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## THEME

*Bioorganometallic Safety Assessment of Ferrocene-Based*

*Candidates: Rat Cytotoxicity Benchmarked Against Computational  
Toxicology*

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# إسراء

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لينت



## Abstract

The growing pharmaceutical interest in ferrocene derivatives is attributed to their unique physicochemical and redox properties, which may enhance biological activity and therapeutic potential. However, toxicological safety assessment remains essential prior to their biomedical application. Therefore, the present study aimed to evaluate the acute toxicological profile and biological safety of three ferrocene derivatives: **N-Ferrocenylmethylaniline (FMA)**, **N-Ferrocenylmethyl-N-acetylaniline (FCMphA)**, and **N-Ferrocenylmethyl-N-benzoylaniline (FCMphB)**, using complementary **in vivo** and **in silico** approaches.

The *in vivo* study was performed on **adult Wistar** rats of both **sexes exposed** to graded doses (**0.1, 1, 10, and 100 mg/kg**). Toxicological evaluation was based on **clinical observations**, **body weight evolution**, **food and water intake**, **relative organ weights**, **hematological** and **biochemical analyses**, and **histopathological examination** of major organs, including **the liver**, **kidneys**, and **brain**, in order to determine dose-dependent toxic effects and organ-specific alterations.

In parallel, the **in silico** investigation involved **ADMET** prediction and **molecular docking** analysis to evaluate pharmacokinetic properties, drug-likeness, absorption profile, biological distribution, and toxicity prediction of the tested compounds. Molecular docking was additionally conducted to investigate ligand–protein interactions with selected target proteins, including **CYP3A4** and **KEAP1**, to better understand the relationship between molecular structure and biological behavior.

The experimental findings revealed the absence of mortality at all administered doses, indicating relatively low acute toxicity of the tested compounds. Nevertheless, several dose-related clinical, physiological, biochemical, and histopathological alterations were observed.

Among the investigated derivatives, **FCMphA** demonstrated the highest toxicological effect, showing more pronounced organ alterations and biological disturbances. In contrast, **FCMphB** exhibited the best biological tolerance and a comparatively safer toxicological profile, while **FMA** induced moderate and mostly reversible effects. Furthermore, the computational analyses supported the experimental observations, revealing differences in absorption, blood–

brain barrier permeability, and protein-binding affinity among the compounds. Docking results showed strong molecular interactions, particularly for **FCMphB**, suggesting a favorable pharmacological profile.

In conclusion, the tested ferrocene derivatives demonstrated promising pharmaceutical potential with relatively low acute toxicity; however, structural modifications significantly influenced their toxicological and biological behavior. The integration of experimental and computational findings highlights the importance of comprehensive toxicological evaluation to support the safe development of ferrocene-based therapeutic candidates.

**Keywords:** Ferrocene derivatives; acute toxicity; in vivo study; in silico analysis; molecular docking; ADMET; Wistar rats; toxicological assessment; lipophilicity; bioorganometallic chemistry.

## ملخص:

يُعزى الاهتمام الصيدلاني المتزايد بمشتقات الفيروسين إلى خصائصها الفيزيائية-الكيميائية والفريدة من نوعها، خاصة خصائص الأكسدة والاختزال، والتي قد تسهم في تعزيز النشاط البيولوجي والإمكانات العلاجية. ومع ذلك، يبقى تقييم السلامة السمية أمراً أساسياً قبل تطبيقها في المجال الطبي الحيوي. لذلك، هدفت هذه الدراسة إلى تقييم السمية الحادة والسلامة البيولوجية لثلاثة مشتقات من الفيروسين :

**N-Ferrocenylmethylaniline (FMA)**،

**N-Ferrocenylmethyl-N-acetylaniline (FCMphA)** و

، و **N-Ferrocenylmethyl-N-benzoylaniline (FCMphB)**،

، وذلك باستخدام منهجين متكاملين: داخل الجسم الحي (In vivo) والحاسوبي (In silico).

أُجريت الدراسة داخل الجسم الحي على جرذان ويستر البالغة من كلا الجنسين، حيث عُرِضت لجرعات متدرجة (0.1، 1، 10، و100 ملغ/كغ). واستند التقييم السمي إلى الملاحظات السريرية، وتطور وزن الجسم، واستهلاك الغذاء والماء، والأوزان النسبية للأعضاء، والتحاليل الدموية والكيميائية الحيوية، إضافةً إلى الفحص النسيجي المرضي للأعضاء الرئيسية، بما في ذلك الكبد والكلَى والدماغ، بهدف تحديد التأثيرات السمية المعتمدة على الجرعة والتغيرات الخاصة بكل عضو.

بالتوازي مع ذلك، شملت الدراسة الحاسوبية التنبؤ بخصائص ADMET وتحليل الإرساء الجزيئي (Molecular Docking) لتقييم الخصائص الحركية الدوائية، ومدى ملاءمة المركبات كأدوية، وخصائص الامتصاص، والتوزيع الحيوي، والتنبؤ بالسمية للمركبات المدروسة. كما أُجري الإرساء الجزيئي لدراسة تفاعلات الليغاند-البروتين مع بروتينات مستهدفة مختارة، من بينها CYP3A4 وKEAP1، بهدف فهم العلاقة بين البنية الجزيئية والسلوك البيولوجي.

أظهرت النتائج التجريبية عدم تسجيل أي حالات وفاة عند جميع الجرعات المعطاة، مما يشير إلى انخفاض نسبي في السمية الحادة للمركبات المختبرة. ومع ذلك، لوحظت عدة تغيرات سريرية، وفسيولوجية، وكيميائية حيوية، ونسجية مرضية مرتبطة بالجرعة. ومن بين المشتقات المدروسة، أظهر FCMphA أعلى تأثير سمي، حيث تسبب في تغيرات عضوية واضطرابات بيولوجية أكثر وضوحاً. في المقابل، أظهر FCMphB أفضل قدرة على التحمل البيولوجي وملفًا سميًا أكثر أمانًا نسبيًا، بينما أحدث FMA تأثيرات متوسطة وقابلة للعكس في معظمها. علاوةً على ذلك، دعمت التحليلات الحاسوبية النتائج التجريبية، إذ كشفت عن اختلافات في الامتصاص، ونفاذية الحاجز الدموي الدماغي، وألفة الارتباط بالبروتينات بين المركبات.

كما أظهرت نتائج الإرساء الجزيئي تفاعلات جزيئية قوية، خاصة بالنسبة لـ FCMphB، مما يشير إلى ملف دوائي

واعد.

في الختام، أظهرت مشتقات الفيروسين المختبرة إمكانات صيدلانية واعدة مع مستوى منخفض نسبيًا من السمية الحادة، إلا أن التعديلات البنوية أثرت بشكل ملحوظ على سلوكها السمي والبيولوجي. وتبرز أهمية دمج النتائج التجريبية والحاسوبية في إجراء تقييم سمي شامل لدعم التطوير الآمن لمركبات علاجية قائمة على الفيروسين.

**الكلمات المفتاحية:** مشتقات الفيروسين؛ السمية الحادة؛ الدراسة داخل الجسم الحي؛ التحليل الحاسوبي؛ الإرساء الجزيئي؛ ADMET؛ جردان ويستر؛ التقييم السمي؛ محبة الدهون؛ الكيمياء العضوية-الفلزية الحيوية

## LIST OF ABBREVIATIONS

**ADMET** : Absorption, Distribution,  
Metabolism, Excretion, and Toxicity

**ALT** :Alanine Aminotransferase

**AST** : Aspartate Aminotransferase

**ATN**:Acute Tubular Necrosis

**ATP**:Adenosine Triphosphate

**BBB**: Blood–Brain Barrier

**CNS** :Central Nervous System

**CT** : Creatinine

**CYP3A4**:Cytochrome P450 3A4

**CYP450** : Cytochrome P450

**D1**: 0.1mg/kg Dose

**D2**: 1 mg/kg Dose

**D3**: 10 mg/kg Dose

**D4**:100mg/kg Dose

**DILI**:Drug-Induced Liver Injury

**EDTA** : Ethylene diamine tetraacetic Acid

**FBS** : Fasting Blood Glucose

**FAM**:N-Ferrocenylmethylaniline,

**KEAP1**:Kelch-like ECH-associated Protein 1

**FCMphA** : N-Ferrocenylmethyl-N-  
acetylaniline

**FCMphB** : N-Ferrocenylmethyl-N-  
benzoylaniline

**Fe**: iron

**Fe<sup>2+</sup>**:Ferrous ion

**Fe<sup>3+</sup>** : Ferrous ion

**fL**: Femtoliter

**GFR**:Glomerular Filtration Rate

**g** : Gram

**g/dL** : Gram per Deciliter

**g/L**: Gram per Liter

**g/ml**: gram per Milliliter

**GRAN** : Granulocytes

**H&E** : Hematoxylin and Eosin

**Hb/HGB** : Hemoglobin

**HCT** : Hematocrit

**IR**: Infrared Spectroscopy

**kcal/mol** : Kilocalories per Mole

**LD<sub>50</sub>** : Median Lethal Dose

**LOAEL:** Lowest Observed Adverse Effect  
Level

**LYM :** Lymphocytes

**MCH :** Mean Corpuscular Hemoglobin

**MCHC :** Mean Corpuscular Hemoglobin  
Concentration

**MCV :** Mean Corpuscular Volume

**mg/kg :** Milligram per Kilogram

**MMFF94:** Merck Molecular Force Field

**NMR:** Nuclear Magnetic Resonance

**NMDR:** Non-Monotonic Dose–Response

**NOAEL :**No Observed Adverse Effect  
Level

**OECD:** Organisation for Economic Co-  
operation and Development

**PDB :** Protein Data Bank

**PDB ID :** Protein Data Bank Identifier

**pg :**Picogram

**PLT :** Platelets

**$\pi$ – $\pi$  Stacking :**Pi–Pi Stacking Interaction

**PyMOL:**Molecular visualization Software

**RBC :** Red Blood Cells

**ROS:** Reactive Oxygen Species

**rpm :** Revolutions per Minute

**SAR :** Structure–Activity Relationship

**TPSA :** Topological Polar Surface Area

**U/L :** Units per Liter

**Vd:**volume of Distrubution

**Vp:** Plasma volume

**Vt:** Total volume

**WBC :** White Blood Cells

**$\eta^5$  (Eta-5) :** Hapticity notation describing the  
coordination of iron to the cyclopentadienyl  
rings in ferrocene complexes. involved in  
redox reactions.

**rpm :** Revolutions per Minute

**U/L:** Units per Liter

**$\mu$ m :**Micrometer

**°C:** Degree Celsius

**$\times 10^9/L$  :** Cell concentration unit

## LIST OF FIGURES

| Number          | Title                                                                                                                                                                                                  | Page      |
|-----------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| <b>Figure1</b>  | Ferrocene powder under vacuum.                                                                                                                                                                         | <b>3</b>  |
| <b>Figure2</b>  | Structure corrected iron.                                                                                                                                                                              | <b>4</b>  |
| <b>Figure3</b>  | IR spectra of ferrocene.                                                                                                                                                                               | <b>5</b>  |
| <b>Figure4</b>  | Oxidation reaction of ferrocene to ferrocenium ion.                                                                                                                                                    | <b>5</b>  |
| <b>Figure5</b>  | Structure crystalline of ferrocene.                                                                                                                                                                    | <b>6</b>  |
| <b>Figure6</b>  | General diagram of the pathway of toxic substances in the body.                                                                                                                                        | <b>12</b> |
| <b>Figure7</b>  | Atypical dose-response curve.                                                                                                                                                                          | <b>13</b> |
| <b>Figure8</b>  | Histopathological evaluation of hepatic Tissue following acute exposure to a ferrocene derivative ( <b>FMA</b> ) across increasing dose levels in male and female rats, Magnification $\times 40$ .    | <b>70</b> |
| <b>Figure9</b>  | Histopathological evaluation of renal tissue following acute exposure to a ferrocene derivative ( <b>FMA</b> ) across increasing dose levels in male and female rats, Magnification $\times 40$ .      | <b>72</b> |
| <b>Figure10</b> | Histopathological evaluation of brain tissue following acute exposure to a ferrocene derivative ( <b>FMA</b> ) across increasing dose levels in male and female rats, Magnification $\times 40$ .      | <b>74</b> |
| <b>Figure11</b> | Histopathological evaluation of hepatic Tissue following acute exposure to a ferrocene derivative ( <b>FCMphA</b> ) across increasing dose levels in male and female rats, Magnification $\times 40$ . | <b>76</b> |
| <b>Figure12</b> | Histopathological evaluation of renal tissue following acute exposure to a ferrocene derivative ( <b>FCMphA</b> ) across increasing dose levels in male and female rats, Magnification $\times 40$ .   | <b>77</b> |
| <b>Figure13</b> | Histopathological evaluation of brain tissue following acute exposure to a ferrocene derivative ( <b>FCMphA</b> ) across increasing dose levels in male and female rats, Magnification $\times 40$ .   | <b>79</b> |
| <b>Figure14</b> | Histopathological evaluation of hepatic Tissue following acute exposure to a ferrocene derivative ( <b>FCMphB</b> ) across increasing dose levels in male and female rats, Magnification $\times 40$ . | <b>81</b> |

|                 |                                                                                                                                                                                                                     |           |
|-----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| <b>Figure15</b> | Histopathological evaluation of renal tissue following acute exposure to a ferrocene derivative ( <b>FCMphB</b> ) across increasing dose levels in male and female rats, Magnification $\times 40$ .                | <b>82</b> |
| <b>Figure16</b> | Histopathological evaluation of brain tissue following acute exposure to a ferrocene derivative ( <b>FCMphB</b> ) across increasing dose levels in male and female rats, Magnification $\times 40$ .                | <b>84</b> |
| <b>Figure17</b> | Two-dimensional (left) and three-dimensional (right) representations of the binding mode of <b>FMA</b> within the active sites of (a) Cytochrome P450 3A4 (PDB ID: 1TQN) and (b) KEAP1 Kelch domain (PDB ID: 4L7B). | <b>89</b> |

## List of Tables

| Number         | Title                                                                                                                                                                                                                     | Page      |
|----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| <b>Table1</b>  | Toxicity of chemicals applied by different routes of exposure.                                                                                                                                                            | <b>11</b> |
| <b>Table2</b>  | Forms of intoxication.                                                                                                                                                                                                    | <b>14</b> |
| <b>Table3</b>  | Main toxic effects observed in the liver.                                                                                                                                                                                 | <b>15</b> |
| <b>Table4</b>  | Various categories of neurotoxic effects                                                                                                                                                                                  | <b>16</b> |
| <b>Table5</b>  | Clinical signs of acute toxicity observed within 1–4 hours following single-dose administration of <b>FMA</b> at graded dose levels (0.1–100 mg/kg) in male and female experimental animals.                              | <b>31</b> |
| <b>Table6</b>  | Clinical signs of subacute toxicity observed during the 14-day post-treatment period following single-dose administration of <b>FMA</b> at graded dose levels (0.1–100 mg/kg) in male and female experimental animals.    | <b>32</b> |
| <b>Table7</b>  | Clinical signs of acute toxicity observed within 1–4 hours following single-dose administration of <b>FCMphA</b> at graded dose levels (0.1–100 mg/kg) in male and female experimental animals.                           | <b>35</b> |
| <b>Table8</b>  | Clinical signs of subacute toxicity observed during the 14-day post-treatment period following single-dose administration of <b>FCMphA</b> at graded dose levels (0.1–100 mg/kg) in male and female experimental animals. | <b>38</b> |
| <b>Table9</b>  | Clinical signs of acute toxicity observed within 1–4 hours following single-dose administration of <b>FCMphB</b> at graded dose levels (0.1–100 mg/kg) in male and female experimental animals.                           | <b>40</b> |
| <b>Table10</b> | Clinical signs of subacute toxicity observed during the 14-day post-treatment period following single-dose administration of <b>FCMphB</b> at graded dose levels (0.1–100 mg/kg) in male and female experimental animals. | <b>44</b> |
| <b>Table11</b> | Effects of graded doses of <b>FMA</b> (0.1, 1, 10, and 100 mg/kg) on weekly body weight, food intake, and water consumption in male and female rats over a 14-day observation period.                                     | <b>47</b> |
| <b>Table12</b> | Effects of graded doses of <b>FCMphA</b> (0.1, 1, 10, and 100 mg/kg) on weekly body weight, food intake, and water consumption in male and female rats over a 14-day observation period.                                  | <b>49</b> |
| <b>Table13</b> | Effects of graded doses of <b>FCMphB</b> (0.1, 1, 10, and 100 mg/kg) on weekly body weight, food intake, and water consumption in male and female rats over a 14-day observation period.                                  | <b>51</b> |

|                |                                                                                                                                                                                                                  |           |
|----------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| <b>Table14</b> | Relative organ weight indices of major organs (liver, kidneys, heart, stomach, brain, lungs, spleen, and testes) in male and female rats following acute exposure to graded doses of <b>FMA</b> (0.1–100 mg/kg). | <b>54</b> |
| <b>Table15</b> | Relative organ weight indices of major organs in male and female rats after acute administration of graded doses of <b>FCMphA</b> (0.1–100 mg/kg).                                                               | <b>56</b> |
| <b>Table16</b> | Relative organ weight indices of major organs in male and female rats following acute exposure to graded doses of <b>FCMphB</b> (0.1–100 mg/kg).                                                                 | <b>57</b> |
| <b>Table17</b> | Hematological parameters in male and female rats following acute administration of graded doses of <b>FMA</b> (0.1–100 mg/kg).                                                                                   | <b>59</b> |
| <b>Table18</b> | Hematological alterations in male and female rats following acute exposure to graded doses of <b>FCMphA</b> (0.1–100 mg/kg).                                                                                     | <b>61</b> |
| <b>Table19</b> | Hematological profile of male and female rats following acute administration of graded doses of <b>FCMphB</b> (0.1–100 mg/kg).                                                                                   | <b>63</b> |
| <b>Table20</b> | Serum biochemical parameters in male and female rats following acute exposure to graded doses of <b>FMA</b> (0.1–100 mg/kg).                                                                                     | <b>65</b> |
| <b>Table21</b> | Serum biochemical profile in male and female rats following acute administration of graded doses of <b>FCMphA</b> (0.1–100 mg/kg).                                                                               | <b>67</b> |
| <b>Table22</b> | Serum biochemical profile in male and female rats following acute administration of graded doses of <b>FCMphB</b> (0.1–100 mg/kg).                                                                               | <b>68</b> |
| <b>Table23</b> | Predicted ADMET and drug-likeness profiles of <b>FMA</b> , <b>FCMphA</b> , and <b>FCMphB</b> .                                                                                                                   | <b>85</b> |
| <b>Table24</b> | Molecular docking results of ferrocene derivatives against CYP3A4 and KEAP1 proteins.                                                                                                                            | <b>87</b> |

## SOMMAIRE

|                                  |          |
|----------------------------------|----------|
| <b>Acknowledgements</b>          |          |
| <b>Dedication</b>                |          |
| <b>Abstract</b>                  |          |
| <b>List of Abbreviations</b>     |          |
| <b>List of Figures</b>           |          |
| <b>List of Tables</b>            |          |
| <b>General Introduction.....</b> | <b>1</b> |
| <b>PART I: LITERATURE REVIEW</b> |          |

|                                                                |          |
|----------------------------------------------------------------|----------|
| <b>Chapter 1: Study of Ferrocene.....</b>                      | <b>3</b> |
| I.1.1. Definition of Ferrocene.....                            | 3        |
| I.1.1.1. General Definition.....                               | 3        |
| I.1.1.2 . Molecular Formula and Chemical Structure.....        | 3        |
| I.1.2. Properties of Ferrocene.....                            | 4        |
| I.1.2.1. Physical Properties.....                              | 4        |
| I.1.2.2. Chemical Properties.....                              | 4        |
| I.1.2.3. Spectroscopic Properties.....                         | 4        |
| I.1.2.4. Electrochemical Properties.....                       | 5        |
| I.1.2.5. Crystal Structure.....                                | 6        |
| I.1.3. Methods of Preparing Ferrocene and Its Derivatives..... | 6        |
| I.1.3.1. Chemical Synthesis.....                               | 6        |
| I.1.3.2. Preparation of Ferrocene Derivatives.....             | 6        |
| I.1.4. Potential Applications of Ferrocene.....                | 7        |

|                                                           |           |
|-----------------------------------------------------------|-----------|
| I.1.4.1. Pharmaceutical Applications.....                 | 7         |
| A. Anticancer activity .....                              | 7         |
| B. Antimalarial activity .....                            | 7         |
| C. Antimicrobial activity.....                            | 7         |
| D. Enzyme inhibitor .....                                 | 8         |
| E. Antioxidant activity.....                              | 8         |
| I.1.4.2. Industrial Applications Ferrocene.....           | 8         |
| I.1.5. Toxicological Background of Ferrocene.....         |           |
| I.1.5.1. Toxicological Profile in Previous Studies.....   | 8         |
| I.1.5.2. Reason for Studying Toxicity.....                | 8         |
| <b>Chapter 2: General Toxicology.....</b>                 | <b>10</b> |
| I.2.1. Definition of Toxicity.....                        | 10        |
| I.2.2. Routes of Exposure.....                            | 10        |
| I.2.3. Toxicokinetics.....                                | 11        |
| I.2.3.1. Absorption.....                                  | 11        |
| I.2.3.2. Distribution and Bioavailability.....            | 11        |
| I.2.3.3. Biotransformation (Metabolism).....              | 11        |
| I.2.3.4. Excretion.....                                   | 12        |
| I.2.4. Toxic Effects.....                                 | 12        |
| I.2.4.1. Concept of Toxic Effect.....                     | 12        |
| I.2.4.2. Dose–Effect and Dose–Response Relationships..... | 12        |
| I.2.5. Different Forms of Toxicity.....                   | 13        |

|                                                                            |    |
|----------------------------------------------------------------------------|----|
| I.2.5.1. Acute Toxicity.....                                               | 13 |
| I.2.5.2. Subacute and Subchronic Toxicity .....                            | 13 |
| A. Subacute Toxicity.....                                                  | 14 |
| B. Subchronic Toxicity.....                                                | 14 |
| I.2.5.3. Chronic Toxicity.....                                             | 14 |
| I.2.6. Toxicity Indicators in Animals.....                                 | 14 |
| I.2.7. Toxic Manifestations by Biological Systems and Target Organs.....   | 15 |
| I.2.7.1. Hepatotoxicity.....                                               | 15 |
| I.2.7.2. Nephrotoxicity.....                                               | 15 |
| I.2.7.3. Neurotoxicity.....                                                | 15 |
| I.2.7.4. Cardiotoxicity.....                                               | 16 |
| I.2.8. Toxicodynamics.....                                                 | 16 |
| I.2.8.1. Interaction with Cellular Targets.....                            | 16 |
| I.2.8.2. Oxidative Stress and Free Radical Formation.....                  | 16 |
| I.2.8.3. Cell Injury and Genotoxicity .....                                | 16 |
| I.2.8.4. Apoptosis and Necrosis.....                                       | 17 |
| I.2.9. Factors Determining Toxicity.....                                   | 17 |
| I.2.9.1. Dose and Duration of Exposure.....                                | 17 |
| I.2.9.2. Biological Factors (Age, Sex, Genetics).....                      | 17 |
| I.2.9.3. Chemical Properties (Stability, Solubility, Oxidation State)..... | 17 |

---

## PART II: PRACTICAL PART

|                                                         |    |
|---------------------------------------------------------|----|
| <b>Chapter1: Materials and Methods</b> .....            | 20 |
| II.1.1. In Vivo Study.....                              | 20 |
| II.1.1.1. Experimental Animals.....                     | 20 |
| II.1.1.2. Experimental Design.....                      | 20 |
| II.1.1.3. Acute Toxicity Study.....                     | 21 |
| II.1.1.4. Clinical Observations.....                    | 22 |
| II.1.1.5. Body Weight, Food and Water Intake.....       | 22 |
| II.1.1.6. Sacrifice and Sample Collection.....          | 22 |
| II.1.1.7. Hematological Analysis.....                   | 23 |
| II.1.1.8. Biochemical Analysis.....                     | 23 |
| II.1.1.9. Histopathological Study.....                  | 23 |
| II.1.2. In Silico Study.....                            | 24 |
| II.1.2.1. Ligand Preparation.....                       | 24 |
| II.1.2.2. ADMET and Drug-Likeness Prediction.....       | 25 |
| II.1.2.3. Target Protein Selection and Preparation..... | 25 |
| II.1.2.4. Molecular Docking Protocol.....               | 27 |
| <b>Chapter2: Results and Discussion</b> .....           | 29 |
| II.2.1. In Vivo Study.....                              | 29 |
| II.2.1.1. Clinical Signs.....                           | 29 |

|                                                                                             |    |
|---------------------------------------------------------------------------------------------|----|
| A. Clinical Signs of Acute Toxicity of FMA.....                                             | 29 |
| B. Clinical Signs of Acute Toxicity of FCMphA.....                                          | 33 |
| C. Clinical Signs of Acute Toxicity of FCMphB.....                                          | 39 |
| II.2.1.2. Weekly body weights, food and water consumption.....                              | 45 |
| A. Weekly body weights, food and water consumption of rats across FMA doses.....            | 45 |
| B. Weekly body weights, food and water consumption of rats across FCMphA doses...           | 47 |
| C. Weekly body weights, food and water consumption of rats across FCMphB doses...           | 49 |
| II.2.1.3. Organ Weight Index.....                                                           | 51 |
| A. Organ Weight Index of Rats Across FMA Doses.....                                         | 51 |
| B. Organ Weight Index of Rats Across FCMphA Doses.....                                      | 54 |
| C. Organ Weight Index of Rats Across FCMphB Doses.....                                      | 56 |
| II.2.1.4. Hematological Assessment.....                                                     | 57 |
| A. Hematological Assessment Across FMA Doses.....                                           | 57 |
| B. Hematological Assessment Across FCMphA Doses.....                                        | 59 |
| C. Hematological Assessment Across FCMphB Doses.....                                        | 61 |
| II.2.1.5. Biochemical Assessment.....                                                       | 63 |
| A. Biochemical Assessment Across FMA Doses.....                                             | 63 |
| B. Biochemical Assessment Across FCMphA Doses.....                                          | 65 |
| C. Biochemical Assessment Across FCMphB Doses.....                                          | 67 |
| II.2.1.6. Cross pathology and histopathological analysis of the vital organs of the rats... | 69 |

|                                                        |           |
|--------------------------------------------------------|-----------|
| A. Histopathological Analysis Across FMA Doses.....    | 69        |
| B. Histopathological Analysis Across FCMphA Doses..... | 74        |
| C. Histopathological Analysis Across FCMphB Doses..... | 79        |
| II.2.2. In Silico Study.....                           | 84        |
| II.2.2.1. ADMET and Drug-Likeness Assessment.....      | 84        |
| II.2.2.2. Molecular Docking Study.....                 | 86        |
| <b>Conclusion.....</b>                                 | <b>91</b> |
| <b>Bibliographic references.....</b>                   | <b>93</b> |
| <b>Annexe</b>                                          |           |

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# ***GENERAL INTRODUCTION***

## General Introduction

Organometallic compounds, owing to their unique physicochemical characteristics, structural diversity, ligand-exchange capability, and remarkable redox and catalytic behaviors, represent a promising class of potential therapeutic agents. Traditionally employed in homogeneous catalysis, these compounds have increasingly attracted attention as candidates for biomedical and pharmaceutical applications (Cybulski et al., 2024).

