electrospray Ionization
- **HPO** : Hypothalamic Pituitary Ovarian
- **ICM** : Inner Cell Mass
- **IGFs** : Insulin-like Growth Factors
- **IUGR** : Intrauterine Growth Restriction
- **LC-MS** : Liquid Chromatography – Mass Spectrometry
- **LD50** : Lethal Dose 50%
- **LH** : Luteinizing Hormone
- **NMR** : Nuclear Magnetic Resonance Spectrometry
- **TLC** : Thin Layer Chromatography Assay
- **TOF-MS** : Time of Flight – Mass Spectrometry
- **U. indica** : *Urginea indica*
- **U. wightii** : *Urginea wightii*
- **Var** : Variation

Chapter II : Material & Methods.