Among them, ferrocene and its derivatives, characterized by their distinctive sandwich structure, constitute one of the most prominent examples of this class. Ferrocene exhibits exceptional stability toward air, heat, and light, in addition to its relatively low cost (Wang et al., 2020).

Over the past decades, ferrocene derivatives have gained growing scientific interest because of their ability to improve the pharmacological performance of conventional organic molecules. The incorporation of a ferrocene moiety into the chemical structure of a drug may enhance cellular permeability, increase metabolic stability, and promote the generation of reactive oxygen species (ROS), a mechanism considered fundamental to anticancer and antimicrobial activities. Furthermore, several studies have demonstrated that the lipophilic nature of these derivatives facilitates their penetration through biological membranes, including the blood–brain barrier (Patra & Gasser, 2017).

Ferrocene and its derivatives are also extensively employed in the design and development of novel bioorganometallic compounds, where the ferrocenyl group frequently serves as a substitute for functional groups, particularly aromatic moieties, in biologically active molecules.

Such modifications contribute to the generation of compounds with innovative structural characteristics and improved biological activities compared to the parent molecules (Cybulski et al., 2024).

Despite the promising therapeutic potential of ferrocene derivatives, the evaluation of their biological safety remains an essential prerequisite prior to pharmaceutical application. In this context, the present study aims to investigate the acute and subacute toxicity of three ferrocene-based derivatives, namely **FMA**, **FCMphA**, and **FCMphB**, using Wistar rats as an experimental model. The study further examines the clinical, physiological, biochemical, and histopathological

effects associated with exposure to these compounds. In addition, computational analyses involving ADMET profiling and molecular docking were performed to support the experimental findings, with the objective of identifying the safest and most effective compound and elucidating the relationship between structural modifications and the toxicological and pharmacological behavior of these derivatives.



**PART I:**  
**LITERATURE REVIEW**

# **CHAPTER 1: STUDY OF FERROCENE**

## PART I: LITERATURE REVIEW

### Chapter 1: Study of Ferrocene

#### I.1.1. Definition of Ferrocene

##### I.1.1.1. General Definition

Ferrocene is a prototypical metallocene in which a central iron cation is symmetrically coordinated between two cyclopentadienyl ligands. This class of compounds is commonly referred to as “sandwich compounds” due to their distinctive geometry (**Figure1**) (Kealy & Pauson, 1951).



**Figure 1:** Ferrocene powder under vacuum (Kopp, 2010).

##### I.1.1.2. Molecular Formula and Chemical Structure

Ferrocene, with the formula  $(\eta^5\text{-C}_5\text{H}_5)_2\text{Fe}$ , is an organometallic compound featuring a highly stable sandwich structure in which an iron center is positioned between two parallel cyclopentadienyl rings. Each ring binds through  $\eta^5$ -coordination, allowing full electron delocalization. The compound follows the 18-electron rule, which accounts for its exceptional stability, further supported by effective overlap between ligand  $\pi$ -orbitals and metal d-orbitals(**Figure2**) (Kealy & Pauson, 1951).

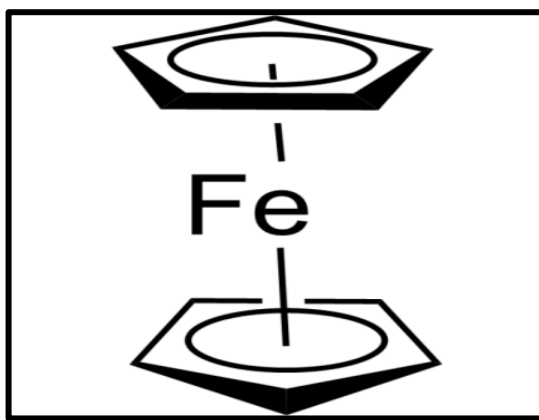


**Figure 2** : Structure corrected iron (Bitencourt et al., 2015)

## I.1.2. Properties of Ferrocene

### I.1.2.1. Physical Properties

Ferrocene is an orange crystalline solid that exhibits diamagnetic behavior. It melts at approximately 174°C and is capable of sublimation at temperatures above 100°C. Its high thermal stability is one of its most remarkable features (Lanez, 2019).

### I.1.2.2. Chemical Properties

Chemically, ferrocene behaves similarly to aromatic compounds such as benzene. It readily undergoes electrophilic substitution reactions on its cyclopentadienyl rings, allowing the synthesis of a wide range of derivatives (Lanez, 2019).

### I.1.2.3. Spectroscopic Properties

Spectroscopic techniques such as infrared (IR) and nuclear magnetic resonance (NMR) are widely used for the characterization of ferrocene and its derivatives. Due to its high symmetry, ferrocene exhibits relatively simple spectra.

In IR spectroscopy, characteristic absorption bands correspond to C–H stretching and ring vibrations. In NMR spectroscopy, all hydrogen atoms are chemically equivalent, resulting in a single resonance signal (Figure3)(Lanez, 2019).

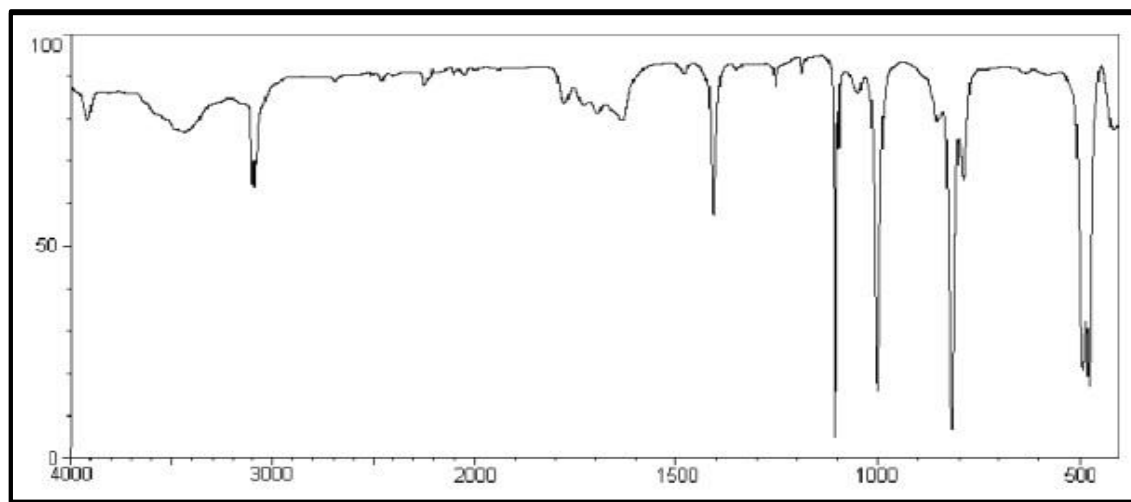


**Figure3:** IR spectra of ferrocene (Lanez, 2019).

#### I.1.2.4. Electrochemical Properties

Ferrocene exhibits a highly reversible redox behavior, making it an important reference system in electrochemistry. The  $\text{Fc}^+/\text{Fc}$  redox couple is widely used as a standard in cyclic voltammetry studies. Changes in peak potentials and current intensities provide valuable information about interactions between ferrocene derivatives and biological molecules such as DNA and proteins( **Figure4** )(Lanez, 2019).

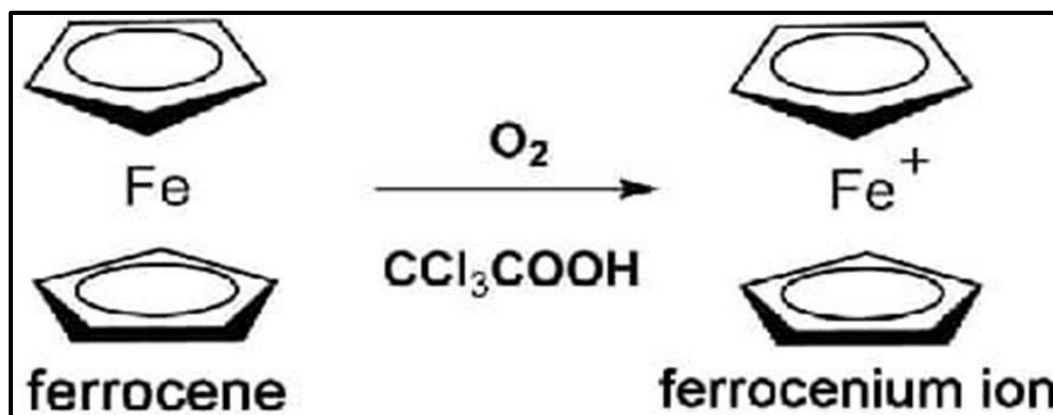


**Figure4:**Oxidation reaction of ferrocene to ferrocenium ion (Okada & Tsuchida, 2019).

### I.1.2.5. Crystal Structure

The molecular structure of ferrocene has been confirmed using X-ray crystallography. The two cyclopentadienyl rings may adopt either eclipsed or staggered conformations, with a low rotational energy barrier between them(**Figure5**) (**Fish & Rosenblum, 1965**).

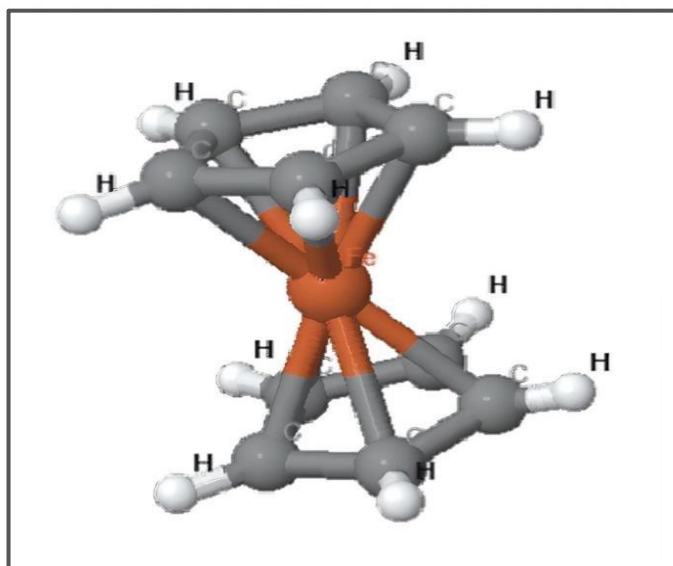


**Figure5:** Structure crystalline of ferrocene. (**Visconti et al., 2017**)

## I.1.3. Methods of Preparing Ferrocene and Its Derivatives

### I.1.3.1. Chemical Synthesis

A widely used intermediate in the synthesis of ferrocene derivatives is ferrocenylmethyl trimethylammonium iodide. This compound is obtained through aminomethylation of ferrocene and serves as a versatile precursor for further functionalization. (**Lanez, 2019**).

### I.1.3.2. Preparation of Ferrocene Derivatives

Modification of the ferrocene nucleus is usually achieved by introducing functional groups onto one or both cyclopentadienyl rings in order to improve the biological activity or the physicochemical properties of the compound. Recent reviews have shown that the most common approaches rely on acylation, alkylation, and coupling reactions with organic bases or heterocyclic rings to obtain derivatives with enhanced pharmacological activity (**Rauf et al., 2023**).

In addition, some synthetic methods involve replacing the ferrocenylmethyl group with biologically active organic moieties such as amines or selenoamides, allowing the preparation of new derivatives with anticancer or antimicrobial properties, which contributes to the development of novel compounds with applications in medicinal and organic chemistry (Gutiérrez-Hernández et al., 2012).

#### **I.1.4. Potential Applications of Ferrocene**

##### **I.1.4.1. Pharmaceutical Applications**

Ferrocene derivatives have gained considerable attention in medicinal chemistry due to their stability, low toxicity, and redox properties.

##### **A. Anticancer activity:**

Ferrocene derivatives have attracted considerable attention in anticancer research due to their unique redox properties and ability to interact with multiple biological targets involved in tumor progression. In particular, Ferrocifen a ferrocene–phenol hybrid, represents a well-studied example that highlights the therapeutic potential of this class of compounds. Unlike classical platinum-based drugs that mainly exert their effects through DNA damage, Ferrocifen displays broader mechanism of action involving not only DNA but also proteins and key enzymes such as thioredoxin reductase . This multi-target activity is associated with significant anticancer effects, especially against drug-resistant cancer cells. Consequently, Ferrocifen remains under preclinical investigation, further supporting the relevance of ferrocene-based frameworks as promising candidates for the development of novel anticancer agents (Wang et al., 2020).

##### **B. Antimalarial activity:**

Ferrocene is recognized as an important organometallic scaffold in medicinal chemistry, and its incorporation into hybrid molecules with other pharmacophores frequently leads to compounds with enhanced biological properties. In particular, many studies have highlighted ferrocene-based derivatives as promising antimalarial agents, especially when combined with established pharmaceutical frameworks, showing improved activity against malaria parasites and making them attractive candidates for antimalarial drug development (Peter & Aderibigbe, 2019).

##### **C. Antimicrobial activity:**

Ferrocene-based compounds also possess significant antimicrobial activity, including antibacterial, antifungal, and antiviral effects. The underlying mechanism is often associated with the production of ROS, which can damage essential biomolecules such as proteins, lipids, and nucleic acids within microbial cells. These findings suggest that ferrocene derivatives could serve as promising candidates for the development of new antimicrobial agents (Ludwig et al., 2019).

#### **D. Enzyme inhibitor:**

Another important aspect of ferrocene's biological activity is its ability to act as an enzyme inhibitor. Several studies have reported that ferrocene derivatives can inhibit key enzymes such as histone deacetylases (HDACs) and tyrosine kinases, which are involved in cell proliferation and cancer progression. This inhibition contributes to cell cycle arrest and suppression of tumor growth, highlighting the therapeutic potential of these compounds (Angelova & Stavrakov, 2025).

#### **E. Antioxidant activity:**

ferrocene exhibits a dual redox behavior, allowing it to function either as a pro-oxidant or an antioxidant depending on the biological context. This property is related to its reversible oxidation to the ferricenium ion, which plays a central role in modulating oxidative stress within cells (Ludwig et al., 2019).

#### **I.1.4.2. Industrial Applications**

Ferrocene derivatives are widely applied in materials science, catalysis, and supramolecular chemistry due to their redox properties and structural versatility (Rauf et al., 2023).

### **I.1.5. Toxicological Background of Ferrocene**

#### **I.1.5.1. Toxicological Profile in Previous Studies**

Ferrocene is generally considered a stable and low-toxicity compound. However, structural modifications can influence its biological effects (Lanez, 2019).

#### **I.1.5.2. Reason for Studying Toxicity**

Evaluating toxicity is essential before pharmaceutical applications. Ferrocene derivatives may interact with DNA and biological systems, leading to beneficial or adverse effects.

Understanding these effects is crucial for safe clinical use (Lanez, 2019).

## **CHAPTER 2: GENERAL TOXICOLOGY**

## Chapter 2: General Toxicology

### I.2.1. Definition of Toxicity

Chemicals that have the potential to produce dysfunction at the molecular, cellular, or organ level upon contact with or after penetration into a living body are said to be toxic. The nature of the substance, the dose, the duration of exposure, and a number of individual factors, including sex, age, nutritional status, and hormonal state, as well as environmental factors and concurrent or prior exposure to other chemical substances, all influence this toxicity (Tron et al., 2002; Viau & Tardif, 2003).

### I.2.2. Routes of Exposure

The main tissue or system through which a xeno-biotic interacts with the body, and from which it can be taken up to cause systemic toxicity, is the exposure route. The common situations of environmental exposure include ingestion (oral), inhalation, and contact with the skin.

Additionally, for research and treatment purposes, intramuscular, intravenous, and subcutaneous injections can serve as methods of exposure.

The primary pathways through which a potentially harmful chemical can enter the body, listed in order of effectiveness for systemic delivery, include injection, inhalation, absorption from the intestinal tract, and skin absorption. The connection between route and exposure, biotransformation, and likelihood of toxicity can be intricate and is affected by the amount and length of dosing (Table 1).

**Table 1** Toxicity of chemicals applied by different routes of exposure. (Dekant & Vamvakas, 2011).

| Chemical                     | Species | Route of application | LD <sub>50</sub> , mg/kg |
|------------------------------|---------|----------------------|--------------------------|
| DDT                          | rat     | intravenous          | 68                       |
|                              | rat     | oral                 | 113                      |
|                              | rat     | skin contact         | 1931                     |
| Atropine sulfate             | rat     | intravenous          | 41                       |
|                              | rat     | oral                 | 620                      |
| 1-Chloro-2,4-dinitro-benzene | rat     | oral                 | 1070                     |
|                              | rat     | intraperitoneal      | 280                      |
|                              | rabbit  | skin contact         | 130                      |
| Dieldrin                     | rat     | oral                 | 46                       |
|                              | rat     | intravenous          | 9                        |
|                              | rat     | skin contact         | 10                       |

The path of exposure significantly affects toxicity due to its impact on the bioavailability of the toxic substance. The peak tissue concentrations reached, the time taken to reach maximum tissue levels, and consequently the length of the effect are governed by the absorption rate and the degree of distribution throughout the system (Dekant & Vamvakas, 2011).

### I.2.3. Toxicokinetics

#### I.2.3.1. Absorption

Absorption refers to the entry of toxic substances into the bloodstream through routes such as ingestion, inhalation, or dermal exposure. Factors such as solubility and membrane permeability influence this process.

#### I.2.3.2. Distribution

Once absorbed, toxic substances are distributed throughout the body via the circulatory system. Bioavailability refers to the fraction of the substance that reaches systemic circulation and is available to exert its toxic effect.

#### I.2.3.3. Biotransformation (Metabolism)

Biotransformation involves enzymatic modification of toxic substances, primarily in the liver.

**Phase I Reactions:** These include oxidation, reduction, and hydrolysis reactions, often producing more reactive metabolites.

**Phase II Reactions:** These involve conjugation with molecules such as glucuronide or sulfate, increasing water solubility and facilitating excretion.

#### I.2.3.4. Excretion

Excretion is the elimination of toxic substances and their metabolites from the body, mainly through urine, bile, feces, or exhaled air (**Figure 6**) (**Patra & Gasser, 2017**).

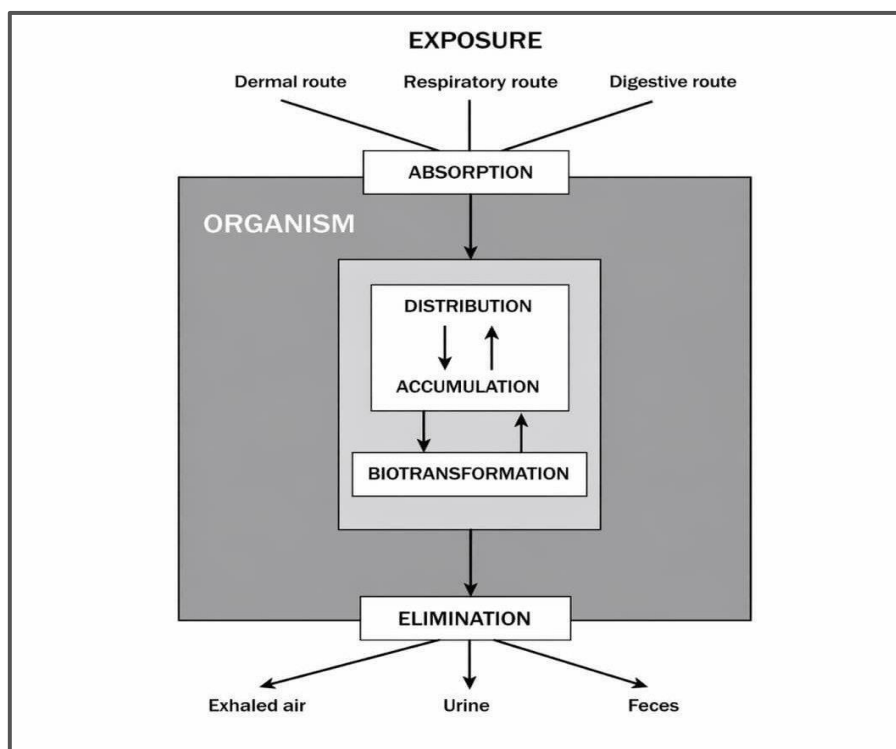


**Figure 6:** General diagram of the pathway of toxic substances in the body (**Tiarti & Riahi, 2021**).

### I.2.4. Toxic Effects

#### I.2.4.1. Concept of Toxic Effect

A chemical substance is considered hazardous if it has the intrinsic capacity to cause negative consequences in living things. This is known as a toxic impact. Accordingly, the dose, the mode of absorption, the kind and intensity of lesions, and the amount of time needed for a lesion to appear all have an impact on the side effect (**Gilles, 2004**).

#### I.2.4.2. Dose–Effect and Dose–Response Relationships

The relationship between the dose and the outcome at the individual level is known as the "dose–effect" relationship. It rises with a dose increase. It is possible to create a dose-effect curve for the target molecule, the cell, or the entire organism. Some toxic effects, like cancer or death, are "all-or-none" impacts rather than gradual (Holmberg et al., 2000).

The dose–response relationship describes the correlation between the amount of a substance and the magnitude of its toxic effect. It is a fundamental concept used to determine safe exposure levels and toxic thresholds. Typically, this relationship follows a sigmoidal curve, with parameters such as threshold dose, maximum effect, and slope providing critical information for risk assessment (Figure 7) (Patra & Gasser, 2017).

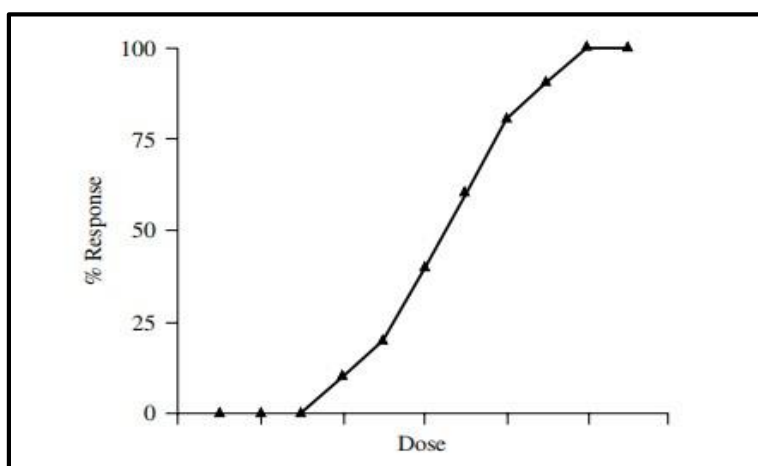


Figure 7 : A typical dose-response curve (Hodgson, 2004).

### 1.2.5. Different Forms of Toxicity

Toxic effects are scientifically categorized based on the duration of exposure and the frequency of administration into four primary forms: acute, subacute, sub-chronic, and chronic toxicity.

#### 1.2.5.1. Acute Toxicity

Is defined as the harmful effects that appear after a single exposure or within a short period not exceeding 24 hours, and it is used to determine the median lethal dose and evaluate the immediate hazard of a substance (Seidle et al., 2010).

#### 1.2.5.2. Subacute and Subchronic Toxicity

**A. Subacute toxicity**

Results from repeated exposure to a substance over a short period, usually ranging from 14 to 28 days, and is used to detect early effects on vital organs (**Chen et al., 2015**).

**B. Subchronic Toxicity**

Refers to the effects resulting from continuous exposure for a period ranging from one to three months, allowing the assessment of progressive cumulative effects within the body (**Kale et al., 2019**).

**I.2.5.3. Chronic Toxicity**

Chronic toxicity is defined as the effects produced by long-term exposure to relatively low amounts of a substance, which may lead to permanent or irreversible disorders in tissues and organs(**Table2**) ( **Tauheed et al., 2021**).

**Table2:** Forms of intoxication (**Gilles, 2004**).

| Form of intoxication | Frequency of administration | Duration of exposure |
|----------------------|-----------------------------|----------------------|
| Acute                | Single                      | < 24 hour            |
| Subacute             | Repeated                    | ≤ 1 month            |
| Subchronic           | Repeated                    | From 1 to 3 months   |
| Chronic              | Repeated                    | > 3 months           |

**I.2.6. Toxicity Indicators in Animals**

Toxicity in experimental animals is evaluated through a set of clinical and functional indicators used to determine the severity of the harmful effects of the tested substance, including observable clinical signs such as lethargy and impaired movement, which are considered among the earliest manifestations of systemic toxicity (**Silva et al., 2021**).Changes in body weight are also regarded as one of the most sensitive indicators in toxicological studies because they reflect

metabolic disturbances resulting from chemical exposure. In addition, food and water intake measurements are used to detect early physiological alterations associated with toxicity.

Furthermore, relative organ weights, biochemical analyses, and histopathological examination help identify the target organs and confirm the occurrence of cellular damage (OECD, 2025).

## I.2.7. Toxic Manifestations by Biological Systems and Target Organs

### I.2.7.1. Hepatotoxicity

The liver is highly susceptible to toxic damage due to its central role in metabolism and detoxification (Table3).

**Table3:** Main toxic effects observed in the liver (Lu, 1992)

| Hepatic lesions    | Characteristics                                                                                                                                              |
|--------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Steatosis</b>   | Corresponds to the accumulation of fats within liver tissue. Toxic substances act by blocking the elimination of hepatic triglycerides into the bloodstream. |
| <b>Necrosis</b>    | Refers to the destruction of hepatocytes and generally corresponds to acute lesions.                                                                         |
| <b>Cholestasis</b> | Decrease or cessation of bile flow due to alteration of biliary excretion.                                                                                   |
| <b>Cirrhosis</b>   | Presence of collagen infiltrations within the hepatic tissue.                                                                                                |
| <b>Hepatitis</b>   | Clinical manifestations of liver inflammation.                                                                                                               |

### I.2.7.2. Nephrotoxicity

The kidneys are vulnerable to toxins because of their role in filtration and excretion (Lanez, 2019).

### I.2.7.3. Neurotoxicity

Neurotoxic substances affect the nervous system, potentially leading to cognitive and motor dysfunction (Table 4) (Lanez, 2019).

**Table 4:** Various categories of neurotoxic effects (Gilles, 2004).

| Toxic effects                     | Characteristics                                                                                             |
|-----------------------------------|-------------------------------------------------------------------------------------------------------------|
| Central nervous system depression | Manifests following exposure to solvents such as toluene and xylene.                                        |
| Peripheral neuropathy             | A disorder of the peripheral nervous system that can be caused by solvents such as n-hexane.                |
| Tetanus                           | Consists of muscle contractions and is caused by a biological toxin produced by <i>Clostridium tetani</i> . |
| Muscle paralysis                  | Caused by a biological toxin produced by <i>Clostridium botulinum</i> .                                     |

#### I.2.7.4. Cardiotoxicity

Cardiotoxicity, also known as toxicologic cardiomyopathy, results from structural and functional alterations in the heart, leading to decreased cardiac output, impaired contractility, and regional hypoperfusion. Cardiomyocyte death and cardiac tissue remodeling contribute to the development of myocardial hypertrophy and heart failure, while cardiotoxicity is associated with a complex interplay of genetic and environmental factors (Klaassen, 2008)

### I.2.8. Toxicodynamics

#### I.2.8.1. Interaction with Cellular Targets

Toxicodynamics focuses on how toxic substances interact with cellular components such as proteins, DNA, and membranes, leading to functional impairment.

#### I.2.8.2. Oxidative Stress and Free Radical Formation

Many toxic compounds induce oxidative stress by generating reactive oxygen species (ROS), which can damage cellular macromolecules.

#### I.2.8.3. Cell Injury and Genotoxicity

Toxic agents may cause reversible or irreversible cell injury. Genotoxic substances specifically damage genetic material, potentially leading to mutations and cancer.

#### **I.2.8.4. Apoptosis and Necrosis**

Cell death can occur via apoptosis (programmed cell death) or necrosis (uncontrolled cell death), depending on the nature and severity of the toxic insult (**Patra & Gasser, 2017**).

### **I.2.9. Factors Determining Toxicity**

#### **I.2.9.1. Dose and Duration of Exposure**

Higher doses and prolonged exposure generally increase toxicity (**Lanez, 2019**).

#### **I.2.9.2. Biological Factors (Age, Sex, Genetics)**

Individual susceptibility varies based on physiological and genetic differences (**Lanez, 2019**).

#### **I.2.9.3. Chemical Properties (Stability, Solubility, Oxidation State)**

Chemical characteristics influence how a substance behaves in biological systems and its overall toxicity (**Patra & Gasser, 2017**).



***PART II:***  
***PRACTICAL PART***

# **CHAPTER 1:**

## **MATERIALS AND METHODS**

## PART II: PRACTICAL PART

### Chapter1: Materials and Methods

#### *II.1.1. In vivo study*

##### **II.1.1.1. Experimental Animals**

A total of adult Wistar rats of both sexes, weighing between 200–250 g and aged approximately 8–10 weeks, were obtained from the animal house of Pasteur Institute, Algeria.

The animals were housed and maintained in the animal facility of the Department of Molecular and Cellular Biology, Faculty of Natural and Life Sciences, University of El-Oued, Algeria.

The animals were kept under standard laboratory conditions, including a controlled temperature of  $22 \pm 2^{\circ}\text{C}$ , relative humidity of 50–60%, and a 12 h light/12 h dark cycle. Standard pellet diet and tap water were provided *ad libitum* throughout the experimental period (No, 2002).

Prior to experimentation, the animals were acclimatized for one week under the same laboratory conditions to ensure physiological stability and to minimize stress-related variations (Christopher, Ganeson, Chinni, & Parasuraman, 2022). Only healthy animals showing no signs of disease were included in the study.

All experimental procedures were carried out in accordance with internationally accepted guidelines for the care and use of laboratory animals, particularly those recommended by the OECD, and followed the ethical standards approved by the local ethics committee (No, 2002).

##### **II.1.1.2. Experimental Design**

The animals were randomly divided into thirteen experimental groups, each consisting of six rats (three males and three females), in order to evaluate both dose-dependent and sex-related responses to the tested compounds(Beery & Zucker, 2011).

The groups were organized as follows:

- **Group I (Control group):** received only the vehicle and served as the untreated control.
- **Group II (FMA-D1):** received FMA at a dose of 0.1 mg/kg body weight.
- **Group III (FMA-D2):** received FMA at a dose of 1 mg/kg body weight.

- **Group IV (FMA-D3):** received **FMA** at a dose of 10 mg/kg body weight.
- **Group V (FMA-D4):** received **FMA** at a dose of 100 mg/kg body weight.
- **Group VI (FCMphA-D1):** received **FCMphA** at a dose of 0.1 mg/kg body weight.
- **Group VII (FCMphA-D2):** received **FCMphA** at a dose of 1 mg/kg body weight.
- **Group VIII (FCMphA-D3):** received **FCMphA** at a dose of 10 mg/kg body weight.
- **Group IX (FCMphA-D4):** received **FCMphA** at a dose of 100 mg/kg body weight.
- **Group X (FCMphB-D1):** received **FCMphB** at a dose of 0.1 mg/kg body weight.
- **Group XI (FCMphB-D2):** received **FCMphB** at a dose of 1 mg/kg body weight.
- **Group XII (FCMphB-D3):** received **FCMphB** at a dose of 10 mg/kg body weight.
- **Group XIII (FCMphB-D4):** received **FCMphB** at a dose of 100 mg/kg body weight.

All treatments were administered as a single dose, and the animals were observed for a period of 14 days following administration for the evaluation of acute toxicity.

#### **II.1.1.3. Acute Toxicity Study**

The acute toxicity study was conducted following a single-dose administration protocol in accordance with internationally recognized guidelines for toxicity testing (**No, 2002**). All treatments were administered orally using an appropriate vehicle, ensuring accurate delivery of the tested compounds.

Each animal received a single dose of the assigned compound according to its respective group. The oral route was selected as it represents a common pathway of exposure and is widely used in toxicological evaluations to assess systemic effects of chemical substances (**Nair & Jacob, 2016**).

Following administration, the animals were carefully observed during the first hours for any immediate signs of toxicity, including changes in behavior, posture, and physiological responses. Thereafter, all animals were monitored daily for a period of 14 days in order to detect any delayed toxic effects.

#### **II.1.1.4. Clinical Observations**

Following the administration of the tested compounds, all animals were closely observed for clinical signs of toxicity in order to assess the general health status and detect any adverse effects. Observations were carried out continuously during the first hours post-treatment, and thereafter recorded daily throughout the 14-day experimental period **(Odedunmoye, Olorunnisola, Adeleke, Asibor, & Odunmorayo, 2026)**.

Particular attention was given to changes in behavioral patterns, including locomotor activity, posture, grooming, and response to external stimuli. In addition, physical parameters such as the condition of the fur, skin, eyes, and mucous membranes were carefully examined. Signs of autonomic dysfunction, including salivation, piloerection, and alterations in respiration, were also monitored **(van Ravenzwaay, Lewis, Sewell, Marty, & Botham, 2026)**.

The occurrence of more severe toxic manifestations, such as tremors, convulsions, lethargy, or mortality, was recorded when observed.

#### **II.1.1.5. Body Weight, Food and Water Intake**

Body weight of the animals was recorded at the beginning of the experiment and monitored regularly throughout the 14-day observation period.

In addition to body weight, food and water intake were measured daily for each group. All measurements were recorded systematically during the experimental period.

#### **II.1.1.6. Sacrifice and Sample Collection**

At the end of the experimental period (14 days), all animals were sacrificed under appropriate conditions to minimize suffering, in accordance with ethical guidelines for the use of laboratory animals **(No, 2002)**.

After 16 hours of fasting, rats were decapitated under light anesthesia using chloroform. Blood samples were collected immediately during the decapitation process and transferred into appropriately labeled tubes. Part of the collected blood was placed in tubes containing anticoagulant (EDTA) for hematological analysis, while the remaining portion was collected in plain tubes, allowed to clot, and centrifuged at 2500 rpm for 10 minutes to obtain serum for subsequent biochemical analysis.

The obtained serum was stored at  $-20^{\circ}\text{C}$  until further use. Fasting blood glucose levels were measured using a glucometer.

After blood collection, major organs were carefully excised, including the liver, kidneys, heart, spleen, lungs, and brain. The organs were rinsed with physiological saline to remove adhering blood, blotted dry, and weighed individually.

The relative organ weight was calculated using the following formula:

$$\text{Relative organ weight (\%)} = (\text{organ weight} / \text{body weight}) \times 100$$

#### **II.1.1.7. Hematological Analysis**

Hematological parameters were analyzed using blood samples collected in tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant.

Using Coulter's technique and a Medonic automatic hematological analyzer (Coulter Beckman, USA), the following hematological parameters were calculated: red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration (Hb), hematocrit (HCT), and platelet count (PLT).

#### **II.1.1.8. Biochemical Analysis**

Biochemical parameters were evaluated using serum obtained after blood coagulation and centrifugation. The analysis was performed to assess the functional status of major organs following exposure to the tested compounds.

The measured parameters included alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as indicators of liver function, as well as urea and creatinine levels as indicators of kidney function. In addition, serum glucose was determined to evaluate metabolic status.

All biochemical analyses were carried out using standard diagnostic methods and commercially available kits, following the manufacturer's instructions (**Endale et al., 2021**).

#### **II.1.1.9. Histopathological Study**

Liver, kidney, heart, spleen, lungs, and brain tissues were collected immediately after sacrifice and fixed in 10% neutral buffered formalin for at least 24–48 hours.

Following fixation, the tissues were processed using standard histological procedures, including dehydration in graded ethanol series, clearing in xylene, and embedding in paraffin wax.

Paraffin-embedded tissues were sectioned at a thickness of approximately 4–5  $\mu\text{m}$  using a microtome, and the obtained sections were mounted on glass slides.

The tissue sections were then stained with hematoxylin and eosin (H&E) for microscopic examination (Suvarna, Layton, & Bancroft, 2018).

Histopathological evaluation was performed using a light microscope to assess structural and morphological changes in the examined organs.

### ***II.1.2. In Silico Study***

#### **II.1.2.1. Ligand Preparation**

The ferrocene-based derivatives (FMA, FCMphA, and FCMphB) were employed as ligands in the present in silico study. Their chemical structures were initially sketched using ChemDraw software to ensure an accurate depiction of the organometallic core along with the corresponding functional substituents.

Subsequently, the obtained two-dimensional (2D) representations were transformed into three-dimensional (3D) structures using Open Babel software (O'Boyle et al., 2011). This transformation was necessary to generate spatially relevant conformations that better reflect the real molecular geometry required for computational modeling.

The generated 3D conformers were then subjected to geometry optimization and energy minimization using the Merck Molecular Force Field (MMFF94) integrated within Avogadro software (version 1.2.0) (Hanwell et al., 2012). This step was essential to stabilize the molecular structures by reducing steric strain and ensuring energetically favorable configurations.

During the preparation process, all ligand structures were carefully examined to verify correct atomic connectivity, appropriate valency, and proper stereochemical configuration.

Particular attention was directed toward the ferrocene unit, given its distinctive sandwich architecture consisting of an iron atom positioned between two cyclopentadienyl rings, a feature that plays a critical role in defining the electronic properties and conformational behavior of these compounds (Astruc, 2017).

Furthermore, the protonation states of the ligands were adjusted to physiological pH (7.4) to more accurately mimic biological environments, following standard practices in computational pharmacokinetics. A final energy minimization step was performed to ensure complete structural relaxation prior to further analyses.

Finally, the optimized ligand structures were saved in PDB format and subsequently utilized for ADMET prediction and molecular docking studies.

### II.1.2.2. ADMET and Drug-Likeness Prediction

The pharmacokinetic behavior and toxicity profiles of the synthesized ferrocene-based derivatives (**FMA**, **FCMphA**, and **FCMphB**) were investigated using in silico prediction tools prior to performing molecular docking studies.

ADMET parameters were analyzed through widely used web-based platforms, including SwissADME, ProTox-II, and admetSAR (**Banerjee, Eckert, Schrey, & Preissner, 2018; Daina, Michielin, & Zoete, 2017a**). The absorption profile of the compounds was evaluated by predicting gastrointestinal (GI) absorption and aqueous solubility, which are critical determinants of oral bioavailability. In addition, distribution-related properties such as blood–brain barrier (BBB) permeability and P-glycoprotein (P-gp) substrate recognition were assessed to better understand the compounds' ability to reach target tissues.

The metabolic stability of the ligands was examined by predicting their interactions with key cytochrome P450 isoenzymes, particularly CYP3A4, CYP2D6, and CYP2C9, which are known to play major roles in drug biotransformation. Toxicological endpoints were also evaluated, including hepatotoxicity, mutagenicity (AMES test), carcinogenic potential, and acute oral toxicity (LD<sub>50</sub>), as provided by the respective computational servers.

Furthermore, the drug-likeness of the investigated compounds was determined based on Lipinski's rule of five and Veber's criteria, which are commonly applied to estimate oral drug suitability (**Christopher A Lipinski, Lombardo, Dominy, & Feeney, 1997; Veber et al., 2002**).

The overall ADMET and drug-likeness profiles were subsequently used as a preliminary screening step to identify the most promising candidates for further molecular docking analysis.

### II.1.2.3. Target Protein Selection and Preparation

The crystallographic structures of the selected target proteins were used as molecular receptors for the docking study. The corresponding three-dimensional (3D) structures were retrieved from the RCSB Protein Data Bank (PDB) (Berman et al., 2000). The human cytochrome P450 3A4 enzyme was obtained using PDB ID CYP3A4 (PDB: 1TQN), while the Kelch domain of Kelch-like ECH-associated protein 1 (KEAP1) was retrieved using PDB ID KEAP1 Kelch

domain (PDB: 4L7B). These structures were selected based on their availability as high-resolution crystallographic models and their suitability for molecular docking analysis.

Prior to docking simulations, all protein structures were carefully prepared using AutoDockTools (ADT) version 1.5.7, following standard molecular docking preparation protocols (**Morris et al., 2009**). The initial preparation step involved the removal of crystallographic water molecules, co-crystallized ligands, and other non-essential heteroatoms present in the PDB files. This step was performed to eliminate potential interference with ligand binding interactions and to ensure a clean and well-defined receptor environment.

Subsequently, polar hydrogen atoms were added to the protein structures to enable proper modeling of hydrogen bonding interactions between the receptor and ligand molecules.

The addition of these hydrogens is essential for accurately representing the physiological protonation state of amino acid residues involved in binding. Kollman partial charges were then assigned to all atoms in the protein structures to ensure appropriate electrostatic calculations during docking simulations. In addition, correct atom types were defined according to the AutoDock force field parameters to improve the reliability and consistency of the docking results.

Following charge assignment and hydrogen addition, the prepared protein structures were converted into PDBQT format, which is required for compatibility with AutoDock-based docking software. This format includes information related to atomic coordinates, partial charges, and atom types necessary for docking calculations.

The identification of the active binding sites was carried out based on the spatial coordinates of the co-crystallized ligands originally present in the downloaded structures. These regions were used to define the docking grid box, ensuring that the search space adequately covered the functional binding pocket of each protein. When necessary, reported binding residues described in the literature were also considered to refine the positioning of the docking grid (**Sevrioukova & Poulos, 2010**).

During the docking simulations, the receptor proteins were treated as rigid structures, maintaining fixed atomic coordinates throughout the calculations. In contrast, the ligand molecules (**FMA**, **FCMphA**, and **FCMphB**) were treated as fully flexible, allowing rotation around all rotatable bonds. This approach enabled the exploration of multiple conformations and orientations of the ligands within the binding sites, thereby facilitating the identification of the most energetically favorable ligand–receptor interactions.

#### II.1.2.4. Molecular Docking Protocol

Molecular docking simulations were performed to evaluate the binding affinity and interaction modes of the synthesized ferrocene-based derivatives (**FMA**, **FCMphA**, and **FCMphB**) toward the selected target proteins, namely cytochrome P450 3A4 (CYP3A4) and the Kelch domain of Kelch-like ECH-associated protein 1 (KEAP1), corresponding to PDB IDs CYP3A4 (PDB: 1TQN) and KEAP1 Kelch domain (PDB: 4L7B), respectively.

Docking calculations were carried out using AutoDock Vina software following standard molecular docking procedures (**Trott & Olson, 2010a**). Both ligands and target proteins were previously prepared and converted into PDBQT format prior to docking analysis.

The docking grid box was centered on the active binding site of each receptor based on the position of the co-crystallized ligand present in the crystallographic structure. Grid box dimensions were carefully adjusted to encompass the entire binding pocket and to allow sufficient conformational space for ligand flexibility during the docking process.

The docking parameters were set to generate multiple binding poses for each ligand–receptor complex. For each simulation, the best conformations were selected based on the lowest binding free energy values expressed in kcal/mol. Lower binding energy values were considered indicative of stronger ligand–receptor interactions and higher binding stability.

The optimal docking conformations were further analyzed using Discovery Studio Visualizer and PyMOL software to examine the molecular interactions established between the ligands and the active sites of the target proteins. Key interactions, including hydrogen bonds, hydrophobic contacts, van der Waals interactions, and  $\pi$ – $\pi$  stacking interactions, were identified and recorded.

Finally, the docking results were visualized using both two-dimensional interaction diagrams and three-dimensional representations of ligand–receptor complexes, allowing a comprehensive analysis of ligand orientation, binding modes, and interaction patterns within the active sites.

## **CHAPTER 2:**

# **RESULTS AND DISCUSSION**

## ***Chapter 2: Results and discussion .***

### ***II.2.1. In vivo study***

#### **II.2.1.1. Clinical signs**

##### **A. Clinical signs of acute toxicity of FMA**

**Overall Safety Profile and Lethality Assessment** The precise evaluation of acute and extended toxicity is a critical step in the preclinical pipeline for any novel chemical entity, particularly organometallic compounds. The results of the single-dose oral administration of the **FMA** compound at escalating concentrations (0.1 to 100 mg/kg) revealed a complete absence of mortality in both sexes during both the acute (4 hours) and extended (14 days) observation periods. Based on these data, it can be concluded that the median lethal dose (LD50) of the compound significantly exceeds the maximum tested limit (LD50>100 mg/kg).

The stability of baseline physiological parameters including respiratory rate, sensory reflexes, salivation, and urination indicates the absence of severe acute toxicity and implies that the compound does not interfere with vital autonomic centers in the brainstem. This preliminary safety profile supports the established evidence that incorporating a ferrocene core into organic molecules generally improves their biocompatibility and metabolic stability while minimizing the direct systemic toxicity often seen with free transition metals (Patra & Gasser, 2017).

**Acute Biphasic Neurobehavioral Responses** Despite the absence of lethal toxicity, careful clinical observation during the first four hours revealed transient, dose-dependent neurobehavioral alterations, which were noticeably more pronounced in male subjects. This response followed a biphasic trajectory. At lower doses (D1 and D2), signs of central nervous system (CNS) depression were observed, manifesting as a hunched posture, ptosis (half-closed eyes), and reduced locomotor activity. This effect is largely attributed to the high lipophilicity of the organometallic compound, which facilitates rapid passive diffusion across the blood-brain barrier (BBB), leading to temporary CNS modulation (Pardridge, 2012).

As the dose escalated to D3, the behavioral trajectory inverted, displaying neuromuscular excitability characterized by trembling. This dynamic shift can be mechanistically explained by the neural tissue concentration reaching a threshold that temporarily alters voltage-gated ion channels

or neurotransmitter release kinetics. The pivotal observation is the absence of severe neurological manifestations (such as convulsions or ataxia) and the retention of the animals' responsiveness to external stimuli, confirming that the effect was a reversible modulatory response rather than destructive neurotoxicity.

**Delayed Toxicity and Sexual Dimorphism** The notable differences observed between males and females during the extended 14-day observation period represent a highly significant finding, highlighting the phenomenon of sexual dimorphism in toxicological responses.

**First: Delayed Neurotoxicity in Females** Females exhibited delayed neurological sensitivity, presenting as transient partial paralysis at the maximum dose (D4) on days 5, 7, and 8.

Toxicokinetically, this discrepancy can be explained by the higher proportion of adipose tissue typically found in females, which increases the volume of distribution (Vd) for highly lipophilic compounds. This leads to a slower clearance rate and the temporary "trapping" of the compound in lipid-rich tissues, such as the myelin sheaths of peripheral nerves, culminating in transient neuropathy (**Zucker & Prendergast, 2020**). The fact that this paralysis was partial and entirely reversible with females fully recovering after day 8 provides definitive evidence against irreversible Wallerian degeneration, underscoring the eventual success of hepatic clearance mechanisms.

**Second: Delayed Dermatological Effects in Males** Conversely, delayed effects in males were confined to pronounced dermatological alterations, presenting as patchy fur starting on day 9, which was more persistent in the highest dose group. This phenomenon can be interpreted as drug-induced telogen effluvium. Systemic metabolic stress or the localized accumulation of the lipophilic drug in the sebaceous glands and keratinocytes can prematurely force actively growing hair follicles (anagen phase) into the shedding phase (telogen phase) (**Phillips, Slomiany, & Allison, 2017**). The persistence of this symptom at the higher dose reflects a clear dose-tissue response relationship.

**Toxicological Conclusions and Perspectives** This study delineates a highly favorable toxicological profile for the **FMA** compound. The compound demonstrates a broad safety margin regarding lethal toxicity, with no adverse effects on baseline autonomic functions. However, the transient behavioral manifestations and the delayed partial paralysis observed at the maximum dose (100 mg/kg) establish this concentration as the Lowest Observed Adverse Effect Level (LOAEL).

The complete reversibility of all observed symptoms indicates that **FMA** possesses pharmacokinetics amenable to effective systemic clearance. To optimize future preclinical trials, it is highly recommended to account for sex-specific pharmacokinetic differences and to utilize lower

therapeutic doses, which are expected to fall safely below the threshold for these transient neurological and dermatological side effects (**Table 05**)( **Table 06**).

**Table 05.** Clinical signs of acute toxicity observed within 1–4 hours following single-dose administration of **FMA** at graded dose levels (0.1–100 mg/kg) in male and female experimental animals.

|                | Control |        | Male             |      |           |      | Female |      |      |      |
|----------------|---------|--------|------------------|------|-----------|------|--------|------|------|------|
| Response       | Male    | Female | D1               | D2   | D3        | D4   | D1     | D2   | D3   | D4   |
| Skin color     | N       | N      | N                | N    | N         | N    | N      | N    | N    | N    |
| Temperature    | N       | N      | N                | N    | N         | N    | N      | N    | N    | N    |
| Fur condition  | N       | N      | N                | N    | N         | N    | N      | N    | N    | N    |
| Posture        | N       | N      | Hunched          | N    | Trembling | N    | N      | N    | N    | N    |
| Urination      | N       | N      | N                | N    | N         | N    | N      | N    | N    | N    |
| Salivation     | N       | N      | N                | N    | N         | N    | N      | N    | N    | N    |
| Activity       | N       | N      | Active           |      | Inactive  |      | N      | N    | N    | N    |
| Respiration    | N       | N      | N                | N    | N         | N    | N      | N    | N    | N    |
| Eyes           | N       | N      | Half-closed eyes |      |           |      | N      | N    | N    | N    |
| Nose/Mouth     | N       | N      | N                | N    | N         | N    | N      | N    | N    | N    |
| Touch response | N       | N      | N                | N    | N         | N    | N      | N    | N    | N    |
| Pain response  | N       | N      | N                | N    | N         | N    | N      | N    | N    | N    |
| Tremors        | A       | A      | A                | A    | A         | A    | A      | A    | A    | A    |
| Convulsion     | A       | A      | A                | A    | A         | A    | A      | A    | A    | A    |
| Ataxia         | A       | A      | A                | A    | A         | A    | A      | A    | A    | A    |
| Paralysis      | A       | A      | A                | A    | A         | A    | A      | A    | A    | A    |
| Mortality      | None    | None   | None             | None | None      | None | None   | None | None | None |

**Table 06.** Clinical signs of subacute toxicity observed during the 14-day post-treatment period following single-dose administration of **FMA** at graded dose levels (0.1–100 mg/kg) in male and female experimental animals.

|                | Control |        | Male             |                  |                  |                            | Female |      |                       |      |
|----------------|---------|--------|------------------|------------------|------------------|----------------------------|--------|------|-----------------------|------|
| Response       | Male    | Female | D1               | D2               | D3               | D4                         | D1     | D2   | D3                    | D4   |
| Skin color     | N       | N      | N                | N                | N                | N                          | N      | N    | N                     | N    |
| Temperature    | N       | N      | N                | N                | N                | N                          | N      | N    | N                     | N    |
| Fur condition  | N       | N      | Patchy<br>Day: 9 | Patchy<br>Day: 9 | Patchy<br>Day: 9 | Patchy<br>Day: 9,11,<br>13 | N      | N    | N                     | N    |
| Posture        | N       | N      | N                | N                | N                | N                          | N      | N    | N                     | N    |
| Urination      | N       | N      | N                | N                | N                | N                          | N      | N    | N                     | N    |
| Salivation     | N       | N      | N                | N                | N                | N                          | N      | N    | N                     | N    |
| Activity       | N       | N      | N                | N                | N                | N                          | N      | N    | N                     | N    |
| Respiration    | N       | N      | N                | N                | N                | N                          | N      | N    | N                     | N    |
| Eyes           | N       | N      | N                | N                | N                | N                          | N      | N    | N                     | N    |
| Nose/Mouth     | N       | N      | N                | N                | N                | N                          | N      | N    | N                     | N    |
| Touch response | N       | N      | N                | N                | N                | N                          | N      | N    | N                     | N    |
| Pain response  | N       | N      | N                | N                | N                | N                          | N      | N    | N                     | N    |
| Tremors        | A       | A      | A                | A                | A                | A                          | A      | A    | A                     | A    |
| Convulsion     | A       | A      | A                | A                | A                | A                          | A      | A    | A                     | A    |
| Ataxia         | A       | A      | A                | A                | A                | A                          | A      | A    | A                     | A    |
| Paralysis      | A       | A      | A                | A                | A                | A                          | A      | A    | Partial<br>Day: 5,7,8 | A    |
| Mortality      | None    | None   | None             | None             | None             | None                       | None   | None | None                  | None |

## B. Clinical signs of acute toxicity of FCMphA

**Physiological Stability and the Absence of Lethal Toxicity** The acute clinical observations of **FCMphA** delineate a highly favorable initial safety profile. The complete absence of mortality across all experimental cohorts confirms that the median lethal dose (LD50) of this compound substantially exceeds the maximum administered dose limit. Furthermore, the stability of core physiological parameters—including integumentary condition (skin color and fur), respiratory rate, secretory functions (salivation and urination), and sensory reflexes indicates an absence of acute systemic toxicity. Importantly, this stability suggests that **FCMphA** does not disrupt the vital autonomic centers within the brainstem. This baseline safety profile reinforces a prevailing paradigm in bio-organometallic chemistry: the strategic incorporation of a robust transition metal core, such as ferrocene, into an organic ligand framework can significantly mitigate the acute systemic toxicity typically associated with free, uncoordinated heavy metals (**Patra & Gasser, 2017**).

**Dose-Dependent CNS Depression and Pharmacokinetic Sexual Dimorphism** Despite its overall physiological safety, behavioral assessments revealed that **FCMphA** exerts a mild, dose-dependent depressant effect on the central nervous system (CNS), manifesting primarily as reduced locomotor activity (inactivity). From a toxicological standpoint, the most mechanistically intriguing aspect of this observation is the pronounced sexual dimorphism in the dose threshold required to elicit this response. Specifically, CNS depression was observed in males at the sub-maximal dose (D3), whereas it only became apparent in females at the maximum dose (D4).

This gender-based discrepancy is indicative of fundamental differences in toxicokinetics. Given the inherently lipophilic nature of ferrocene derivatives, the higher relative proportion of adipose tissue typically present in females leads to a proportionally larger physiological volume of distribution (Vd) for the compound:  $V_d = V_p + V_t$

This expanded distribution into peripheral lipid compartments effectively sequesters a fraction of the lipophilic drug, thereby attenuating the peak concentration of the free fraction available to passively diffuse across the blood-brain barrier (BBB) at equivalent weight-adjusted doses (**Zucker & Prendergast, 2020**). Additionally, well-documented sex-specific disparities in the expression profiles of hepatic cytochrome P450 (CYP450) enzymes in rodents may facilitate a

more rapid metabolic clearance of the compound in females, further delaying the onset of neurological symptoms(Waxman & Holloway, 2009).

Structure-Activity Relationship (SAR) and Comparative Neurotoxicity A comparative analysis between the acute toxicity profiles of **FCMphA** and the previously evaluated **FMA** derivative highlights a critical Structure-Activity Relationship (SAR). While **FMA** induced a biphasic neurological response—characterized by early depression (hunched posture, ptosis) followed by neuromuscular excitability (tremors)—**FCMphA** elicited a purely sedative/depressant effect at elevated doses, with a complete absence of postural abnormalities, tremors, convulsions, or ataxia.

This phenotypic divergence suggests that the specific structural modifications introduced into the organic ligand of **FCMphA** profoundly altered its pharmacodynamic interactions. It is highly probable that these structural variations shifted the compound's partition coefficient (logP) or altered its steric conformation, effectively abolishing its off-target affinity for voltage-gated ion channels or excitatory neurotransmitter receptors in the peripheral and central nervous systems. Understanding these parameters of BBB transport and receptor interaction illustrates how minor structural tuning in organometallic drug design can successfully eliminate unwanted neuromuscular excitability, thereby widening the neurotoxicological safety margin(Pardridge, 2012).

Toxicological Conclusion In summary, **FCMphA** exhibits an acute safety window that surpasses that of **FMA** in terms of neurological stability. Its acute side effects are strictly limited to transient, high-dose behavioral sedation, devoid of the severe neurotoxic manifestations or autonomic failure that frequently plague novel neuroactive compounds. This profile positions **FCMphA** as a highly promising pharmacological candidate for further preclinical development, emphasizing the necessity of tailoring future dosing regimens to account for the observed pharmacokinetic differences between sexes to accurately establish the No Observed Adverse Effect Level (NOAEL) ( Table 07).

**Table 07.** Clinical signs of acute toxicity observed within 1–4 hours following single-dose administration of **FCMphA** at graded dose levels (0.1–100 mg/kg) in male and female experimental animals.

|                | Control |        | Male |      |          |      | Female |      |      |          |
|----------------|---------|--------|------|------|----------|------|--------|------|------|----------|
| Response       | Male    | Female | D1   | D2   | D3       | D4   | D1     | D2   | D3   | D4       |
| Skin color     | N       | N      | N    | N    | N        | N    | N      | N    | N    | N        |
| Temperature    | N       | N      | N    | N    | N        | N    | N      | N    | N    | N        |
| Fur condition  | N       | N      | N    | N    | N        | N    | N      | N    | N    | N        |
| Posture        | N       | N      | N    | N    | N        | N    | N      | N    | N    | N        |
| Urination      | N       | N      | N    | N    | N        | N    | N      | N    | N    | N        |
| Salivation     | N       | N      | N    | N    | N        | N    | N      | N    | N    | N        |
| Activity       | N       | N      | N    |      | Inactive | N    | N      | N    | N    | Inactive |
| Respiration    | N       | N      | N    | N    | N        | N    | N      | N    | N    | N        |
| Eyes           | N       | N      | N    | N    | N        |      | N      | N    | N    | N        |
| Nose/Mouth     | N       | N      | N    | N    | N        | N    | N      | N    | N    | N        |
| Touch response | N       | N      | N    | N    | N        | N    | N      | N    | N    | N        |
| Pain response  | N       | N      | N    | N    | N        | N    | N      | N    | N    | N        |
| Tremors        | A       | A      | A    | A    | A        | A    | A      | A    | A    | A        |
| Convulsion     | A       | A      | A    | A    | A        | A    | A      | A    | A    | A        |
| Ataxia         | A       | A      | A    | A    | A        | A    | A      | A    | A    | A        |
| Paralysis      | A       | A      | A    | A    | A        | A    | A      | A    | A    | A        |
| Mortality      | None    | None   | None | None | None     | None | None   | None | None | None     |

The acute clinical assessment (1–4 hours post-administration) of **FCMphA** reveals a robust initial safety profile, characterized by zero mortality and the preservation of core autonomic functions across all experimental cohorts. The absence of severe neurological anomalies—such as tremors, convulsions, or ataxia—confirms that the compound does not induce acute excitotoxicity

or severe brainstem disruption. However, similar to other lipophilic organometallic compounds, **FCMphA** exhibited a dose-dependent, transient central nervous system (CNS) depressant effect, manifesting as behavioral inactivity.

Mechanistically, this sedation highlights the compound's ability to passively diffuse across the blood-brain barrier (BBB) and temporarily modulate neural activity(**Banks, 2016**).

Notably, a distinct sexual dimorphism was observed in the threshold required to elicit this response: males exhibited inactivity at the sub-maximal dose (D3), whereas females only displayed this sign at the maximum dose (D4). This discrepancy is driven by gender-specific toxicokinetics. Because females generally possess a higher fractional volume of adipose tissue, the physiological volume of distribution for highly lipophilic xenobiotics is significantly expanded. By sequestering a larger proportion of the lipophilic drug in peripheral adipose compartments, the peak plasma concentration available to penetrate the BBB is markedly attenuated in females, thereby raising the threshold required to trigger acute CNS depression(**Zucker & Prendergast, 2020**).

While **FCMphA** shares the non-lethal acute profile of its analog (**FMA**), the extended 14-day observation period uncovered a broader and more complex spectrum of delayed multi-systemic effects. The emergence of dark ocular discharge on day 2 is a classic presentation of chromodacryorrhea—the hypersecretion of porphyrin-rich tears from the Harderian gland, which serves as a highly sensitive, non-specific physiological biomarker of acute systemic stress in rodents(**editor, n.d.**) (**Klaassen, 2013**).

More critically, the observation of epistaxis (nosebleeds) on days 2 and 5 in high-dose females points to delayed mucosal and vascular toxicity. The introduction of specific functional groups to the ferrocene core in **FCMphA** may impart secondary redox-active properties. In the systemic circulation, transition metal complexes can undergo redox cycling, transferring electrons to endogenous peroxides to generate highly reactive oxygen species (ROS), such as hydroxyl radicals. This localized oxidative stress can disrupt the integrity of the delicate mucosal capillary beds or transiently interfere with the coagulation cascade, leading to the observed epistaxis(**Jomova & Valko, 2011**). The complete reversibility of these bleeding episodes suggests mucosal irritation rather than permanent coagulopathy or bone marrow suppression.

Dermatological alterations presented as patchy fur in male subjects across all dose groups (days 3–11), indicating a consistent, dose-independent sensitivity of the integumentary system to **FCMphA**. Hair follicles are highly metabolic micro-organs characterized by rapid cellular proliferation. Systemic stress, altered hormonal signaling, or the direct accumulation of the

organometallic compound within keratinocytes can prematurely arrest the active growth phase (anagen) of the hair cycle, forcing follicles synchronously into the resting and shedding phases, a condition known as telogen effluvium (Stenn & Paus, 2001). The appearance of alopecia at even the lowest dose (D1) suggests that the specific structural conformation of **FCMphA** possesses a high affinity for cutaneous tissues or a low threshold for disrupting follicular metabolic homeostasis.

Consistent with the delayed toxicological profile of highly lipophilic organometallics, females at the highest dose (D4) exhibited partial paralysis on days 5, 7, and 8. This delayed neurotoxicity is pharmacokinetically driven by the slow release of the compound from deep lipid reservoirs (such as peripheral adipose tissue) and its subsequent accumulation in the lipid-rich myelin sheaths surrounding peripheral nerves. When the localized tissue concentration temporarily exceeds the toxicological threshold due to a slow elimination rate, it can disrupt voltage-gated ion channels, resulting in transient demyelinating neuropathy (Pajouhesh & Lenz, 2005). The complete resolution of the partial paralysis after day 8 confirms that **FCMphA** does not cause irreversible axonal damage (Wallerian degeneration) and that hepatic and renal clearance mechanisms are ultimately capable of detoxifying and eliminating the compound (Patra & Gasser, 2017).

Collectively, the toxicological evaluation of **FCMphA** reveals a compound that is acutely safe and non-lethal, yet possesses a broader delayed toxicity footprint compared to its analogs. While its acute effects are limited to transient CNS sedation, its subacute profile involves multi-systemic interactions, including mucosal and vascular fragility (epistaxis), systemic stress responses (chromodacryorrhea), follicular disruption, and reversible peripheral neuropathy at extreme doses. All adverse effects demonstrated complete reversibility, underscoring the compound's eventual systemic clearance. These findings emphasize the absolute necessity of rigorous dose optimization in future therapeutic applications to safely harness the compound's efficacy while remaining well below the threshold of these multi-systemic off-target effects (Table 08).

**Table 08.** Clinical signs of subacute toxicity observed during the 14-day post-treatment period following single-dose administration of **FCMphA** at graded dose levels (0.1–100 mg/kg) in male and female experimental animals.

|                   | Control |        | Male                      |                         |                         |                         | Female                      |      |                           |      |
|-------------------|---------|--------|---------------------------|-------------------------|-------------------------|-------------------------|-----------------------------|------|---------------------------|------|
| Response          | Male    | Female | D1                        | D2                      | D3                      | D4                      | D1                          | D2   | D3                        | D4   |
| Skin color        | N       | N      | N                         | N                       | N                       | N                       | N                           | N    | N                         | N    |
| Temperature       | N       | N      | N                         | N                       | N                       | N                       | N                           | N    | N                         | N    |
| Fur condition     | N       | N      | Patchy<br>Day: 8,<br>9 11 | Patchy<br>Day:<br>9, 11 | Patchy<br>Day:<br>3, 11 | Patchy<br>Day:<br>8, 11 | N                           | N    | N                         | N    |
| Posture           | N       | N      | N                         | N                       | N                       | N                       | N                           | N    | N                         | N    |
| Urination         | N       | N      | N                         | N                       | N                       | N                       | N                           | N    | N                         | N    |
| Salivation        | N       | N      | N                         | N                       | N                       | N                       | N                           | N    | N                         | N    |
| Activity          | N       | N      | Inactiv<br>e<br>Day : 3   | N                       | N                       | N                       | N                           | N    | N                         | N    |
| Respiration       | N       | N      | N                         | N                       | N                       | N                       | N                           | N    | N                         | N    |
| Eyes              | N       | N      | N                         | N                       | N                       | N                       | Dark<br>discharge<br>Day: 2 | N    | N                         | N    |
| Nose/Mouth        | N       | N      | N                         | N                       | N                       | N                       | N                           | N    | Noseble<br>ed<br>Day: 2,5 | N    |
| Touch<br>response | N       | N      | N                         | N                       | N                       | N                       | N                           | N    | N                         | N    |
| Pain response     | N       | N      | N                         | N                       | N                       | N                       | N                           | N    | N                         | N    |
| Tremors           | A       | A      | A                         | A                       | A                       | A                       | A                           | A    | A                         | A    |
| Convulsion        | A       | A      | A                         | A                       | A                       | A                       | A                           | A    | A                         | A    |
| Ataxia            | A       | A      | A                         | A                       | A                       | A                       | A                           | A    | A                         | A    |
| Paralysis         | A       | A      | A                         | A                       | A                       | A                       | A                           | A    | Partial<br>Day:<br>5,7,8  | A    |
| Mortality         | None    | None   | None                      | None                    | None                    | None                    | None                        | None | None                      | None |

### C. Clinical signs of acute toxicity of FCMphB

The acute clinical evaluation of **FCMphB** (1–4 hours post-administration) reveals an impeccable initial safety profile, distinguishing it as the most acutely tolerated compound among the tested derivatives. The complete absence of mortality across all dose groups (0.1–100 mg/kg) firmly establishes that the median lethal dose unequivocally exceeds the maximum administered concentration. Furthermore, the absolute stability of all monitored physiological parameters including respiratory rate, thermoregulation, secretory functions (salivation and urination), and sensory reflexes—indicates that **FCMphB** induces zero acute disruption to the autonomic nervous system or brainstem centers. This pristine acute safety record strongly supports the pharmacological principle that precise ligand modifications around a ferrocene core can virtually eliminate the immediate systemic toxicity often associated with metallodrugs, optimizing their biocompatibility (Patra & Gasser, 2017).

From a toxicological and medicinal chemistry perspective, the most remarkable finding is the complete absence of behavioral alterations or central nervous system (CNS) modulation.

Unlike its structural analogs where **FMA** induced a biphasic neurobehavioral response (depression followed by tremors) and **FCMphA** caused dose-dependent sedation **FCMphA** provoked no inactivity, excitability, or postural changes whatsoever.

This profound phenotypic divergence highlights a critical Structure-Activity Relationship (SAR). The specific functional groups integrated into the **FCMphB** molecule likely altered its fundamental physicochemical properties, such as reducing its overall lipophilicity or increasing its topological polar surface area. These structural tuning strategies are known to restrict rapid, passive diffusion across the tight endothelial junctions of the blood-brain barrier (BBB). Consequently, **FCMphB** fails to reach pharmacologically active concentrations within the CNS during the acute phase, completely circumventing the rapid-onset neurotoxicity observed with its more lipophilic counterparts (Banks, 2016; C A Lipinski, Lombardo, Dominy, & Feeney, 2001).

In addition to behavioral normalcy, the total absence of neuromotor anomalies such as tremors, convulsions, paralysis, or ataxia confirms the compound's high target specificity. Highly lipophilic or poorly optimized xenobiotics often exhibit indiscriminate affinity for voltage-gated ion channels or peripheral cholinergic receptors, triggering acute excitotoxicity or motor incoordination. The clean neuromuscular profile of **FCMphB** demonstrates that its structural conformation effectively prevents these off-target interactions in the immediate post-dosing window, maintaining complete integrity of the peripheral nervous system and neuromuscular junctions (editor, n.d.) (Klaassen, 2013).

In summary, **FCMphB** demonstrates a superior acute toxicological profile characterized by absolute clinical silence. It effectively bypasses the acute CNS depression and neuromuscular excitability seen in its structural analogs, likely due to optimized physicochemical properties that limit rapid brain penetrance and off-target receptor binding. This exceptional acute tolerability provides a highly favorable therapeutic window, positioning **FCMphB** as an ideal candidate for further pharmacological development, pending the evaluation of its subacute and long-term systemic clearance profiles (**Table 09**).

**Table 09.** Clinical signs of acute toxicity observed within 1–4 hours following single-dose administration of **FCMphB** at graded dose levels (0.1–100 mg/kg) in male and female experimental animals.

|                | Control |        | Male |      |      |      | Female |      |      |      |
|----------------|---------|--------|------|------|------|------|--------|------|------|------|
| Response       | Male    | Female | D1   | D2   | D3   | D4   | D1     | D2   | D3   | D4   |
| Skin color     | N       | N      | N    | N    | N    | N    | N      | N    | N    | N    |
| Temperature    | N       | N      | N    | N    | N    | N    | N      | N    | N    | N    |
| Fur condition  | N       | N      | N    | N    | N    | N    | N      | N    | N    | N    |
| Posture        | N       | N      | N    | N    | N    | N    | N      | N    | N    | N    |
| Urination      | N       | N      | N    | N    | N    | N    | N      | N    | N    | N    |
| Salivation     | N       | N      | N    | N    | N    | N    | N      | N    | N    | N    |
| Activity       | N       | N      | N    | N    | N    | N    | N      | N    | N    | N    |
| Respiration    | N       | N      | N    | N    | N    | N    | N      | N    | N    | N    |
| Eyes           | N       | N      | N    | N    | N    | N    | N      | N    | N    | N    |
| Nose/Mouth     | N       | N      | N    | N    | N    | N    | N      | N    | N    | N    |
| Touch response | N       | N      | N    | N    | N    | N    | N      | N    | N    | N    |
| Pain response  | N       | N      | N    | N    | N    | N    | N      | N    | N    | N    |
| Tremors        | A       | A      | A    | A    | A    | A    | A      | A    | A    | A    |
| Convulsion     | A       | A      | A    | A    | A    | A    | A      | A    | A    | A    |
| Ataxia         | A       | A      | A    | A    | A    | A    | A      | A    | A    | A    |
| Paralysis      | A       | A      | A    | A    | A    | A    | A      | A    | A    | A    |
| Mortality      | None    | None   | None | None | None | None | None   | None | None | None |

The acute clinical evaluation of **FCMphB** (1–4 hours post-administration), as detailed in **Table 9**, reveals an impeccable initial safety profile. The compound distinguishes itself as the most acutely tolerated derivative among the tested series. The complete absence of mortality across all dose groups (0.1–100 mg/kg) in both male and female cohorts establishes unequivocally that the median lethal dose significantly exceeds the maximum administered concentration. Furthermore, the absolute stability of all monitored physiological parameters including respiratory rate, integumentary condition, secretory functions (salivation and urination), and sensory reflexes—indicates that **FCMphB** induces zero acute disruption to the autonomic nervous system. This pristine acute safety record corroborates the pharmacological principle that the rational design of coordination spheres around transition metals can effectively mask the inherent toxicity of the metal ion, thereby enhancing the systemic biocompatibility of the resulting metallodrug(**Mjos & Orvig, 2014**).

From a neurotoxicological perspective, the most remarkable finding is the complete absence of behavioral alterations or central nervous system (CNS) modulation. Unlike many neuroactive xenobiotics, **FCMphB** provoked no inactivity, tremors, convulsions, or postural changes whatsoever.

The total absence of neuromotor anomalies confirms the compound's high target specificity and reduced off-target liabilities. Poorly optimized drug candidates often exhibit indiscriminate affinity for secondary pharmacological targets, such as peripheral voltage-gated ion channels or G-protein-coupled receptors, which typically triggers acute excitotoxicity or motor incoordination. The clean neuromuscular profile of **FCMphB** demonstrates that it successfully evades these off-target interactions, preserving the intricate physiological signaling at the neuromuscular junctions during the immediate post-dosing window(**Bowes et al., 2012**).

This profound phenotypic divergence highlights a critical Structure-Activity Relationship (SAR). The specific functional groups integrated into the **FCMphB** ligand likely altered its fundamental physicochemical properties. In predictive toxicology, modifications that increase the topological polar surface area (TPSA) or adjust the lipophilicity profile are known to significantly restrict a molecule's passive diffusion across the highly selective endothelial tight junctions of the blood-brain barrier. Consequently, **FCMphB** fails to reach neurotoxic concentrations within the CNS parenchyma during the acute phase, completely circumventing the rapid-onset

neurobehavioral alterations often observed with structurally related, highly lipophilic agents(Abbott, Patabendige, Dolman, Yusof, & Begley, 2010; Gleeson, 2008).

In summary, **FCMphB** demonstrates a superior acute toxicological profile characterized by absolute clinical silence. By effectively bypassing the acute CNS depression and neuromuscular excitability seen in alternative derivatives, it provides a highly favorable initial therapeutic window. This exceptional acute tolerability positions **FCMphB** as a highly promising pharmacological candidate, warranting further investigation into its subacute systemic clearance and long-term tissue accumulation profiles.

**Subacute Survivability and Global Safety Profile** The subacute clinical evaluation over the 14-day post-treatment period confirms the exceptional global safety profile of **FCMphB**. The complete absence of delayed mortality across all dose cohorts (0.1–100 mg/kg) underscores the non-lethal nature of the compound and its metabolites. Furthermore, the stability of core physiological parameters throughout the observation window indicates that the bio-organometallic framework does not undergo deleterious systemic degradation or induce chronic organ failure. This supports the premise that carefully functionalized ferrocene derivatives exhibit high thermodynamic stability in biological media, preventing the delayed release of toxic free iron ions into the systemic circulation (Gasser et al., 2011).

A highly intriguing finding in the subacute phase is the manifestation of dermatological alterations (patchy fur on day 5) exclusively at the lowest dose (D1), affecting both male and female subjects uniformly, while being completely absent at higher dose levels (D2, D3, and D4).

In classical toxicology, dose-response relationships are typically linear; however, this phenomenon is a classic example of a non-monotonic dose-response (NMDR) curve, often characterized by a U-shaped or inverted U-shaped trajectory.

Mechanistically, at low systemic concentrations, the lipophilic organometallic compound may exhibit a highly specific affinity for receptors regulating the anagen phase of the hair follicle, triggering premature follicular arrest and subsequent telogen effluvium. Conversely, at higher systemic concentrations, the compound may either trigger rapid receptor down-regulation (desensitization) or activate alternative compensatory clearance pathways that paradoxically

protect the cutaneous microenvironment. Such non-monotonic behaviors are increasingly recognized in the pharmacological profiling of complex, receptor-modulating xenobiotics(Vandenberg et al., 2012).

The neurotoxicological data reveal a delayed, sex-specific, and non-linear response. Partial paralysis was observed exclusively in females at the sub-maximal dose (D3) on days 5, 7, and 8, whereas males remained entirely unaffected. The gender disparity is primarily driven by variations in body composition; the higher adipose tissue volume in females acts as a deep depot for lipophilic drugs. This promotes the slow, sustained release of the compound into the lipid-rich myelin sheaths of peripheral nerves, eventually surpassing the local toxic threshold and causing reversible demyelinating neuropathy(Cavaletti et al., 2019; Franconi & Campesi, 2014).

Crucially, this delayed partial paralysis was absent in females at the highest dose (D4). This paradoxical absence of toxicity at the maximum dose can be attributed to nonlinear pharmacokinetics, specifically the auto-induction of hepatic drug-metabolizing enzymes.

Supratherapeutic doses of certain xenobiotics are known to potently induce the expression of hepatic cytochrome P450 (CYP450) enzymes. Consequently, the maximum dose (D4) likely triggered a massive up-regulation of its own metabolic clearance, drastically reducing the compound's biological half-life and preventing the sustained tissue accumulation necessary to induce peripheral neuropathy. The complete recovery of the D3 females by the end of the observation period further confirms the reversible, non-degenerative nature of this neurotoxic episode(Lin & Lu, 1998).

The 14-day subacute profile of **FCMphB** highlights a highly tolerable compound characterized by non-lethal, completely reversible, and highly specific off-target effects. The presence of non-monotonic dermatological responses and auto-induced neuroprotective clearance at high doses provides invaluable pharmacokinetic insights. These findings strongly support the viability of **FCMphB** as a therapeutic candidate and underscore the critical need for precise, sex-specific dose calibration in future preclinical efficacy models to maintain concentrations within the optimal therapeutic window (Table 10).

**Table 10.** Clinical signs of subacute toxicity observed during the 14-day post-treatment period following single-dose administration of **FCMphB** at graded dose levels (0.1–100 mg/kg) in male and female experimental animals.

|                   | Control |        | Male             |      |      |      | Female           |      |                          |      |
|-------------------|---------|--------|------------------|------|------|------|------------------|------|--------------------------|------|
| Response          | Male    | Female | D1               | D2   | D3   | D4   | D1               | D2   | D3                       | D4   |
| Skin color        | N       | N      | N                | N    | N    | N    | N                | N    | N                        | N    |
| Temperature       | N       | N      | N                | N    | N    | N    | N                | N    | N                        | N    |
| Fur condition     | N       | N      | Patchy<br>Day: 5 | N    | N    | N    | Patchy<br>Day: 5 | N    | N                        | N    |
| Posture           | N       | N      | N                | N    | N    | N    | N                | N    | N                        | N    |
| Urination         | N       | N      | N                | N    | N    | N    | N                | N    | N                        | N    |
| Salivation        | N       | N      | N                | N    | N    | N    | N                | N    | N                        | N    |
| Activity          | N       | N      | N                | N    | N    | N    | N                | N    | N                        | N    |
| Respiration       | N       | N      | N                | N    | N    | N    | N                | N    | N                        | N    |
| Eyes              | N       | N      | N                | N    | N    | N    | N                | N    | N                        | N    |
| Nose/Mouth        | N       | N      | N                | N    | N    | N    | N                | N    | N                        | N    |
| Touch<br>response | N       | N      | N                | N    | N    | N    | N                | N    | N                        | N    |
| Pain response     | N       | N      | N                | N    | N    | N    | N                | N    | N                        | N    |
| Tremors           | A       | A      | A                | A    | A    | A    | A                | A    | A                        | A    |
| Convulsion        | A       | A      | A                | A    | A    | A    | A                | A    | A                        | A    |
| Ataxia            | A       | A      | A                | A    | A    | A    | A                | A    | A                        | A    |
| Paralysis         | A       | A      | A                | A    | A    | A    | A                |      | Partial<br>Day:<br>5,7,8 | A    |
| Mortality         | None    | None   | None             | None | None | None | None             | None | None                     | None |

### II.2.1.2. Weekly body weights, food and water consumption

#### A. Weekly body weights, food and water consumption of rats across FMA doses

The subacute clinical evaluation over the 14-day post-treatment period confirms the exceptional global safety profile of **FMA**. The complete absence of delayed mortality across all dose cohorts (0.1–100 mg/kg) underscores the non-lethal nature of the compound and its metabolites. Furthermore, the stability of core physiological parameters throughout the observation window indicates that the bio-organometallic framework does not undergo deleterious systemic degradation or induce chronic organ failure. This supports the premise that carefully functionalized ferrocene derivatives exhibit high thermodynamic stability in biological media, preventing the delayed release of toxic free iron ions into the systemic circulation (**Gasser, Ott, & Metzler-Nolte, 2011**).

A highly intriguing finding in the subacute phase is the manifestation of dermatological alterations (patchy fur on day 5) exclusively at the lowest dose (D1), affecting both male and female subjects uniformly, while being completely absent at higher dose levels (D2, D3, and D4).

In classical toxicology, dose-response relationships are typically linear; however, this phenomenon is a classic example of a non-monotonic dose-response (NMDR) curve, often characterized by a U-shaped or inverted U-shaped trajectory.

Mechanistically, at low systemic concentrations, the lipophilic organometallic compound may exhibit a highly specific affinity for receptors regulating the anagen phase of the hair follicle, triggering premature follicular arrest and subsequent telogen effluvium. Conversely, at higher systemic concentrations, the compound may either trigger rapid receptor down-regulation (desensitization) or activate alternative compensatory clearance pathways that paradoxically protect the cutaneous microenvironment. Such non-monotonic behaviors are increasingly recognized in the pharmacological profiling of complex, receptor-modulating xenobiotics (**Vandenberg et al., 2012**)(**Cavaletti et al., 2019; Franconi & Campesi, 2014**).

The neurotoxicological data reveal a delayed, sex-specific, and non-linear response. Partial paralysis was observed exclusively in females at the sub-maximal dose (D3) on days 5, 7,

and 8, whereas males remained entirely unaffected. The gender disparity is primarily driven by variations in body composition; the higher adipose tissue volume in females acts as a deep depot for lipophilic drugs. This promotes the slow, sustained release of the compound into the lipid-rich myelin sheaths of peripheral nerves, eventually surpassing the local toxic threshold and causing reversible demyelinating neuropathy (Cavaletti et al., 2019; Franconi & Campesi, 2014).

Crucially, this delayed partial paralysis was absent in females at the highest dose (D4). This paradoxical absence of toxicity at the maximum dose can be attributed to nonlinear pharmacokinetics, specifically the auto-induction of hepatic drug-metabolizing enzymes.

Supratherapeutic doses of certain xenobiotics are known to potently induce the expression of hepatic cytochrome P450 (CYP450) enzymes. Consequently, the maximum dose (D4) likely triggered a massive up-regulation of its own metabolic clearance, drastically reducing the compound's biological half-life and preventing the sustained tissue accumulation necessary to induce peripheral neuropathy. The complete recovery of the D3 females by the end of the observation period further confirms the reversible, non-degenerative nature of this neurotoxic episode (Lin & Lu, 1998).

The 14-day subacute profile of **FMA** highlights a highly tolerable compound characterized by non-lethal, completely reversible, and highly specific off-target effects. The presence of non-monotonic dermatological responses and auto-induced neuroprotective clearance at high doses provides invaluable pharmacokinetic insights. These findings strongly support the viability of **FMA** as a therapeutic candidate and underscore the critical need for precise, sex-specific dose calibration in future preclinical efficacy models to maintain concentrations within the optimal therapeutic window (Table 11).

**Table 11.** Effects of graded doses of **FMA** (0.1, 1, 10, and 100 mg/kg) on weekly body weight, food intake, and water consumption in male and female rats over a 14-day observation period.

| Parameters |                        | Week  | Control   | D1        | D2        | D3        | D4        |
|------------|------------------------|-------|-----------|-----------|-----------|-----------|-----------|
| Female     | Weight (g)             | Week0 | 141.1±2.5 | 130.8±1.7 | 147.3±6.4 | 141.9±1.5 | 144.2±4   |
|            |                        | Week1 | 154.5±1.4 | 150.5±5   | 152.1±2.1 | 146.6±1.3 | 163.3±5.3 |
|            |                        | Week2 | 187.6±3.6 | 164.6±2.1 | 150.4±1.2 | 147.5±4.2 | 151.6±2.9 |
|            | Food consumption (g)   | Week1 | 10.5±3    | 12.5±2.1  | 13.6±1.5  | 10.4±1.3  | 9.4±3.2   |
|            |                        | Week2 | 15.6±2.4  | 13.5±3.1  | 14.3±4.1  | 11.5±1.6  | 10.4±4.3  |
|            | Water consumption (ml) | Week1 | 55±8      | 55±11     | 50±9      | 105±7     | 100±12    |
|            |                        | Week2 | 55±6      | 55±7      | 55±10     | 67±9      | 90±10     |
| Male       | Weight (g)             | Week0 | 146.5±3.1 | 137.4±1.5 | 144.7±4.1 | 145.8±5.5 | 133.5±2.1 |
|            |                        | Week1 | 169.2±5.1 | 184.9±5   | 117.5±6.1 | 155.7±3.5 | 115.7±2.3 |
|            |                        | Week2 | 200.7±1.8 | 188.1±5.2 | 105.6±7.1 | 157.4±3.2 | 120.5±1.4 |
|            | Food consumption (g)   | Week1 | 11.1±2.6  | 10.7±4.6  | 9.1±2.1   | 8.4±6.7   | 9.6±5.1   |
|            |                        | Week2 | 14.2±1.8  | 10.5±2.3  | 10.2±1.9  | 10.6±2.1  | 9.8±3.9   |
|            | Water consumption (ml) | Week1 | 50±10     | 50±9      | 100±4     | 120±6     | 122±7     |
|            |                        | Week2 | 50±5      | 50±8      | 116±2     | 100±8     | 99±5      |

#### B. Weekly body weights, food and water consumption of rats across FCMphA doses

The longitudinal monitoring of somatic growth and nutritional indices reveals that **FCMphA** exerts a profound, dose-dependent metabolic penalty, culminating in severe systemic toxicity at the highest dose (D4). Unlike the mild growth attenuation seen at lower doses, the D4 female cohort exhibited a drastic absolute weight loss, dropping significantly below their baseline weight. This severe cachectic state, coupled with pronounced hypophagia, is a classic presentation of xenobiotic-induced anorexia. When the physiological burden of metabolizing a heavy organometallic load exceeds the organism's homeostatic capacity, a systemic inflammatory response is triggered. This "sickness behavior" involves the release of pro-inflammatory cytokines that directly suppress the hypothalamic appetite centers. Consequently, the organism is forced into a severe catabolic state, mobilizing its somatic and lipid reserves to fuel the energy-demanding pathways of hepatic and renal clearance, effectively explaining the dramatic weight collapse(Aviello, Cristiano, Luckman, & D'Agostino, 2021) .

A critical physiological divergence in this study is the dramatic escalation in water consumption (polydipsia) observed at D3 and D4, which paradoxically accompanies the severe

reduction in food intake. In mammalian physiology, fluid and dry matter intake are tightly coupled; thus, this uncoupling is a hallmark of osmoregulatory failure. The massive compensatory water intake points directly to drug-induced polyuria and acute renal tubular stress.

The excretion of highly lipophilic metallic metabolites places a tremendous oxidative burden on the renal proximal tubules and collecting ducts, disrupting the mobilization of aquaporin channels. This creates a functional state of drug-induced nephrogenic diabetes insipidus, where the kidneys fail to concentrate urine. In response, the animals are compelled to dramatically increase their water intake as a vital behavioral adaptation to avert fatal systemic dehydration during the active clearance of the compound (**Bockenhauer & Bichet, 2015; Mehta et al., 2015**)

Interestingly, the toxicological profile of **FCMphA** reveals a reverse sexual dimorphism compared to typical lipophilic agents. In this instance, female rats exhibited a much more severe catabolic collapse at the maximum dose than their male counterparts. This shift highlights the complex interplay between the specific chemical conformation of the **FCMphA** ligand and sex-dependent pharmacokinetics. The larger adipose compartment in females, which typically acts as a protective buffer for lipophilic drugs, may have reached its saturation point at the D4 dose. Once this depot capacity is exceeded, a rapid mobilization of the toxicant into the systemic circulation occurs. Modern toxicological studies emphasize that sex-specific disparities in the expression of hepatic efflux transporters and phase II conjugation enzymes often lead to the differential accumulation of reactive intermediates, rendering females uniquely susceptible to the metabolic collapse induced by this specific structural derivative (**Pardridge, 2012; Zucker & Prendergast, 2020**).

Collectively, the metabolic and nutritional data establish clear boundaries for the safety profile of **FCMphA**. The compound possesses a remarkably narrow therapeutic window at high concentrations. The D4 dose is unequivocally established as highly toxic, characterized by severe anorexia, catabolic weight loss (especially in females), and osmoregulatory distress. These findings strongly emphasize the absolute necessity of restricting future therapeutic or preclinical applications of **FCMphA** to lower dose ranges (D1–D2) to safely harness its pharmacological potential without triggering profound metabolic and renal disruption (**Table 12**).

**Table 12.** Effects of graded doses of **FCMphA** (0.1, 1, 10, and 100 mg/kg) on weekly body weight, food intake, and water consumption in male and female rats over a 14-day observation period.

| Parameters |                        | Week  | Control   | D1        | D2        | D3        | D4        |
|------------|------------------------|-------|-----------|-----------|-----------|-----------|-----------|
| Female     | Weight (g)             | Week0 | 141.1±2.5 | 159.2±4.2 | 144.3±2.4 | 153.7±5.5 | 145.4±5.2 |
|            |                        | Week1 | 154.5±1.4 | 166.9±6.4 | 149.8±8.1 | 159.3±6.3 | 108.3±1.3 |
|            |                        | Week2 | 187.6±3.6 | 172.7±6.1 | 153.4±4.2 | 170.6±4.1 | 113.7±8.3 |
|            | Food consumption (g)   | Week1 | 10.5±3    | 11.3±1.1  | 10.5±2.3  | 11.2±3.3  | 9.1±1.3   |
|            |                        | Week2 | 15.6±2.4  | 12.1±2.3  | 11.5±4.5  | 10.3±1.2  | 9.5±1.5   |
|            | Water consumption (ml) | Week1 | 55±8      | 45±10     | 65±10     | 110±8     | 99±13     |
|            |                        | Week2 | 55±6      | 50±11     | 60±9      | 100±11    | 91±9      |
| Male       | Weight (g)             | Week0 | 146.5±3.1 | 154.3±2.5 | 170.5±6   | 139.7±3.5 | 155.3±4.7 |
|            |                        | Week1 | 169.2±5.1 | 167.4±4.5 | 167.4±3.2 | 144.5±3.3 | 150.5±2.3 |
|            |                        | Week2 | 200.7±1.8 | 165.7±4.1 | 143.8±5.2 | 146.8±1.1 | 151.2±1.9 |
|            | Food consumption (g)   | Week1 | 11.1±2.6  | 10.6±2.1  | 11.1±2.5  | 9.5±3.1   | 9.9±1.2   |
|            |                        | Week2 | 14.2±1.8  | 10.5±2.2  | 13.2±3.1  | 10.8±3.3  | 9.5±1.3   |
|            | Water consumption (ml) | Week1 | 50±10     | 50±12     | 60±6      | 96±6      | 111±7     |
|            |                        | Week2 | 50±5      | 50±9      | 64±7      | 101±9     | 115±11    |

### C. Weekly body weights, food and water consumption of rats across FCMphB doses

The longitudinal evaluation of somatic growth and nutritional indices reveals that **FCMphB** possesses a vastly superior systemic tolerability profile compared to its structural analogs (**FMA** and **FCMphA**). The most prominent indicator of this biocompatibility is the remarkable stability of food consumption across all dose cohorts in both sexes. Unlike the severe xenobiotic-induced anorexia and subsequent catabolic collapse observed with previous derivatives, **FCMphB** did not trigger the profound neuroimmune "sickness behavior." The preservation of normal appetite indicates that the specific structural configuration of **FCMphB** does not provoke the massive release of pro-inflammatory cytokines, which typically suppress the hypothalamic satiety centers during systemic toxic stress. Consequently, the animals were able to maintain a relatively normal trajectory of body weight gain, successfully fueling the metabolic demands of drug clearance without depleting their somatic reserves.

Despite the overall metabolic stability, a highly intriguing physiological anomaly was observed in the female D1 cohort. These animals exhibited a progressive decrease in body weight over the 14-day period, a phenomenon that was paradoxically absent at higher doses (D2–D4).

This specific pattern is a quintessential representation of a non-monotonic dose-response (NMDR) relationship. Mechanistically, at very low systemic concentrations, the lipophilic organometallic compound may act as an endocrine modulator, exhibiting a specific affinity for metabolic or hormonal receptors that transiently suppress somatic growth. However, at higher concentrations, this effect is negated—either through the rapid down-regulation and desensitization of these specific receptors or via the robust auto-induction of hepatic clearance enzymes that rapidly eliminate the compound before it can exert continuous receptor-mediated effects. Such non-monotonic behaviors underscore the complex, receptor-specific interactions of metallodrugs at trace concentrations(**Lagarde et al., 2015**).

While food intake remained stable, the water consumption data revealed a moderate, dose-dependent escalation (polydipsia) at the highest dose (D4) in both sexes. Reaching approximately 110 ml in females and 97 ml in males during Week 2, this elevated fluid intake indicates a mild physiological adaptation rather than the severe osmoregulatory failure seen with **FCMphA**. The active renal excretion of lipophilic organometallic metabolites inherently requires increased urinary output (mild polyuria) to prevent the crystallization or tubular accumulation of the xenobiotic. Because the animals maintained their food intake and body weight, this polydipsia represents a highly successful and controlled homeostatic compensation. The kidneys effectively cleared the compound while the animals behaviorally matched the increased fluid turnover, thereby preserving systemic hydration without experiencing acute nephrotoxic distress(**Radi, 2019**). Collectively, the nutritional and metabolic data for **FCMphB** establish a highly favorable safety margin. The compound successfully evades the severe catabolic penalty and anorexia associated with heavy metal toxicity. This benign physiological profile is a testament to the principles of rational bio-organometallic design. By fine-tuning the coordination sphere and physicochemical properties of the ferrocene derivative, the metabolic burden on the liver and kidneys is significantly minimized. **FCMphB** demonstrates that it is possible to achieve effective systemic distribution while maintaining strict metabolic and osmoregulatory homeostasis, positioning it as an exceptionally safe and viable candidate for advanced preclinical efficacy trials (**Table 13**) (**Anthony et al., 2020**).

**Table 13.** Effects of graded doses of **FCMphB** (0.1, 1, 10, and 100 mg/kg) on weekly body weight, food intake, and water consumption in male and female rats over a 14-day observation period.

| Parameters |                        | Week  | Control   | D1        | D2        | D3        | D4        |
|------------|------------------------|-------|-----------|-----------|-----------|-----------|-----------|
| Female     | Weight (g)             | Week0 | 141.1±2.5 | 172.7±4.1 | 147.5±7.4 | 129.5±5.5 | 151.2±3.4 |
|            |                        | Week1 | 154.5±1.4 | 144.4±6.4 | 161.5±3.2 | 137.5±3.3 | 160.3±2.4 |
|            |                        | Week2 | 187.6±3.6 | 145.5±5.1 | 160.6±6.2 | 140.0±1.1 | 158.8±1.8 |
|            | Food consumption (g)   | Week1 | 10.5±3    | 11.7±1.1  | 11.1±1.3  | 10.3±2.2  | 10.4±0.4  |
|            |                        | Week2 | 15.6±2.4  | 14.1±3.4  | 10.2±1.4  | 10.0±2.6  | 11.0 ±0.3 |
|            | Water consumption (ml) | Week1 | 55±8      | 65±6      | 69±11     | 69±7      | 98±12     |
|            |                        | Week2 | 55±6      | 60±10     | 65±10     | 65±9      | 110±10    |
| Male       | Weight (g)             | Week0 | 146.5±3.1 | 155.6±3.7 | 139.7±4.4 | 139.9±3.5 | 143.3±3.2 |
|            |                        | Week1 | 169.2±5.1 | 169.1±5.5 | 152.1±2.1 | 152.6±1.6 | 149.2±3.3 |
|            |                        | Week2 | 200.7±1.8 | 170.7±3.2 | 156.2±4.8 | 160.4±1.2 | 145.5±1.9 |
|            | Food consumption (g)   | Week1 | 11.1±2.6  | 11.5±3.4  | 11.9±2.3  | 9.4±0.8   | 9.9±1.3   |
|            |                        | Week2 | 14.2±1.8  | 12.2±2.5  | 11.8±2.1  | 10.3±2.6  | 10.7±0.5  |
|            | Water consumption (ml) | Week1 | 50±10     | 50±5      | 69±10     | 65±6      | 97±13     |
|            |                        | Week2 | 50±5      | 56±4      | 60±9      | 65±7      | 93±8      |

### II.2.1.3. Organ weights index

#### A. Organ weights index of rats across FMA doses

**FMA** administration resulted in selective and dose-dependent alterations in organ weight indices, particularly in male animals.

In female rats, organ weights remained largely stable, with only minor fluctuations observed across all groups. A slight reduction in liver weight at D4 ( $2.28 \pm 0.32$ ) may suggest mild hepatic involvement, although not statistically significant.

In male rats, more notable changes were observed. Liver weight showed a significant decrease at D3 and D4, indicating potential hepatic atrophy or reduced metabolic activity.

Conversely, heart weight increased significantly at D4, suggesting possible cardiac hypertrophy or compensatory adaptation.

Other organs, including kidneys, brain, lungs, and spleen, showed no major deviations, indicating that **FMA** toxicity may be targeted rather than systemic.

The evaluation of relative organ weights is a fundamental macroscopic biomarker in predictive toxicology, providing critical insights into target-organ toxicity, generalized edema, or organ-specific atrophy. The data reveal that major vital organs including the brain, lungs, kidneys, stomach, spleen, and testes remained macroscopically unaffected across all dose groups in both sexes. The preservation of the brain and testicular indices is particularly significant, indicating that **FMA** does not induce gross neuro-edema or acute gonadal toxicity. This stability suggests that the xenobiotic insult is highly targeted rather than causing a systemic, non-specific organomegaly or generalized vascular leakage (Nirogi *et Al.*, 2014).

A profound and highly significant anomaly was observed in the hepatic index of male rats, which exhibited a marked, dose-dependent decrease at D3 and D4. In toxicological pathology, a significant reduction in relative liver weight (hepatic atrophy) is a highly alarming indicator. Given the severe catabolic weight loss and anorexia previously established at these doses, this hepatic shrinkage is primarily driven by the massive depletion of hepatocellular glycogen and lipid reserves. As the primary site of xenobiotic metabolism, the liver requires immense energetic resources to clear heavy organometallic loads. When nutritional intake is suppressed, the liver rapidly cannibalizes its own metabolic stores.

Furthermore, this male-specific vulnerability underscores a profound sex-dimorphism in drug-induced liver injury (DILI). Male rodents typically exhibit a different profile of cytochrome P450 isoenzymes, which may rapidly oxidize the **FMA** compound into highly reactive, electrophilic intermediates. The accumulation of these reactive species can induce localized hepatocellular necrosis or apoptosis, exacerbating the loss of liver mass. Females, potentially utilizing alternative, less reactive conjugation pathways, were remarkably spared from this macroscopic hepatic collapse (Pessayre *et al.*, 2012; Ruggieri, Anticoli, D'Ambrosio, Giordani, & Viora, 2016).

In stark contrast to the hepatic atrophy, male rats at the highest dose (D4) exhibited a dramatic and statistically significant surge in their relative heart weight. An acute, macroscopic enlargement of the heart in response to a xenobiotic is a classic hallmark of drug-induced cardiotoxicity. This sudden increase is unlikely to be true muscular hypertrophy given the short 14-day timeframe; rather, it strongly suggests acute myocardial edema or profound inflammatory infiltration.

Mechanistically, transition metal complexes can trigger severe localized oxidative stress within the myocardium a tissue highly susceptible to reactive oxygen species (ROS) due to its immense mitochondrial density and relatively low baseline antioxidant defenses. Alternatively, this cardiac enlargement may represent an acute compensatory response to xenobiotic-induced hemodynamic stress, such as severe pulmonary hypertension or vascular endothelial dysfunction.

Regardless of the exact pathway, this significant cardiac swelling establishes the heart as a critical, high-dose toxicity target for **FMA** in males, strictly limiting its therapeutic window(Laverty et al., 2011).

The relative organ weight profiling defines a highly specific, sex-dependent toxicological signature for **FMA** at elevated doses. While the compound largely spares the neuro-renal and reproductive axes, it induces severe target-organ toxicity in males, characterized by catabolic hepatic atrophy and acute myocardial edema (cardiotoxicity). These macroscopic findings correlate perfectly with the systemic catabolic collapse previously observed, decisively establishing the upper safety limits of **FMA** and necessitating rigorous cardiac and hepatic monitoring in any future preclinical applications (**Table 14**).

**Table 14.** Relative organ weight indices of major organs (liver, kidneys, heart, stomach, brain, lungs, spleen, and testes) in male and female rats following acute exposure to graded doses of **FMA** (0.1–100 mg/kg).

| Parameters |                | Control   | D1        | D2        | D3                     | D4                     |
|------------|----------------|-----------|-----------|-----------|------------------------|------------------------|
| Female     | <b>Liver</b>   | 2.91±0.12 | 2.61±0.14 | 2.99±0.21 | 2.69±0.13              | 2.28±0.32              |
|            | <b>Kidneys</b> | 0.26±0.03 | 0.23±0.10 | 0.22±0.31 | 0.27±0.21              | 0.24±0.11              |
|            | <b>Heart</b>   | 0.31±0.02 | 0.29±0.09 | 0.33±0.12 | 0.34±0.08              | 0.25±0.12              |
|            | <b>Stomach</b> | 0.78±0.22 | 0.69±0.19 | 0.71±0.08 | 0.77±0.15              | 0.67±0.23              |
|            | <b>Brain</b>   | 0.76±0.10 | 0.70±0.21 | 0.77±0.06 | 0.73±0.31              | 0.76±0.22              |
|            | <b>Lungs</b>   | 0.88±0.32 | 0.81±0.43 | 0.78±0.40 | 0.88±0.22              | 0.83±0.29              |
|            | <b>Spleen</b>  | 0.20±0.11 | 0.22±0.05 | 0.21±0.10 | 0.32±0.12              | 0.22±0.11              |
|            | <b>Liver</b>   | 2.73±0.30 | 2.93±0.14 | 2.98±0.22 | 2.31±0.18 <sup>a</sup> | 2.22±0.11 <sup>a</sup> |
| Male       | <b>Kidneys</b> | 0.49±0.15 | 0.51±0.12 | 0.43±0.22 | 0.46±0.12              | 0.41±0.17              |
|            | <b>Heart</b>   | 0.20±0.05 | 0.21±0.13 | 0.22±0.12 | 0.20±0.18              | 0.33±0.06 <sup>c</sup> |
|            | <b>Stomach</b> | 0.51±0.33 | 0.55±0.32 | 0.55±0.12 | 0.54±0.24              | 0.52±0.19              |
|            | <b>Brain</b>   | 0.70±0.14 | 0.72±0.12 | 0.77±0.22 | 0.76±0.12              | 0.73±0.20              |
|            | <b>Lungs</b>   | 0.82±0.41 | 0.88±0.16 | 0.79±0.23 | 0.80±0.33              | 0.88±0.22              |
|            | <b>Spleen</b>  | 0.17±0.13 | 0.20±0.11 | 0.22±0.14 | 0.20±0.11              | 0.18±0.10              |
|            | <b>Testes</b>  | 2.13±0.52 | 2.13±0.10 | 2.11±0.11 | 2.14±0.26              | 2.15±0.34              |

Values are expressed as (Mean ± SEM)(<sup>a</sup> Significantly different from control group ( $P < 0.05$ ), <sup>c</sup> Significantly different from control group ( $P < 0.001$ ))

### B. Organ weights index of rats across FCMphA doses.

The macroscopic evaluation of relative organ weights provides a definitive structural snapshot of **FCMphA**'s toxicity profile. A highly encouraging finding is the absolute stability of the cardiac, cerebral, pulmonary, renal, and gonadal indices across all dose groups in both male and female cohorts. Most notably, **FCMphA** completely preserves the myocardial weight index.

This stands in stark contrast to the previously evaluated **FMA** derivative, which induced severe acute cardiac edema (cardiotoxicity) at high doses. This remarkable divergence underscores a highly successful Structure-Activity Relationship (SAR) optimization. The specific structural modifications introduced into the **FCMphA** ligand have successfully abolished off-target oxidative or hemodynamic stress on the myocardium. Furthermore, the stability of the brain and kidney indices confirms the absence of generalized vascular leakage or systemic neuro-edema, confirming that the compound's toxicity is highly organ-specific rather than systemic.

The most profound macroscopic anomaly in this study is the highly significant surge in the hepatic index exclusively observed in male rats at the maximum dose (D4), where the relative liver weight spiked to 3.40. In modern toxicological pathology, such striking drug-induced hepatomegaly in the absence of generalized edema is a classic manifestation of xenobiotic-induced hepatocellular hypertrophy. Highly lipophilic organometallic compounds impose a massive metabolic burden on the liver. To efficiently detoxify and clear this heavy molecular load, hepatocytes undergo robust adaptive hypertrophy, characterized by a massive intracellular proliferation of the smooth endoplasmic reticulum (SER) and a dramatic up-regulation of cytochrome P450 (CYP450) drug-metabolizing enzymes.

While initially an adaptive, life-saving physiological response designed to enhance clearance capacity, this extreme liver enlargement at the maximum dose borders on early-stage drug-induced liver injury (DILI). If the metabolic pathways become saturated, this hypertrophy may rapidly transition into early microvesicular steatosis (lipid accumulation) or inflammatory cellular infiltration as the liver struggles to process reactive organometallic intermediates (**Andrade et al., 2019; Weaver et al., 2020**).

The presentation of this profound hepatomegaly highlights a stark sexual dimorphism. While male rats exhibited massive liver enlargement at D4, female rats displayed a mild, non-significant downward trend in relative liver weight, indicative of minor catabolic depletion rather than hypertrophy. This discrepancy is deeply rooted in the sex-specific architecture of hepatic metabolism. Male rodents possess a distinct baseline profile of hepatic efflux transporters and specific CYP450 isoforms particularly those involved in the oxidation of heavy xenobiotics which are highly inducible and physically expand liver mass when challenged. Conversely, females may rely on alternative, less mass-expanding conjugation pathways (Phase II metabolism) to clear the identical compound. This highlights that the ultimate toxicological target and the macroscopic physiological response to **FCMphA** are fundamentally governed by the intrinsic, sex-dependent metabolic machinery of the organism (**Floreani, Bizzaro, Shalaby, Taliani, & Burra, 2023**).

The organ weight profiling of **FCMphA** reveals a highly specific, sex-divergent toxicological signature. The rational design of the compound successfully eliminated the acute cardiotoxicity associated with its analogs, significantly improving its cardiovascular safety margin. However, the dose-limiting toxicity is decisively shifted to the liver, manifesting as severe, male-specific adaptive hepatomegaly at supratherapeutic doses. These findings establish the liver as the primary pharmacological target and clearance bottleneck for **FCMphA**, necessitating strict dose

optimization and targeted hepatic monitoring in future therapeutic applications to prevent adaptive hypertrophy from progressing to clinical hepatotoxicity (**Table 15**).

**Table 15.** Relative organ weight indices of major organs in male and female rats after acute administration of graded doses of **FCMphA** (0.1–100 mg/kg).

| Parameters |                | Control   | D1        | D2        | D3        | D4                     |
|------------|----------------|-----------|-----------|-----------|-----------|------------------------|
| Female     | <b>Liver</b>   | 2.91±0.12 | 2.82±0.11 | 2.59±0.32 | 2.35±0.61 | 2.56±0.51              |
|            | <b>Kidneys</b> | 0.26±0.03 | 0.25±0.04 | 0.26±0.04 | 0.26±0.05 | 0.23±0.07              |
|            | <b>Heart</b>   | 0.31±0.02 | 0.33±0.08 | 0.33±0.02 | 0.36±0.06 | 0.31±0.05              |
|            | <b>Stomach</b> | 0.78±0.22 | 0.80±0.31 | 0.77±0.11 | 0.75±0.06 | 0.75±0.12              |
|            | <b>Brain</b>   | 0.76±0.10 | 0.70±0.16 | 0.78±0.14 | 0.77±0.12 | 0.79±0.11              |
|            | <b>Lungs</b>   | 0.88±0.32 | 0.83±0.12 | 0.88±0.34 | 0.81±0.22 | 0.86±0.25              |
|            | <b>Spleen</b>  | 0.20±0.11 | 0.29±0.12 | 0.28±0.18 | 0.22±0.16 | 0.20±0.01              |
| Male       | <b>Liver</b>   | 2.73±0.30 | 2.70±0.21 | 2.88±0.13 | 2.34±0.27 | 3.40±0.16 <sup>a</sup> |
|            | <b>Kidneys</b> | 0.49±0.15 | 0.34±0.13 | 0.45±0.11 | 0.41±0.10 | 0.43±0.12              |
|            | <b>Heart</b>   | 0.20±0.05 | 0.21±0.07 | 0.20±0.11 | 0.20±0.09 | 0.22±0.05              |
|            | <b>Stomach</b> | 0.51±0.33 | 0.54±0.25 | 0.59±0.32 | 0.50±0.16 | 0.56±0.32              |
|            | <b>Brain</b>   | 0.70±0.14 | 0.70±0.09 | 0.70±0.15 | 0.71±0.08 | 0.70±0.11              |
|            | <b>Lungs</b>   | 0.82±0.41 | 0.81±0.24 | 0.88±0.21 | 0.87±0.44 | 0.80±0.22              |
|            | <b>Spleen</b>  | 0.17±0.13 | 0.18±0.16 | 0.22±0.12 | 0.12±0.09 | 0.13±0.11              |
|            | <b>Testes</b>  | 2.13±0.52 | 2.22±0.10 | 2.34±0.11 | 2.15±0.41 | 2.18±0.21              |

Values are expressed as (Mean ± SEM).<sup>(a)</sup> Significantly different from control group ( $P < 0.05$ )

### C. Organ weights index of rats across FCMphB doses

**FCMphB** demonstrated minimal impact on organ weight indices, further supporting its lower toxicity.

In female rats, a slight increase in liver weight across all doses suggests adaptive metabolic activity rather than toxicity. Other organs remained stable.

In male rats, a significant increase in brain weight at D4 was observed, which may indicate neurological involvement or fluid imbalance, although no consistent pattern was seen across other doses.

Overall, organ weight data confirm that **FCMphB** is less disruptive to organ integrity compared to the other compounds (**Table 16**).

**Table 16.** Relative organ weight indices of major organs in male and female rats following acute exposure to graded doses of **FCMphB** (0.1–100 mg/kg).

| Parameters |                | Control   | D1        | D2        | D3        | D4                     |
|------------|----------------|-----------|-----------|-----------|-----------|------------------------|
| Female     | <b>Liver</b>   | 2.91±0.12 | 3.01±0.10 | 3.11±0.10 | 3.21±0.13 | 3.14±0.10              |
|            | <b>Kidneys</b> | 0.26±0.03 | 0.22±0.05 | 0.31±0.09 | 0.25±0.01 | 0.33±0.10              |
|            | <b>Heart</b>   | 0.31±0.02 | 0.32±0.04 | 0.33±0.05 | 0.31±0.03 | 0.28±0.09              |
|            | <b>Stomach</b> | 0.78±0.22 | 0.77±0.08 | 0.70±0.21 | 0.78±0.05 | 0.77±0.09              |
|            | <b>Brain</b>   | 0.76±0.10 | 0.76±0.12 | 0.78±0.22 | 0.75±0.15 | 0.73±0.14              |
|            | <b>Lungs</b>   | 0.88±0.32 | 0.88±0.15 | 0.88±0.25 | 0.83±0.30 | 0.86±0.23              |
|            | <b>Spleen</b>  | 0.20±0.11 | 0.32±0.19 | 0.27±0.11 | 0.23±0.08 | 0.22±0.12              |
| Male       | <b>Liver</b>   | 2.73±0.30 | 2.77±0.23 | 2.81±0.13 | 2.93±0.04 | 2.71±0.24              |
|            | <b>Kidneys</b> | 0.49±0.15 | 0.53±0.12 | 0.42±0.18 | 0.43±0.11 | 0.45±0.19              |
|            | <b>Heart</b>   | 0.20±0.05 | 0.25±0.09 | 0.32±0.11 | 0.20±0.13 | 0.33±0.12              |
|            | <b>Stomach</b> | 0.51±0.33 | 0.47±0.23 | 0.54±0.21 | 0.53±0.32 | 0.51±0.11              |
|            | <b>Brain</b>   | 0.70±0.14 | 0.79±0.20 | 0.82±0.31 | 0.83±0.12 | 0.88±0.03 <sup>b</sup> |
|            | <b>Lungs</b>   | 0.82±0.41 | 0.89±0.10 | 0.80±0.15 | 0.82±0.23 | 0.81±0.34              |
|            | <b>Spleen</b>  | 0.17±0.13 | 0.18±0.09 | 0.21±0.06 | 0.20±0.11 | 0.16±0.12              |
|            | <b>Testes</b>  | 2.13±0.52 | 2.33±0.22 | 2.22±0.26 | 2.12±0.13 | 2.45±0.42              |

Values are expressed as (Mean ± SEM).<sup>(b)</sup> Indicates a statistically significant difference compared to the corresponding group (male/female) at **P < 0.05** )

#### II.2.1.4 Hematological assessment

##### A. Hematological assessment across FMA doses

The evaluation of hematological parameters following acute administration of graded doses of **FMA** (0.1–100 mg/kg) provided critical insights into the systemic tolerability and immune-hematopoietic responses to this organometallic derivative (**Table 17**). Overall, **FMA** demonstrated a favorable biosafety profile, as evidenced by the absolute stability of platelet counts (PLT) across all dose levels in both sexes. This stability confirms that **FMA** does not induce

thrombopoiesis disruption, coagulopathy, or general bone marrow suppression, reinforcing its in vivo safety at therapeutic to moderately high doses(Wu, Tan, Tan, Chen, & Yang, 2025).

However, a distinct, dose-dependent, and sex-dimorphic response was observed in the leukocytic and erythrocytic lineages. In female rats, white blood cell (WBC) and lymphocyte (LYM) counts exhibited only a mild, non-pathological elevation. Conversely, male rats displayed a more pronounced immunological reactivity, culminating in significant leukocytosis at the highest dose (D4), driven primarily by elevated lymphocytes and granulocytes. This pronounced leukocytic mobilization in males suggests a sex-specific adaptive immune activation or a systemic stress response to high-dose xenobiotic clearance. Given the redox-active nature of the ferrocene core ( $\text{Fe}^{2+}/\text{Fe}^{3+}$ ), acute exposure to high concentrations (D4) may transiently elevate systemic reactive oxygen species (ROS), thereby triggering a compensatory mobilization of innate and adaptive immune cells to maintain homeostasis(Lu et al., 2025).

Furthermore, the erythrocytic profile revealed intriguing compensatory dynamics. At higher doses (D3–D4), both sexes exhibited moderate increases in red blood cell (RBC) counts, hematocrit (HCT), and hemoglobin (HGB) levels. This could be attributed to transient hemoconcentration (e.g., mild dehydration) or a compensatory erythropoietic response. Concurrently, a subtle decrease in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) was recorded, suggesting a mild microcytic tendency. This phenomenon indicates that while **FMA** stimulates the release of new erythrocytes to enhance oxygen-carrying capacity, the massive influx of exogenous iron from the ferrocene moiety might temporarily disrupt normal hemoglobin packaging dynamics, or induce mild oxidative alterations to the erythrocyte membrane, resulting in slightly smaller cells without precipitating overt clinical anemia(Huang et al., 2025).

**Table 17.** Hematological parameters in male and female rats following acute administration of graded doses of FMA (0.1–100 mg/kg).

| Parameters |                           | Control        | D1             | D2             | D3             | D4                          |
|------------|---------------------------|----------------|----------------|----------------|----------------|-----------------------------|
| Female     | WBC( $\times 10^9/L$ )    | 7.3 $\pm$ 2.1  | 8.8 $\pm$ 1.7  | 8.6 $\pm$ 2.6  | 8.2 $\pm$ 1.3  | 8.7 $\pm$ 3.5               |
|            | LYM( $\times 10^9/L$ )    | 4.5 $\pm$ 1.1  | 6.5 $\pm$ 2.0  | 6.2 $\pm$ 2.3  | 5.9 $\pm$ 1.3  | 6.0 $\pm$ 2.4               |
|            | GRAN( $\times 10^9/L$ )   | 2.4 $\pm$ 1.3  | 2.0 $\pm$ 1.1  | 3.7 $\pm$ 1.3  | 2.9 $\pm$ 0.6  | 2.3 $\pm$ 1.1               |
|            | RBC( $\times 10^{12}/L$ ) | 7.05 $\pm$ 0.5 | 7.04 $\pm$ 0.1 | 6.83 $\pm$ 0.2 | 8.57 $\pm$ 1.2 | 8.59 $\pm$ 0.3              |
|            | HGB (g/dL)                | 16.7 $\pm$ 0.6 | 15.0 $\pm$ 1.7 | 15.3 $\pm$ 1.4 | 15.5 $\pm$ 3.2 | 15.3 $\pm$ 2.0              |
|            | HCT (%)                   | 40.1 $\pm$ 1.4 | 37.0 $\pm$ 1.6 | 38.9 $\pm$ 3.1 | 45.4 $\pm$ 1.1 | 44.3 $\pm$ 2.1              |
|            | MCV (fL)                  | 56.8 $\pm$ 4.2 | 52.6 $\pm$ 2.1 | 56.9 $\pm$ 6.2 | 53.0 $\pm$ 4.2 | 51.6 $\pm$ 2.4              |
|            | MCH (pg)                  | 23.7 $\pm$ 2.2 | 21.3 $\pm$ 2.4 | 22.5 $\pm$ 2.5 | 21.0 $\pm$ 2.0 | 20.1 $\pm$ 2.2              |
|            | MCHC (g/dL)               | 41.7 $\pm$ 1.7 | 40.5 $\pm$ 1.2 | 39.5 $\pm$ 1.0 | 39.7 $\pm$ 1.3 | 39.0 $\pm$ 1.2              |
|            | PLT ( $\times 10^9/L$ )   | 952 $\pm$ 65   | 993 $\pm$ 45   | 957 $\pm$ 43   | 946 $\pm$ 109  | 998 $\pm$ 56                |
| Male       | WBC( $\times 10^9/L$ )    | 7.7 $\pm$ 3.2  | 11.7 $\pm$ 4.3 | 13.3 $\pm$ 4.1 | 10.2 $\pm$ 3.1 | 14.7 $\pm$ 2.2 <sup>c</sup> |
|            | LYM( $\times 10^9/L$ )    | 4.7 $\pm$ 2.3  | 8.7 $\pm$ 2.2  | 6.8 $\pm$ 2.1  | 7.1 $\pm$ 2.0  | 9.9 $\pm$ 1.4               |
|            | GRAN( $\times 10^9/L$ )   | 2.9 $\pm$ 1.5  | 2.5 $\pm$ 1.1  | 2.6 $\pm$ 1.7  | 2.8 $\pm$ 1.2  | 4.2 $\pm$ 1.1               |
|            | RBC( $\times 10^{12}/L$ ) | 7.5 $\pm$ 0.2  | 7.5 $\pm$ 0.5  | 7.7 $\pm$ 0.6  | 8.4 $\pm$ 1.1  | 7.9 $\pm$ 0.4               |
|            | HGB (g/dL)                | 16.1 $\pm$ 0.5 | 16.2 $\pm$ 1.0 | 15.1 $\pm$ 1.2 | 16.5 $\pm$ 0.4 | 16.5 $\pm$ 1.3              |
|            | HCT (%)                   | 40.3 $\pm$ 1.2 | 39.9 $\pm$ 1.4 | 38.2 $\pm$ 1.1 | 43.4 $\pm$ 3.2 | 42.7 $\pm$ 2.2              |
|            | MCV (fL)                  | 53.8 $\pm$ 2.6 | 53.1 $\pm$ 3.1 | 50.0 $\pm$ 2.4 | 51.5 $\pm$ 2.5 | 54.0 $\pm$ 4.1              |
|            | MCH (pg)                  | 21.5 $\pm$ 1.1 | 21.5 $\pm$ 2.3 | 19.7 $\pm$ 2.2 | 20.6 $\pm$ 1.4 | 20.9 $\pm$ 1.2              |
|            | MCHC (g/dL)               | 40.0 $\pm$ 1.1 | 40.6 $\pm$ 1.6 | 39.5 $\pm$ 3.2 | 40.0 $\pm$ 1.5 | 38.6 $\pm$ 1.7              |
|            | PLT ( $\times 10^9/L$ )   | 877 $\pm$ 86   | 939 $\pm$ 43   | 931 $\pm$ 74   | 991 $\pm$ 38   | 927 $\pm$ 45                |

Values are expressed as (Mean  $\pm$  SEM).(<sup>c</sup> Indicates a statistically significant difference compared to the control group at  $P < 0.001$ .)

### B. Hematological assessment across FCMphA doses

In stark contrast to the relative biosafety profile of **FMA**, the acute administration of the synthesized ferrocene derivative **FCMphA** provoked pronounced, dose-dependent, and highly sex-dimorphic hematological disturbances (**Table 18**). These alterations strongly indicate significant systemic stress and potential hematopoietic toxicity at elevated doses.

In female rats, the leukocytic lineage exhibited a classical toxicological "biphasic" response. The initial profound drop in white blood cells (WBC) and lymphocytes at D3 suggests

acute immunosuppression, likely driven by xenobiotic-induced oxidative stress and subsequent lymphocyte apoptosis. This was abruptly followed by a sharp, reactive leukocytosis at the highest dose (D4). This rebound phenomenon is a well-documented physiological response to widespread tissue damage or severe systemic inflammation, wherein the bone marrow aggressively mobilizes granulocytes and lymphocytes to counteract the toxic insult. Furthermore, the erythrocytic indices in females revealed a progressive decline in RBC, HGB, and HCT levels with stable MCV and MCH values. This specific pattern is indicative of normocytic-normochromic anemia. Coupled with the striking reduction in platelet counts (thrombocytopenia) observed at higher doses (D2–D4), this strongly points toward direct bone marrow suppression (myelotoxicity). The excessive intracellular accumulation of the redox-active ferrocene core at high doses likely triggers severe Fenton-mediated reactive oxygen species (ROS) generation, which damages hematopoietic stem cells, impairs megakaryocyte function, and accelerates the peripheral destruction of circulating platelets and erythrocytes.

Conversely, male rats demonstrated a markedly different, predominantly compensatory and pro-inflammatory hematological profile. The moderate but consistent elevation in WBC counts reflects a sustained systemic immune activation rather than a biphasic collapse.

Intriguingly, males exhibited a transient but significant polycythemia (elevated RBC, HGB, and HCT) at D3. This hemoconcentration could be a physiological adaptation to localized tissue hypoxia or xenobiotic-induced dehydration. Additionally, the extreme variability in male platelet counts—ranging from significant thrombocytosis at D2/D4 to a sharp reduction at D3—highlights severe instability in hemostatic regulation. This sex-dimorphic response suggests that endocrine variations (e.g., the protective antioxidant effects of estrogen versus testosterone-driven metabolic rates) play a crucial role in modulating the systemic clearance, oxidative burden, and ultimate hematotoxicity of **FCMphA**. Collectively, these findings mandate caution regarding the dose-limiting hematological toxicity and bleeding risks associated with high-dose **FCMphA** administration.

**Table 18.** Hematological alterations in male and female rats following acute exposure to graded doses of **FCMphA** (0.1–100 mg/kg).

| Parameters |                           | Control        | D1                         | D2                          | D3                          | D4                          |
|------------|---------------------------|----------------|----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Female     | WBC( $\times 10^9/L$ )    | 7.3 $\pm$ 2.1  | 8.9 $\pm$ 2.2              | 9.9 $\pm$ 2.0 <sup>b</sup>  | 4.6 $\pm$ 3.8               | 15.7 $\pm$ 2.7 <sup>c</sup> |
|            | LYM( $\times 10^9/L$ )    | 4.5 $\pm$ 1.1  | 6.7 $\pm$ 1.2              | 7.0 $\pm$ 1.3 <sup>c</sup>  | 2.8 $\pm$ 2.3               | 10.2 $\pm$ 1.9 <sup>b</sup> |
|            | GRAN( $\times 10^9/L$ )   | 2.4 $\pm$ 1.3  | 1.8 $\pm$ 2.0              | 1.4 $\pm$ 2.2               | 1.7 $\pm$ 1.2               | 5.0 $\pm$ 1.6 <sup>c</sup>  |
|            | RBC( $\times 10^{12}/L$ ) | 7.05 $\pm$ 0.5 | 6.2 $\pm$ 0.8              | 6.6 $\pm$ 0.9               | 6.5 $\pm$ 0.9               | 6.5 $\pm$ 0.4               |
|            | HGB (g/dL)                | 16.7 $\pm$ 0.6 | 14.1 $\pm$ 1.4             | 15.1 $\pm$ 0.3 <sup>a</sup> | 14.7 $\pm$ 0.6              | 15.2 $\pm$ 1.1              |
|            | HCT (%)                   | 40.1 $\pm$ 1.4 | 37.4 $\pm$ 1.1             | 37.4 $\pm$ 3.1              | 36.1 $\pm$ 2.3              | 37.6 $\pm$ 2.5              |
|            | MCV (fL)                  | 56.8 $\pm$ 4.2 | 55.0 $\pm$ 2.6             | 56.6 $\pm$ 3.1              | 55.0 $\pm$ 1.0              | 57.8 $\pm$ 2.3              |
|            | MCH (pg)                  | 23.7 $\pm$ 2.2 | 22.5 $\pm$ 1.2             | 22.9 $\pm$ 2.8              | 22.4 $\pm$ 4.0              | 23.3 $\pm$ 3.0              |
|            | MCHC (g/dL)               | 41.7 $\pm$ 1.7 | 41.0 $\pm$ 1.0             | 40.4 $\pm$ 1.1              | 40.8 $\pm$ 1.0              | 40.4 $\pm$ 1.3              |
|            | PLT ( $\times 10^9/L$ )   | 952 $\pm$ 65   | 1176 $\pm$ 89 <sup>a</sup> | 700 $\pm$ 62 <sup>c</sup>   | 710 $\pm$ 77 <sup>b</sup>   | 740 $\pm$ 83 <sup>b</sup>   |
| Male       | WBC( $\times 10^9/L$ )    | 7.7 $\pm$ 3.2  | 8.2 $\pm$ 2.2              | 8.9 $\pm$ 1.8               | 10.2 $\pm$ 1.7              | 10.6 $\pm$ 1.3              |
|            | LYM( $\times 10^9/L$ )    | 4.7 $\pm$ 2.3  | 4.0 $\pm$ 0.7              | 6.3 $\pm$ 2.1               | 6.7 $\pm$ 2.2               | 6.9 $\pm$ 2.2               |
|            | GRAN( $\times 10^9/L$ )   | 2.9 $\pm$ 1.5  | 2.2 $\pm$ 1.2              | 2.3 $\pm$ 1.1               | 3.2 $\pm$ 2.1               | 3.1 $\pm$ 1.1               |
|            | RBC( $\times 10^{12}/L$ ) | 7.5 $\pm$ 0.2  | 7.7 $\pm$ 0.6              | 7.8 $\pm$ 0.4               | 9.9 $\pm$ 0.1 <sup>c</sup>  | 7.3 $\pm$ 0.8               |
|            | HGB (g/dL)                | 16.1 $\pm$ 0.5 | 16.8 $\pm$ 1.2             | 15.8 $\pm$ 2.0              | 19.3 $\pm$ 0.7 <sup>c</sup> | 14.6 $\pm$ 2.1              |
|            | HCT (%)                   | 40.3 $\pm$ 1.2 | 42.2 $\pm$ 1.3             | 39.3 $\pm$ 1.5              | 49.3 $\pm$ 2.3 <sup>b</sup> | 36.8 $\pm$ 3.1              |
|            | MCV (fL)                  | 53.8 $\pm$ 2.6 | 54.4 $\pm$ 1.8             | 50.4 $\pm$ 2.8              | 53.1 $\pm$ 2.6              | 48.9 $\pm$ 2.3              |
|            | MCH (pg)                  | 21.5 $\pm$ 1.1 | 21.6 $\pm$ 1.3             | 20.3 $\pm$ 2.1              | 21.0 $\pm$ 1.8              | 19.3 $\pm$ 2.7              |
|            | MCHC (g/dL)               | 40.0 $\pm$ 1.1 | 39.8 $\pm$ 1.2             | 40.2 $\pm$ 3.2              | 39.7 $\pm$ 2.2              | 39.5 $\pm$ 2.4              |
|            | PLT ( $\times 10^9/L$ )   | 877 $\pm$ 86   | 1060 $\pm$ 54 <sup>a</sup> | 1387 $\pm$ 89 <sup>c</sup>  | 761 $\pm$ 45                | 1127 $\pm$ 38 <sup>c</sup>  |

Values are expressed as **Mean  $\pm$  SEM**. ( **a**: Control group; **b**: statistically significant difference compared to control group; **c**: statistically significant difference compared to other treated group(s) (**P** < **0.05**))

### C. Hematological assessment across F399CMphB doses

Compared to the pronounced systemic toxicity of **FCMphA**, the acute administration of **FCMphB** exhibited a remarkably benign and biologically limited impact on hematological parameters (**Table 19**). The observed alterations were transient, lacked clear dose-dependency, and remained well within physiological thresholds, underscoring the exceptional in vivo biosafety and hemocompatibility of this specific ferrocene derivative.

In female rats, the leukocytic, erythrocytic, and thrombocytic lineages remained entirely stable across all dose levels. The absolute preservation of white blood cell (WBC), lymphocyte, and granulocyte counts strongly indicates that **FCMphB** does not trigger systemic inflammation, pathological immune activation, or immunosuppression.

Interestingly, males exhibited a transient but significant spike in erythrocytic parameters (RBC, HGB, and HCT) at D2. Given the strict normalcy of the morphological red cell indices (MCV, MCH, MCHC) during this spike, this pattern does not reflect pathological macrocytosis or microcytosis. Instead, it likely represents a mild, transient hemoconcentration (fluid shift) or a strictly adaptive stimulation of erythropoiesis without structural erythrocyte impairment.

Finally, platelet (PLT) counts across both sexes exhibited minor physiological fluctuations but maintained robust stability without any dose-dependent decline. The complete absence of dose-limiting thrombocytopenia confirms that **FCMphB** exerts no suppressive effects on bone marrow megakaryocytes and poses no risk of secondary coagulopathy or bleeding disorders. Collectively, these findings demonstrate that **FCMphB** possesses a highly optimized organometallic structure, successfully uncoupling potent metabolic and endocrine bioactivity from hematological toxicity, thereby presenting a highly promising pharmacological profile (**Cybulski, Michalak, Buchowicz, & Mazur, 2024**)(**Wu et al., 2025**).

**Table 19.** Hematological profile of male and female rats following acute administration of graded doses of FCMphB (0.1–100 mg/kg).

| Parameters    |                                           | Control        | D1             | D2                          | D3                         | D4                          |
|---------------|-------------------------------------------|----------------|----------------|-----------------------------|----------------------------|-----------------------------|
| <b>Female</b> | <b>WBC(<math>\times 10^9/L</math>)</b>    | 7.3 $\pm$ 2.1  | 7.0 $\pm$ 1.1  | 8.7 $\pm$ 1.4               | 7.3 $\pm$ 2.2              | 7.9 $\pm$ 2.5               |
|               | <b>LYM(<math>\times 10^9/L</math>)</b>    | 4.5 $\pm$ 1.1  | 5.3 $\pm$ 0.5  | 6.1 $\pm$ 0.6               | 4.9 $\pm$ 1.5              | 5.7 $\pm$ 1.7               |
|               | <b>GRAN(<math>\times 10^9/L</math>)</b>   | 2.4 $\pm$ 1.3  | 1.4 $\pm$ 0.9  | 2.3 $\pm$ 0.8               | 2.1 $\pm$ 1.1              | 1.9 $\pm$ 1.2               |
|               | <b>RBC(<math>\times 10^{12}/L</math>)</b> | 7.05 $\pm$ 0.5 | 6.4 $\pm$ 1.2  | 7.7 $\pm$ 1.4               | 7.2 $\pm$ 0.6              | 7.2 $\pm$ 0.8               |
|               | <b>HGB (g/dL)</b>                         | 16.7 $\pm$ 0.6 | 15.2 $\pm$ 1.3 | 16.0 $\pm$ 0.7              | 17.2 $\pm$ 1.2             | 16.4 $\pm$ 0.1              |
|               | <b>HCT (%)</b>                            | 40.1 $\pm$ 1.4 | 36.6 $\pm$ 3.3 | 44.3 $\pm$ 2.6              | 41.3 $\pm$ 2.5             | 40.4 $\pm$ 2.1              |
|               | <b>MCV (fL)</b>                           | 56.8 $\pm$ 4.2 | 56.6 $\pm$ 2.2 | 57.0 $\pm$ 1.8              | 57.3 $\pm$ 1.3             | 55.4 $\pm$ 3.2              |
|               | <b>MCH (pg)</b>                           | 23.7 $\pm$ 2.2 | 23.4 $\pm$ 2.7 | 24.0 $\pm$ 2.3              | 23.9 $\pm$ 2.1             | 22.5 $\pm$ 3.1              |
|               | <b>MCHC (g/dL)</b>                        | 41.7 $\pm$ 1.7 | 41.4 $\pm$ 2.1 | 42.1 $\pm$ 1.1              | 41.8 $\pm$ 1.3             | 40.6 $\pm$ 2.0              |
|               | <b>PLT (<math>\times 10^9/L</math>)</b>   | 952 $\pm$ 65   | 952 $\pm$ 76   | 762 $\pm$ 135               | 884 $\pm$ 87               | 940 $\pm$ 54                |
| <b>Male</b>   | <b>WBC(<math>\times 10^9/L</math>)</b>    | 7.7 $\pm$ 3.2  | 9.8 $\pm$ 2.2  | 7.8 $\pm$ 2.6               | 9.8 $\pm$ 3.2              | 12.5 $\pm$ 3.2 <sup>c</sup> |
|               | <b>LYM(<math>\times 10^9/L</math>)</b>    | 4.7 $\pm$ 2.3  | 7.1 $\pm$ 3.3  | 5.1 $\pm$ 3.1               | 6.4 $\pm$ 2.4              | 9.0 $\pm$ 2.9               |
|               | <b>GRAN(<math>\times 10^9/L</math>)</b>   | 2.9 $\pm$ 1.5  | 2.2 $\pm$ 1.7  | 2.5 $\pm$ 1.1               | 3.0 $\pm$ 1.2              | 3.0 $\pm$ 1.0               |
|               | <b>RBC(<math>\times 10^{12}/L</math>)</b> | 7.5 $\pm$ 0.2  | 7.5 $\pm$ 0.5  | 9.9 $\pm$ 2.8               | 7.2 $\pm$ 0.3              | 8.3 $\pm$ 2.2               |
|               | <b>HGB (g/dL)</b>                         | 16.1 $\pm$ 0.5 | 15.8 $\pm$ 0.4 | 19.8 $\pm$ 0.2 <sup>c</sup> | 14.9 $\pm$ 0.8             | 15.6 $\pm$ 0.6              |
|               | <b>HCT (%)</b>                            | 40.3 $\pm$ 1.2 | 39.8 $\pm$ 1.2 | 49.4 $\pm$ 1.8 <sup>c</sup> | 36.7 $\pm$ 2.5             | 40.4 $\pm$ 3.1              |
|               | <b>MCV (fL)</b>                           | 53.8 $\pm$ 2.6 | 52.8 $\pm$ 2.2 | 49.8 $\pm$ 2.8              | 50.6 $\pm$ 2.1             | 48.4 $\pm$ 2.4              |
|               | <b>MCH (pg)</b>                           | 21.5 $\pm$ 1.1 | 20.9 $\pm$ 1.4 | 20.0 $\pm$ 1.0              | 20.5 $\pm$ 1.2             | 18.6 $\pm$ 2.0              |
|               | <b>MCHC (g/dL)</b>                        | 40.0 $\pm$ 1.1 | 39.6 $\pm$ 1.3 | 40.1 $\pm$ 1.5              | 40.5 $\pm$ 1.8             | 38.5 $\pm$ 2.2              |
|               | <b>PLT (<math>\times 10^9/L</math>)</b>   | 877 $\pm$ 86   | 711 $\pm$ 75   | 873 $\pm$ 73                | 1199 $\pm$ 86 <sup>a</sup> | 855 $\pm$ 56                |

Values are expressed as **Mean  $\pm$  SEM**(. <sup>a</sup> indicates a statistically significant difference at **P < 0.05**, <sup>b</sup> at **P < 0.01**, and <sup>c</sup> at **P < 0.001** compared to the control group).

### II.2.1.5. Biochemical Assessment

#### A. Biochemical Assessment Across FMA Doses

The biochemical evaluation of animals exposed to escalating acute doses of **FMA** (0.1–100 mg/kg) reveals a distinct, dose-dependent pattern of hepatotoxicity, which is intricately modulated by the sex of the subjects (**Table 20**). The most prominent anomaly is the significant

elevation of serum transaminases—alanine aminotransferase (ALT) and aspartate aminotransferase (AST)—particularly in male rats exposed to intermediate and high doses (D3 and D4). Mechanistically, ALT is predominantly localized within the hepatic cytosol and serves as a highly sensitive, specific biomarker for acute hepatocellular membrane disruption. Its pronounced leakage into the systemic circulation indicates direct physical damage to the hepatocyte lipid bilayer. Concurrently, the significant rise in AST, an enzyme distributed across both cytosolic and mitochondrial compartments, reflects a more severe degree of cellular destruction involving mitochondrial impairment. The dose-limiting hepatotoxicity of **FMA** at higher concentrations is likely driven by the intracellular accumulation of the redox-active ferrocene core. Once the local buffering capacity is overwhelmed, the dissociation of iron triggers severe Fenton-mediated oxidative stress, causing widespread lipid peroxidation and subsequent enzymatic leakage (Das et al., 2024).

Crucially, the observed sex-dimorphic susceptibility—where males exhibited significantly higher transaminase surges (e.g., AST: 188.9 U/L, ALT: 330.4 U/L at D4) compared to females—is a well-documented phenomenon in xenobiotic toxicology. This variance is fundamentally attributed to sex-specific differences in hepatic biotransformation pathways, particularly the basal expression and activity of Cytochrome P450 (CYP) isoenzymes. Males generally possess a higher intrinsic capacity to rapidly metabolize certain organometallics, paradoxically leading to a faster, more concentrated accumulation of reactive oxygen species (ROS) and toxic intermediates that overwhelm local antioxidant defenses more rapidly than in females (Waxman & Holloway, 2009).

In stark contrast to the profound hepatic distress, renal function parameters (urea and creatinine) remained strictly within physiological reference ranges across all experimental cohorts.

This absolute stability unequivocally confirms the complete preservation of the glomerular filtration rate and renal excretory capacity. It solidifies the conclusion that **FMA** exerts a highly selective, hepatocentric toxicity profile at elevated doses, completely sparing the renal parenchyma from heavy-metal-induced nephrotoxicity (Cho & Suh, 2024).

Regarding metabolic homeostasis, fasting blood glucose (FBS) levels demonstrated a mild, non-pathological elevation at higher doses, most notably in male rats. Since the primary values remained subclinical, this glycemic drift does not indicate direct diabetogenic toxicity.

Instead, it reflects a secondary metabolic consequence of hepatic dysfunction—where impaired glycogen storage and altered gluconeogenesis disrupt carbohydrate homeostasis.

Furthermore, this mild hyperglycemia is a classic manifestation of a glucocorticoid-mediated endocrine stress response to acute xenobiotic exposure. The steadfast normalcy of serum creatinine further corroborates the localized nature of the toxicity, confirming that the acute systemic stress of high-dose FMA is effectively confined to the liver without precipitating multiorgan failure.

**Table 20.** Serum biochemical parameters in male and female rats following acute exposure to graded doses of **FMA** (0.1–100 mg/kg).

| Parameters |            | Control     | D1         | D2         | D3                      | D4                      |
|------------|------------|-------------|------------|------------|-------------------------|-------------------------|
| Female     | FBS (g/L)  | 0.86±0.14   | 0.79±0.16  | 0.94±0.22  | 0.70±0.16               | 1.08±0.23               |
|            | AST (U/L)  | 113.04±11.2 | 112.2±11.5 | 120.4±12.1 | 115.03±13.0             | 115.3±8.2               |
|            | ALT (U/L)  | 230.5±21.4  | 280.3±22.1 | 256.2±23.1 | 227.4±22.4              | 321.2±33.3 <sup>a</sup> |
|            | Urea (g/L) | 0.51±0.1    | 0.7±0.2    | 0.63±0.2   | 0.85±0.2                | 0.60±0.4                |
|            | CT (mg/L)  | 7.1±2.0     | 7.0±1.3    | 6.0±2.1    | 4.0±3.2                 | 7.0±1.4                 |
| Male       | FBS (g/L)  | 0.73±0.37   | 0.84±0.32  | 0.81±0.21  | 1.07±0.20               | 0.86±0.12               |
|            | AST (U/L)  | 98.11±15.2  | 121.3±23.1 | 76.4±25.1  | 176.1±13.5 <sup>a</sup> | 188.9±21.3 <sup>c</sup> |
|            | ALT (U/L)  | 225.7±30.5  | 215.3±32.1 | 218.9±23.1 | 330.4±17.1 <sup>c</sup> | 323.6±34.7 <sup>a</sup> |
|            | Urea (g/L) | 0.6±0.08    | 0.51±0.2   | 0.6±0.1    | 0.55±0.4                | 0.50±0.5                |
|            | CT (mg/L)  | 8.5±2.5     | 7.0±2.1    | 6.0±2.2    | 8.0±2.7                 | 7.5±2.1                 |

Values are expressed as **Mean ± SEM**. (^<sup>a</sup> indicates statistically significant difference at **P < 0.05**, ^<sup>b</sup> at **P < 0.01**, and ^<sup>c</sup> at **P < 0.001** compared to the control group).

### B.Biochemical Assessment Across FCMpha Doses

The administration of **FCMpha** resulted in moderate biochemical alterations, primarily characterized by a dose-dependent hepatic response and notable systemic metabolic shifts (**Table 21**). Serum transaminase levels (ALT and AST) exhibited measurable elevations, particularly in male rats at the highest administered dose (D4: ALT 354.2 U/L, AST 176.7 U/L). Although significant, these elevations represent a pattern often associated with mild to moderate hepatocellular stress rather than massive fulminant necrosis. From a toxicological perspective, this enzymatic leakage reflects a partial compromise of hepatocyte membrane integrity. However, it also indicates a robust adaptive hepatic response to the intense metabolic burden imposed by processing the organometallic compound. The liver actively upregulates its biotransformation

machinery to clear the xenobiotic, generating transient reactive intermediates that cause localized oxidative stress before being neutralized. This suggests that while **FCMphA** is not entirely devoid of hepatic effects, its hepatotoxic potential is relatively controlled, reflecting a transient adaptive phase rather than irreversible structural damage(Skat-Rørdam, Lykkesfeldt, Gluud, & Tveden-Nyborg, 2025)

Renal biochemical markers, on the other hand, revealed a distinct and highly informative physiological dissociation. Serum creatinine levels remained entirely stable and within normal physiological limits across all dose groups, unequivocally confirming that the glomerular filtration rate (GFR) is fully preserved and that structural nephrotoxicity is absent. However, a significant elevation in serum urea levels was observed in male rats at the highest dose (D4: 0.97 g/L). This divergence between elevated urea and stable creatinine is a classic toxicological indicator of "pre-renal azotemia" or an altered nitrogen balance, effectively ruling out intrinsic acute kidney injury (AKI). The isolated rise in urea is most likely attributable to enhanced systemic protein catabolism—a common consequence of the metabolic stress induced by high-dose xenobiotic exposure. Additionally, transient pre-renal factors, such as mild xenobiotic-induced dehydration or reduced renal perfusion secondary to systemic stress, frequently contribute to this specific biochemical pattern(Vaidya, Ferguson, & Bonventre, 2008).

Metabolically, fasting blood glucose (FBS) levels displayed moderate variability with a clear tendency toward elevation in male animals exposed to higher doses. This mild hyperglycemic drift aligns perfectly with the isolated urea elevation, as both are hallmark features of a coordinated endocrine stress response. Acute exposure to high concentrations of **FCMphA** likely stimulates the hypothalamic-pituitary-adrenal (HPA) axis, resulting in the release of endogenous glucocorticoids. These stress hormones simultaneously drive muscle protein breakdown (increasing urea production) and stimulate hepatic gluconeogenesis (increasing blood glucose) to meet the heightened energy demands of xenobiotic detoxification. Ultimately, the steadfast normalcy of serum creatinine alongside these metabolic shifts confirms that **FCMphA** toxicity is primarily centered on metabolic and hepatic adaptation, completely sparing the renal filtration barrier from structural damage(Srinivasan, 2012).

**Table 21.** Serum biochemical profile in male and female rats following acute administration of graded doses of **FCMphA** (0.1–100 mg/kg).

| Parameters |            | Control     | D1         | D2         | D3         | D4                      |
|------------|------------|-------------|------------|------------|------------|-------------------------|
| Female     | FBS (g/L)  | 0.86±0.14   | 0.75±0.12  | 0.57±0.22  | 1.10±0.31  | 0.73±0.21               |
|            | AST (U/L)  | 113.04±11.2 | 111.2±9.4  | 89.9±24.1  | 94.5±23.2  | 117.8±6.1               |
|            | ALT (U/L)  | 230.5±21.4  | 230.7±32.1 | 221.6±37.2 | 231.1±24.4 | 235.2±15.5              |
|            | Urea (g/L) | 0.51±0.1    | 0.56±0.2   | 0.51±0.4   | 0.52±0.1   | 0.45±0.1                |
|            | CT (mg/L)  | 7.1±2.0     | 6.3±2.0    | 7.0±3.1    | 7.09±1.1   | 6.12±2.3                |
| Male       | FBS (g/L)  | 0.73±0.37   | 1.09±0.32  | 1.08±0.28  | 1.0±0.32   | 1.01±0.21               |
|            | AST (U/L)  | 98.11±15.2  | 93.2±9.3   | 128.1±24.2 | 78±25.1    | 176.7±26.1 <sup>c</sup> |
|            | ALT (U/L)  | 225.7±30.5  | 253.1±24.6 | 256.2±23.2 | 221.1±27.7 | 354.2±26.5 <sup>c</sup> |
|            | Urea (g/L) | 0.6±0.08    | 0.8±0.21   | 0.58±0.3   | 0.53±0.2   | 0.97±0.1 <sup>a</sup>   |
|            | CT (mg/L)  | 8.5±2.5     | 6.02±2.9   | 6.78±2.1   | 7.0±1.1    | 6.1±2.1                 |

Values are expressed as **Mean ± SEM**. (a Indicates a statistically significant difference vs Control (**P < 0.05**)).

**C** Indicates a statistically significant difference vs corresponding group (male/female) (**P < 0.05**)).

### C. Biochemical Assessment Across FCMphB Doses

Exposure to the **FCMphB** derivative resulted in a highly distinct biochemical signature, fundamentally differentiating it from the other tested compounds (**Table 22**). While structurally less damaging to the hepatic parenchyma, **FCMphB** proved to be profoundly disruptive to systemic metabolic homeostasis. The most striking anomaly was the marked, dose-dependent escalation in fasting blood glucose (FBS) levels, particularly in male rats, where values surged to a robust hyperglycemic state (reaching 1.92 g/L at D4). Unlike the mild, stress-induced glycemic drift observed with **FMA**, this magnitude of hyperglycemia indicates a severe disruption of carbohydrate metabolism. Mechanistically, the intracellular accumulation of the redox-active ferrocene moiety can catalyze Fenton-mediated reactive oxygen species (ROS) overproduction. This localized oxidative stress is known to heavily disrupt the PI3K/Akt insulin signaling pathway, thereby inducing acute peripheral insulin resistance. Furthermore, the xenobiotic stress likely triggers a massive release of counter-regulatory hormones (glucocorticoids and catecholamines), which aggressively drive hepatic gluconeogenesis and inhibit pancreatic beta-cell insulin secretion, culminating in the observed diabetogenic-like state (**Harrison, Lorenzo, & McClain, 2023**).

Regarding hepatic function, **FCMphB** induced moderate elevations in serum transaminases (ALT and AST) at higher doses (D3 and D4) in male rats. However, the absolute magnitude of these enzymatic leaks was notably less severe than those provoked by **FMA**. This

biochemical profile suggests a state of mild to moderate hepatocellular stress rather than extensive, irreversible necrotic injury. The liver appears to structurally tolerate **FCMphB** better, engaging in a reversible, adaptive biotransformation response. The fact that the liver prioritizes xenobiotic clearance over normal metabolic regulation perfectly explains why the structural damage (ALT/AST) is moderate, while the metabolic output (hyperglycemia) is severely dysregulated (McGill & Jaeschke, 2018).

The renal and nitrogenous biochemical markers exhibited a complex, non-linear physiological pattern that further confirms the absence of nephrotoxicity. While serum creatinine (CT) levels remained impeccably stable across all experimental cohorts—guaranteeing the absolute preservation of the glomerular filtration rate (GFR) and structural renal integrity—urea levels displayed significant fluctuations. Notably, serum urea significantly decreased at D1 in males (0.28 g/L) and at D4 in females (0.30 g/L). In toxicological pathology, a marked decrease in blood urea nitrogen alongside normal creatinine is a classic indicator of altered hepatic nitrogen metabolism, specifically the downregulation of the hepatic urea cycle. As the liver redirects its enzymatic and energy resources toward detoxifying the **FCMphB** compound and driving gluconeogenesis, native protein catabolism and urea synthesis are temporarily suppressed. This biochemical dissociation solidifies the conclusion that the systemic alterations induced by **FCMphB** are entirely functional and metabolic in nature, leaving the renal architecture completely unscathed.

**Table 22.** Serum biochemical profile in male and female rats following acute administration of graded doses of **FCMphB** (0.1–100 mg/kg).

| Parameters |            | Control     | D1                     | D2                     | D3                      | D4                      |
|------------|------------|-------------|------------------------|------------------------|-------------------------|-------------------------|
| Female     | FBS (g/L)  | 0.86±0.14   | 0.89±0.16              | 1.0±0.57               | 1.60±0.13 <sup>b</sup>  | 1.32±11.1 <sup>a</sup>  |
|            | AST (U/L)  | 113.04±11.2 | 99.1±24.2              | 92.3±22.1              | 112.4±13.3              | 117.2±14.2              |
|            | ALT (U/L)  | 230.5±21.4  | 267.3±33.2             | 224.6±29.1             | 234.7±19.2              | 253.4±35.3              |
|            | Urea (g/L) | 0.51±0.1    | 0.51±0.3               | 0.51±0.3               | 0.53±0.2                | 0.30±0.14 <sup>b</sup>  |
|            | CT (mg/L)  | 7.1±2.0     | 5.08±1.1               | 6.5±2.1                | 6.2±2.3                 | 5.9±1.6                 |
| Male       | FBS (g/L)  | 0.73±0.37   | 0.87±0.34              | 1.90±0.23 <sup>c</sup> | 1.60±0.23 <sup>c</sup>  | 1.92±0.39 <sup>c</sup>  |
|            | AST (U/L)  | 98.11±15.2  | 87.8±12.2              | 112.2±15.6             | 187.8±13.3 <sup>c</sup> | 156.7±11.6 <sup>b</sup> |
|            | ALT (U/L)  | 225.7±30.5  | 223.1±27.5             | 252.3±35.4             | 308.5±20.0 <sup>b</sup> | 289.8±24.7 <sup>b</sup> |
|            | Urea (g/L) | 0.6±0.08    | 0.28±0.02 <sup>c</sup> | 0.55±0.1               | 0.75±0.08               | 0.49±2.1                |

|  |           |         |         |         |         |         |
|--|-----------|---------|---------|---------|---------|---------|
|  | CT (mg/L) | 8.5±2.5 | 6.4±2.1 | 6.6±2.2 | 6.0±2.0 | 6.7±2.2 |
|--|-----------|---------|---------|---------|---------|---------|

Values are expressed as **Mean ± SEM**.**(a)** Indicates a statistically significant difference compared to the Control group (**P < 0.05**).**(b)** Indicates a statistically significant difference between male and female groups (**P < 0.05**).**(c)** Indicates a statistically significant difference compared to the corresponding group (male/female) (**P < 0.05**)

### II.2.1.6. Cross pathology and histopathological analysis of the vital organs of the rats

#### A. Histopathological Analysis Across FMA Doses

##### A.1. Liver Histopathology

The microscopic evaluation of the hepatic parenchyma following acute **FMA** exposure (**Figure 8**) provides a sequential, morphological visualization of dose-dependent, sex-dimorphic organometallic hepatotoxicity. The Control sections of both sexes exhibit a pristine physiological microarchitecture, featuring well-organized polygonal hepatocytes with prominent central nuclei, perfectly arranged in radial cords around a non-congested central vein, confirming the absence of baseline pathology.

At the lowest exposure levels (D1 and D2), the morphological integrity of the liver is largely preserved. The appearance of discrete cytoplasmic vacuolation and mild sinusoidal dilatation—predominantly observed in the male D2 cohort—represents a classic, reversible early-stage adaptive response. From a toxicological perspective, this mild vacuolation typically indicates cellular swelling and transient lipid accumulation as the hepatocytes upregulate their smooth endoplasmic reticulum to rapidly metabolize the lipophilic xenobiotic without crossing the threshold into irreversible necrotic damage(**Skat-Rørdam et al., 2025**) .

However, the intermediate dose (D3) represents a critical toxicological inflection point, unequivocally exposing a profound sex-related disparity in hepatotoxic susceptibility. The male D3 sections exhibit distinct signs of irreversible cellular injury, characterized by nuclear pyknosis (irreversible chromatin condensation), focal necrosis, and prominent periportal inflammatory infiltration. In stark contrast, female rats at the same dose demonstrated a much higher structural tolerance, exhibiting only moderate vacuolation and partial architectural disruption. This striking sexual dimorphism is fundamentally driven by sex-specific variations in the hepatic inflammatory and biotransformation pathways. The male liver's intrinsically higher basal expression of specific cytochrome P450 isoforms accelerates the breakdown of **FMA**, paradoxically generating a rapid,

localized surge of reactive toxic intermediates that overwhelm local antioxidant defenses, thereby triggering acute cellular death and aggressive localized leukocyte recruitment (Tiwari et al., 2025).

At the highest administered dose (D4), the xenobiotic burden overwhelms the adaptive capacity in both sexes, culminating in catastrophic structural collapse. The micrographs reveal extensive hepatocellular necrosis, massive vascular congestion, prominent inflammatory cell infiltration, and focal hemorrhage. This severe architectural disintegration is a direct physical consequence of acute intracellular metal toxicity. The massive influx of the redox-active ferrocene core into hepatocytes saturates intracellular storage proteins. The subsequent release of labile reactive iron aggressively catalyzes Fenton-mediated lipid peroxidation, violently rupturing the hepatocyte plasma and organelle membranes. This profound oxidative stress rapidly dictates the transition from apoptosis to unprogrammed necrosis, inducing widespread hemorrhage and the severe acute drug-induced liver injury (DILI) visualized in the D4 panels (Mancardi, Mezzanotte, Arrigo, Barinotti, & Roetto, 2021).

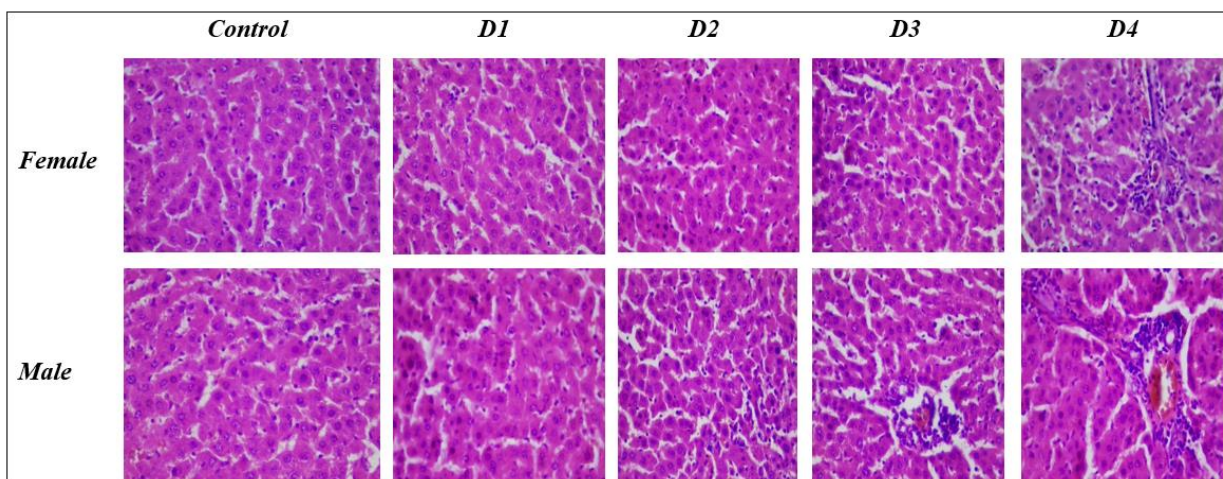


**Figure 8.** Histopathological evaluation of hepatic Tissue following acute exposure to a ferrocene derivative (FMA) across increasing dose levels in male and female rats, Magnification  $\times 40$ .

### A.2. Kidney Histopathology

The microscopic examination of renal tissue sections (Figure 9) following acute administration of escalating doses of FMA reveals a remarkably high threshold of structural tolerance, providing compelling visual confirmation of the previously observed biochemical stability of serum urea and creatinine levels.

In both male and female Control groups, the renal parenchyma exhibits a flawless physiological histoarchitecture. The micrographs clearly display structurally intact glomeruli with well-defined capillary tufts perfectly enveloped by pristine Bowman's capsules. The surrounding proximal and distal convoluted tubules maintain their characteristic morphology, featuring a continuous cuboidal epithelial lining and open, unobstructed luminal spaces. There is an absolute absence of any inflammatory infiltrate, cellular degeneration, or vascular congestion.

As the administered dose of the organometallic xenobiotic increases through the lower and intermediate tiers (D1, D2, and D3), the renal architecture demonstrates exceptional resilience. In both sexes, the glomeruli continue to preserve their structural integrity, and the tubular epithelial cells show no evidence of frank necrosis, desquamation, or cast formation. The absence of these classical nephrotoxic lesions is toxicologically highly significant. It definitively proves that **FMA**, unlike many traditional heavy-metal-based chemotherapeutics, does not inflict direct structural damage to the delicate glomerular filtration barrier nor does it induce acute tubular necrosis (ATN) during its systemic clearance. The lack of tubular damage perfectly corroborates the normal physiological serum creatinine levels, as structural tubular integrity is the primary determinant of renal excretory function(**Racusen & Solez, 1986**) .

However, at the highest suprapharmacological dose (D4), distinct but relatively mild focal alterations become visually apparent in both sexes, representing the upper limit of renal hemodynamic tolerance. The D4 micrographs exhibit focal areas of prominent interstitial vascular congestion and focal parenchymal hemorrhage (visible as deep red pooling of erythrocytes between the tubular structures), without corresponding tubular necrosis or glomerular collapse. This specific morphological presentation—congestion without cellular death—is indicative of severe systemic hemodynamic stress and localized microvascular disruption rather than direct parenchymal cytotoxicity. The massive influx of the redox-active ferrocene core at this extreme dose likely provokes a transient, systemic inflammatory response and oxidative stress, which mechanically alters renal perfusion dynamics and capillary permeability, resulting in the observed focal pooling of blood(**Mansoor et al., 2023**) . Importantly, the male D4 cohort appears to exhibit slightly more pronounced hemorrhagic pooling compared to the female cohort. This subtle sex-dimorphic response mirrors the metabolic trend observed in the liver, suggesting that the accelerated systemic

biotransformation of **FMA** in males generates a more intense, transient microvascular shock in the renal interstitium

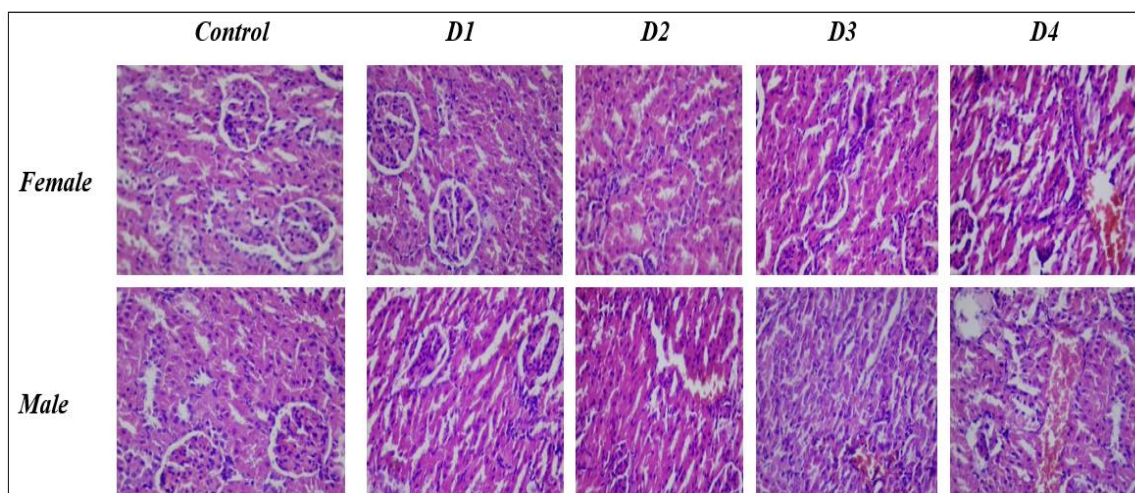


**Figure 9.** Histopathological evaluation of renal tissue following acute exposure to a ferrocene derivative (**FMA**) across increasing dose levels in male and female rats, Magnification  $\times 40$ .

### A.3. Brain Histopathology

The microscopic examination of cerebral tissue sections (**Figure 10**) provides critical insights into the dose-dependent neurotoxic potential of the organometallic ferrocene derivative, **FMA**. Because ferrocene compounds are highly lipophilic, they possess an inherent capacity to cross the blood-brain barrier (BBB) and accumulate within the lipid-rich parenchyma of the central nervous system (CNS).

In the Control groups of both sexes, the cerebral cortex exhibits a perfectly preserved, normal histoarchitecture. The micrographs display an evenly distributed population of healthy neurons characterized by vesicular nuclei and prominent nucleoli, embedded within a homogenous, intact fibrillar neuropil background. There is no evidence of edema, microglial activation, or neurodegeneration, establishing a robust baseline of neurological health.

At the lowest exposure levels (D1), the cerebral architecture remains largely unremarkable. Both male and female cohorts preserve normal neuronal morphology and neuropil integrity, suggesting that at minimal concentrations, **FMA** does not induce acute neurotoxicity or that the endogenous CNS antioxidant buffering systems successfully neutralize any transient oxidative stress generated by the xenobiotic (**Banks, Rhea, Reed, & Erickson, 2024**).

However, as the dose escalates to the intermediate levels (D2 and D3), early to moderate signs of neuropathological distress become visually apparent. The micrographs reveal scattered neuronal alterations, including mild cytoplasmic shrinkage and the distinct appearance of perineuronal vacuolation (clear halos or spaces surrounding the neuronal cell bodies). This specific histological finding is a classic manifestation of localized neuro-edema. Toxicologically, this indicates that the accumulating redox-active iron from the ferrocene core is beginning to induce lipid peroxidation within the neuronal membranes. This oxidative stress alters cellular osmolarity and membrane permeability, leading to the accumulation of fluid around the shrinking neurons, a process often preceding irreversible neurodegeneration (Kawade, Treml, Thomas, Daggett, & Mattingly, 2025).

At the highest suprapharmacological dose (D4), the neurotoxic impact becomes profound and generalized across both sexes. The cerebral tissue exhibits severe degenerative changes characterized by widespread neuronal shrinkage, pyknotic (hyperchromatic and condensed) nuclei, and extensive perineuronal and perivascular vacuolation, giving the neuropil a distinct "spongy" or vacuolated appearance (spongiosis). The presence of these dark, shrunken "red neurons" is a definitive histological hallmark of acute anoxic-ischemic injury or severe oxidative shock. At this extreme concentration, the massive intracellular release of reactive iron ( $\text{Fe}^{2+}$ ) vigorously drives Fenton-type reactions, generating overwhelming amounts of highly toxic hydroxyl radicals. These radicals brutally degrade the lipid-dense myelin sheaths and neuronal membranes, triggering severe neuro-inflammation and widespread apoptotic cell death. Although both sexes are severely affected at D4, the male sections display a slightly more pronounced spongiform degeneration, which aligns with the previously established pattern of higher systemic metabolic sensitivity and faster toxic intermediate generation in males.

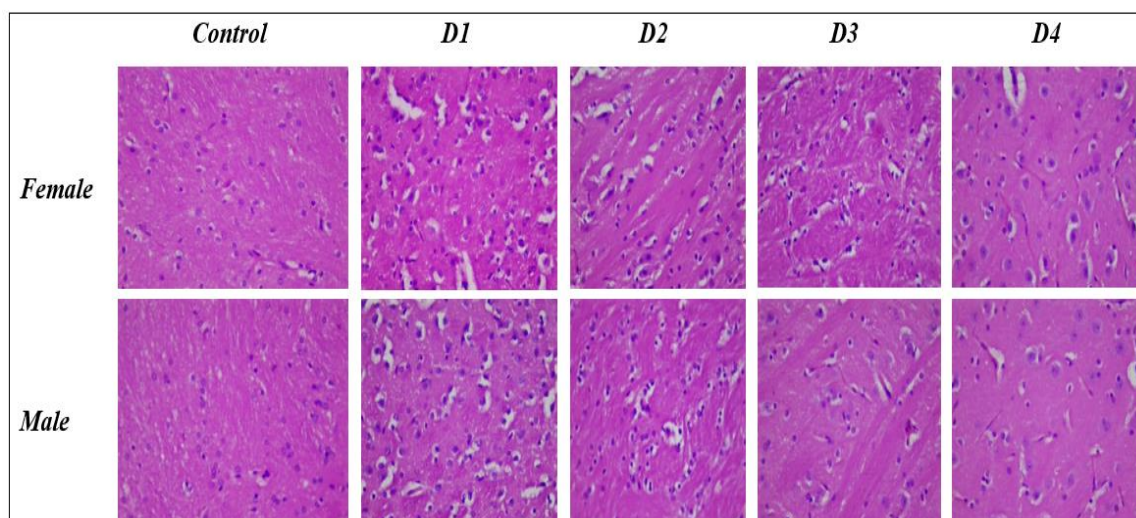


**Figure 10.** Histopathological evaluation of brain tissue following acute exposure to a ferrocene derivative (FMA) across increasing dose levels in male and female rats, Magnification  $\times 40$ .

## B. Histopathological Analysis Across FCMphA Doses

### B.1. Liver Histopathology

A rigorous microscopic evaluation of the hepatic parenchyma following acute exposure to the organometallic derivative **FCMphA** (**Figure 11**) provides a critical morphological foundation for understanding its dose-dependent and sex-dimorphic hepatotoxic profile.

In the Control cohorts of both sexes, the liver exhibits an intact, pristine physiological microarchitecture. The presence of well-organized polygonal hepatocytes, seamlessly radiating hepatic cords around the central vein, and normal, unobstructed sinusoidal spaces confirms a healthy baseline, devoid of any spontaneous pathology, congestion, or inflammation.

At the lowest exposure level (D1), the morphological integrity of the liver remains largely uncompromised. The observation of minimal structural deviation limited to discrete, mild sinusoidal dilation and occasional cytoplasmic vacuolation suggests that at this minimal concentration, the hepatic biotransformation machinery successfully metabolizes and clears the lipophilic xenobiotic without sustaining oxidative damage. From a toxicological standpoint, this mild vacuolation represents a reversible, adaptive hypertrophy of the smooth endoplasmic reticulum, typical of early-stage xenobiotic processing rather than overt cytotoxicity (**Russmann, Kullak-Ublick, & Grattagliano, 2009**).

As the dose increases (D2 and D3), a distinct inflection point in systemic tolerance is crossed. The liver tissue demonstrates progressive, dose-dependent hepatocellular degeneration.

The micrographs display distinct cytoplasmic vacuolation, early architectural disorganization, and prominent nuclear pyknosis (a definitive marker of irreversible chromatin condensation and programmed cell death). Furthermore, D3 reveals clear focal areas of necrosis accompanied by an influx of inflammatory cells, predominantly localizing around the portal triads.

This periportal inflammatory recruitment is a classic toxicological response to localized oxidative stress. The intracellular accumulation of the redox-active ferrocene moiety generates reactive oxygen species (ROS) that outpace local antioxidant buffers, leading to lipid peroxidation of the hepatocyte membranes and triggering aggressive leukocyte chemotaxis (**Skat-Rørdam et al., 2025**).

At the highest suprapharmacological dose (D4), the adaptive and antioxidant defenses are completely overwhelmed, resulting in severe acute liver injury. The D4 micrographs reveal catastrophic structural disruption characterized by widespread confluent necrosis, marked inflammatory infiltration, and extreme vascular congestion. Crucially, the massive pooling of erythrocytes (hemorrhage) clearly visible within the hepatic parenchyma—especially pronounced in the male cohort—perfectly corroborates the severe systemic thrombocytopenia (platelet depletion) previously documented in the hematological assessment of **FCMphA**. The concurrent presence of severe oxidative endothelial damage and a depleted systemic platelet pool mechanically precipitates this extensive intrahepatic hemorrhage. The more severe presentation in males further reinforces the established sex-dimorphism in xenobiotic susceptibility. The intrinsically higher basal activity of male-specific hepatic drug-metabolizing enzymes accelerates the generation of highly reactive, toxic intermediates. This rapid metabolic turnover precipitates a faster, more aggressive necrotic and hemorrhagic shock compared to the slower, estrogen-buffered clearance observed in females.

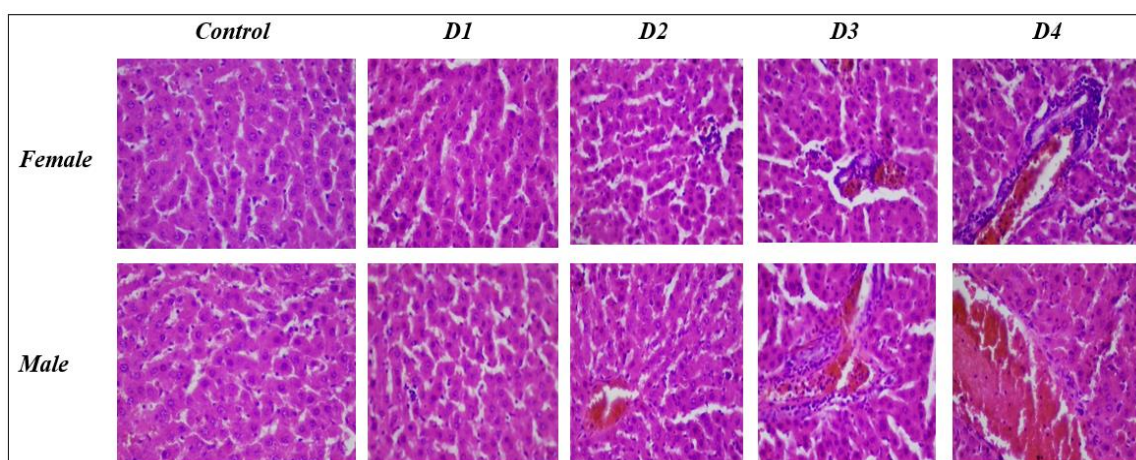


**Figure 11.** Histopathological evaluation of hepatic Tissue following acute exposure to a ferrocene derivative (**FCMphA**) across increasing dose levels in male and female rats, Magnification  $\times 40$ .

### B.2. Kidney Histopathology

The microscopic evaluation of renal tissue following acute **FCMphA** exposure (**Figure 12**) unveils a profound, dose-dependent nephrotoxic profile, culminating in severe acute tubular necrosis (ATN) and hemorrhagic glomerulopathy at suprapharmacological doses.

At the lower exposure tiers (Control, D1, and D2), the renal parenchyma in both sexes exhibits pristine structural integrity. The micrographs demonstrate perfectly delineated glomeruli enclosed by intact Bowman's capsules, and healthy proximal and distal convoluted tubules with preserved epithelial brush borders. This indicates that at lower concentrations, the renal xenobiotic clearance mechanisms efficiently excrete the organometallic compound without inciting localized oxidative damage or cellular degeneration.

However, the transition to the intermediate and highest doses (D3 and D4) marks a catastrophic collapse of renal microarchitecture. The histological sections reveal widespread tubular necrosis, severe epithelial desquamation obstructing the lumina with cellular debris, and prominent glomerular atrophy. Crucially, the D4 micrographs display massive parenchymal hemorrhage (visible as extensive deep red pooling of erythrocytes) and severe vascular congestion, particularly pronounced in the male cohort. This visual evidence of severe intrarenal bleeding perfectly corroborates the profound systemic thrombocytopenia (platelet depletion) previously documented in the hematological assessment of **FCMphA**. The Fenton-mediated oxidative stress generated by the accumulating ferrocene core actively degrades the renal microvascular

endothelium. When this severe endothelial injury is coupled with a depleted systemic platelet pool, the inevitable physical consequence is the extensive hemorrhagic pooling visualized in these sections (Wang et al., 2025)

Furthermore, this severe morphological destruction presents a fascinating toxicological paradox when juxtaposed with the previously noted stability of serum creatinine levels. In toxicological pathology, this phenomenon is widely recognized as the "creatinine blind spot." The kidney possesses a massive "renal functional reserve"; therefore, acute structural damage (such as the tubular necrosis seen here) occurs much earlier and more aggressively than the functional decline. Serum creatinine levels typically do not rise until more than 50% of the total nephron mass is functionally obliterated. Thus, the stable creatinine merely reflects the compensatory hyperfiltration of the surviving nephrons, while the histopathology unmasks the true, severe structural nephrotoxicity of high-dose FCMphA (Bhosale & Kulkarni, 2020) .

Finally, the observation that male rats exhibit slightly more aggressive hemorrhagic and necrotic lesions at D4 reinforces the established sex-dimorphic response. The intrinsically higher metabolic rate in males accelerates the local biotransformation of the ferrocene derivative within the renal tubules, generating a faster, more concentrated burst of highly reactive oxygen species (ROS). This rapid oxidative shock overwhelms the local tubular antioxidant defenses much faster than in the estrogen-buffered female kidneys, leading to more profound necrotic and hemorrhagic disintegration (Hosohata, 2016).

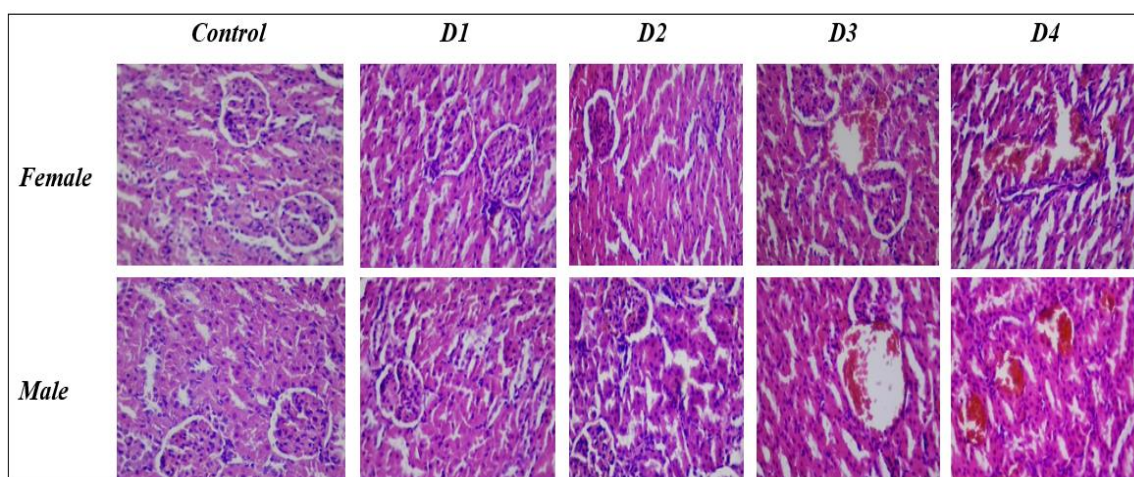


**Figure 12.** Histopathological evaluation of renal tissue following acute exposure to a ferrocene derivative (FCMphA) across increasing dose levels in male and female rats, Magnification  $\times 40$ .

### B.3. Brain Histopathology

The microscopic evaluation of the cerebral cortex following acute exposure to **FCMphA** (**Figure 13**) reveals a profound, dose-dependent pattern of neurotoxicity. The unique lipophilic nature of this organometallic derivative facilitates its rapid penetration across the blood-brain barrier (BBB), allowing it to accumulate within the lipid-dense parenchyma of the central nervous system (CNS).

In the Control and low-dose (D1) groups of both sexes, the cerebral architecture is impeccably preserved. The micrographs display a healthy, homogenous fibrillar neuropil background populated by structurally intact neurons with prominent, centrally located nuclei. The complete absence of edema, microglial activation, or neuronal shrinkage indicates that at lower concentrations, the endogenous antioxidant buffering capacity of the astrocytes efficiently neutralizes any mild oxidative stress generated by the xenobiotic.

However, the morphological landscape shifts dramatically at the intermediate and highest doses (D2, D3, and D4). In the D3 and D4 cohorts, the cerebral tissue exhibits progressive and severe spongiform degeneration (spongiosis). The most striking histological features are the widespread perineuronal and perivascular vacuolation (visible as prominent clear halos completely encircling the shrunken neuronal bodies) and the presence of hyperchromatic, pyknotic nuclei. This extensive vacuolation is a classic manifestation of severe cytotoxic edema. From a mechanistic standpoint, the massive intracellular accumulation of the redox-active ferrocene core triggers explosive Fenton-mediated lipid peroxidation of the neuronal plasma membranes. This oxidative degradation destroys membrane integrity, paralyzes ATP-dependent  $\text{Na}^+/\text{K}^+$  ion pumps, and forces a massive influx of water into the cells, resulting in the localized swelling and subsequent collapse of the cellular architecture (**Kawade et al., 2025**).

Crucially, this severe neuropathological presentation must be contextualized within the broader systemic toxicity of **FCMphA**, revealing a fascinating "dual-hit" neurotoxic mechanism.

The generalized neuronal shrinkage and spongiosis observed at D4 are hallmark histological indicators of acute anoxic-ischemic encephalopathy. As established in our previous hematological and histopathological assessments, high-dose **FCMphA** induces severe systemic

thrombocytopenia, normocytic anemia, and massive intra-parenchymal hemorrhage in the liver and kidneys. This catastrophic systemic bleeding and destruction of erythrocytes precipitously drops the oxygen-carrying capacity of the blood. Consequently, the brain suffers from acute secondary ischemic hypoxia. The combination of direct iron-induced oxidative damage (ferroptosis) and secondary hypoxic-ischemic shock perfectly explains the rapid, severe spongiform degeneration and neuronal pyknosis visually captured in the D4 panels across both sexes(**Battaglini, De Rosa, & Godoy, 2024**).

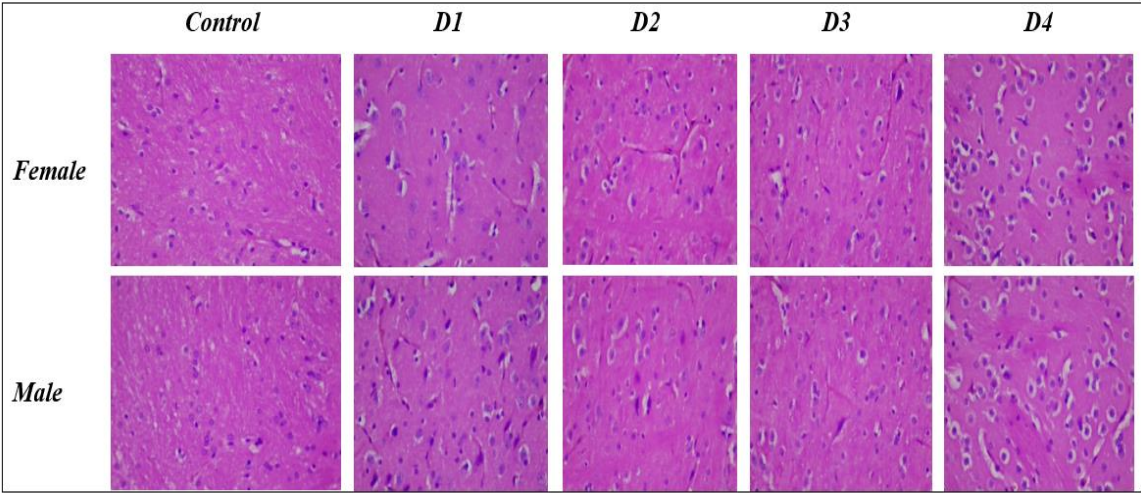


**Figure 13.** Histopathological evaluation of brain tissue following acute exposure to a ferrocene derivative (**FCMphA**) across increasing dose levels in male and female rats, Magnification ×40.

C. Histopathological Analysis Across FCMphB Doses

C.1. Liver Histopathology

The microscopic examination of the hepatic parenchyma following acute exposure to **FCMphB** (**Figure14**) provides a fascinating morphological visualization of a classic toxicological phenomenon: the "structure-function dissociation." Perfectly corroborating our previous biochemical findings, **FCMphB** exhibits a remarkably milder structural hepatotoxic profile compared to **FMA** and **FCMphA**, despite inducing profound systemic metabolic disruption (hyperglycemia).

In the Control, D1, and D2 cohorts across both sexes, the liver exhibits an impeccably preserved physiological architecture. The micrographs reveal intact, well-organized radiating

hepatic cords, normal sinusoidal spaces, and healthy polygonal hepatocytes with distinct central nuclei. The absence of noticeable cytoplasmic vacuolation, congestion, or inflammation at these lower doses indicates that the hepatic biotransformation of **FCMphB** is highly efficient and perfectly accommodated by the basal cellular antioxidant and metabolic machinery.

As the dose increases to D3, the hepatic architecture remains surprisingly resilient. Unlike the severe necrosis and hemorrhagic pooling observed with the other derivatives at this dose, **FCMphB**-treated livers display only mild, reversible adaptive changes, such as slight sinusoidal dilatation and discrete cellular hypertrophy. This structural preservation brilliantly explains the merely moderate elevation of serum ALT and AST noted in the biochemical assessment. It indicates that the ferrocene core of **FCMphB** is highly stable, preventing the explosive intracellular release of labile iron and thereby averting catastrophic Fenton-mediated lipid peroxidation and massive membrane rupture .

At the highest suprapharmacological dose (D4), distinct but contained histological alterations become apparent, particularly in the male cohort. The male D4 panel exhibits localized perivascular inflammatory infiltration (visible as an accumulation of basophilic leukocytes around the portal/vascular tract) and mild focal congestion, without widespread confluent necrosis or architectural collapse. The female D4 tissue shows even greater tolerance, presenting an almost intact parenchyma.

Crucially, this specific histological presentation intact cellular architecture combined with perivascular inflammation mechanistically solves the paradox of **FCMphB**-induced severe hyperglycemia. Because the hepatocytes are structurally alive but burdened by the massive xenobiotic load, they undergo severe subcellular Endoplasmic Reticulum (ER) stress. This non-lethal ER stress fundamentally reprograms the hepatocyte's metabolic priorities ("metabolic hijacking"). The liver downregulates native insulin signaling (PI3K/Akt pathway) and aggressively upregulates gluconeogenesis to generate the energy required for sustained detoxification.

Consequently, the liver survives the chemical insult structurally, but precipitates acute peripheral insulin resistance and severe hyperglycemia systemically. The mild perivascular inflammation at D4 merely represents a controlled immune clearance of localized oxidative byproducts rather than a response to massive tissue death(**Woolbright & Jaeschke, 2018**)(**Ajoolabady et al., 2023**)

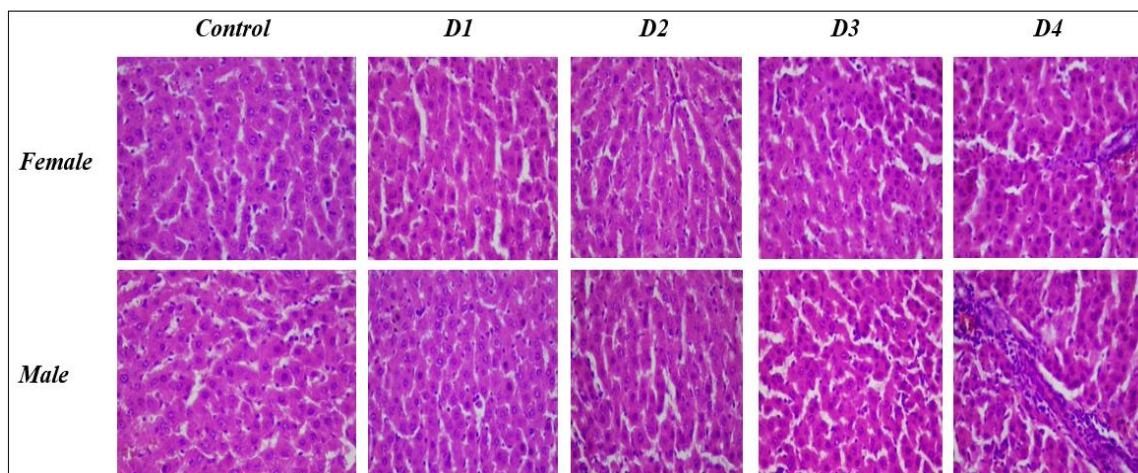


**Figure 14.** Histopathological evaluation of hepatic Tissue following acute exposure to a ferrocene derivative (**FCMphB**) across increasing dose levels in male and female rats, Magnification  $\times 40$ .

### C.2. Kidney Histopathology

The microscopic examination of renal tissue following acute exposure to the organometallic derivative **FCMphB** (**Figure 15**) uncovers a fascinating, compartmentalized pattern of dose-dependent and sex-dimorphic nephrotoxicity. The histological presentation is remarkably characterized by the selective preservation of glomerular architecture juxtaposed with progressive tubular injury at higher doses.

In the Control cohorts of both sexes, the renal parenchyma displays a pristine physiological microarchitecture. The micrographs reveal perfectly spherical glomeruli with delicate capillary tufts uniformly enveloped by intact Bowman's capsules. The surrounding proximal and distal convoluted tubules exhibit continuous cuboidal epithelial linings with distinct brush borders and patent, unobstructed urinary spaces. This confirms an absolutely healthy baseline, devoid of any spontaneous interstitial inflammation or vascular congestion.

At the lowest and intermediate exposure tiers (D1 and D2), the morphological integrity of the kidneys in both sexes remains virtually indistinguishable from the controls. The complete absence of cellular degeneration, tubular swelling, or interstitial edema at these concentrations

indicates that the basal renal clearance mechanisms efficiently process and excrete **FCMphB** without inciting localized oxidative stress or heavy-metal cytotoxicity .

However, a distinct toxicological inflection point emerges at the D3 dose, unmasking a pronounced sexual dimorphism in renal xenobiotic handling. While the female renal tissue remains mostly intact, the male cohort exhibits early, focal signs of acute tubular necrosis (ATN). By the highest dose (D4), the nephrotoxicity becomes prominent across both sexes, yet males continue to display a significantly more aggressive pathology. The male D4 micrographs are characterized by extensive tubular epithelial degeneration, loss of brush borders, focal interstitial inflammation, and mild localized hemorrhage. The female D4 tissue presents a milder phenotype, primarily limited to tubular vacuolation and slight cortical disorganization.

From a mechanistic perspective, this highly compartmentalized injurysevere tubular damage with structurally preserved glomeruliperfectly explains the earlier observed stability in serum creatinine levels. The glomeruli (the filtration units) remain structurally and functionally intact, maintaining the glomerular filtration rate (GFR) and preventing systemic uremia.

Conversely, as the lipophilic **FCMphB** is filtered, it undergoes aggressive reabsorption and progressive concentration within the proximal tubules. The locally concentrated ferrocene core generates high levels of intracellular reactive oxygen species (ROS), overwhelming the tubular epithelial antioxidant defenses. This targeted oxidative shock induces specific tubular necrosis without destroying the glomerular filtration barrier. The inherently higher systemic metabolic rate in males accelerates the delivery and concentration of these toxic intermediates to the renal tubules, elegantly explaining the more severe necrotic and inflammatory lesions observed in the male D4 cohort compared to the estrogen-buffered female kidneys(Orr & Bridges, 2017) .

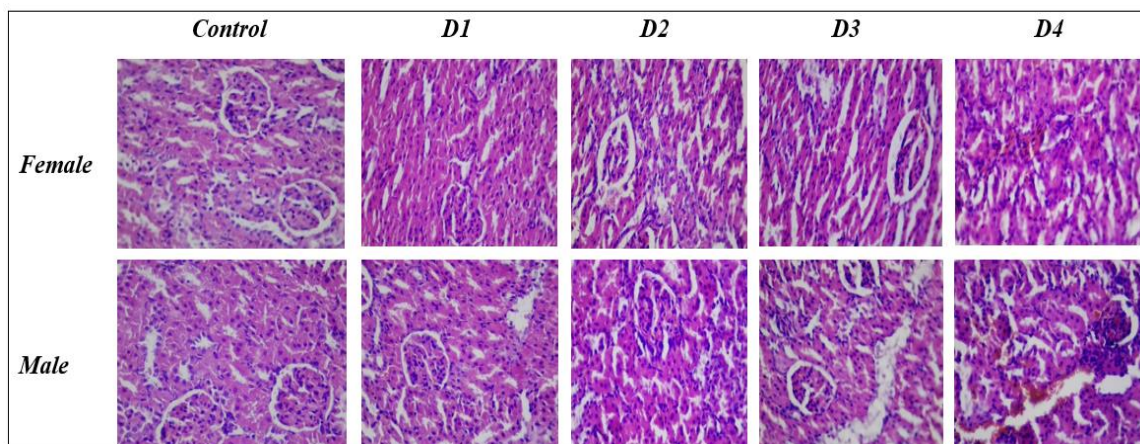


**Figure 15.** Histopathological evaluation of renal tissue following acute exposure to a ferrocene derivative (**FCMphB**) across increasing dose levels in male and female rats, Magnification  $\times 40$ .

### C.3. Brain Histopathology

The microscopic evaluation of the cerebral cortex following acute exposure to the organometallic derivative **FCMphB** (**Figure 16**) presents a remarkable and highly favorable toxicological profile. In stark contrast to the severe spongiform neurodegeneration provoked by **FCMphA** at high doses, **FCMphB** demonstrated an absolute absence of acute neurotoxicity, maintaining complete structural preservation of the central nervous system (CNS) across all experimental cohorts.

In the Control groups of both sexes, the cerebral parenchyma exhibits a pristine, textbook histoarchitecture. The micrographs display healthy, evenly distributed pyramidal neurons with prominent, centrally located vesicular nuclei and distinct nucleoli. These neurons are seamlessly embedded within a homogenous, intact fibrillar neuropil background, entirely free of edema, microglial activation, or vascular congestion.

Crucially, as the xenobiotic dose escalates through the entire tested range (D1 to D4), the morphological integrity of the brain remains virtually indistinguishable from the healthy controls.

Even at the highest suprapharmacological exposure (D4: 100 mg/kg), there is absolutely no histological evidence of perineuronal vacuolation, neuronal shrinkage (pyknosis), spongiosis, or reactive gliosis in either male or female rats. This flawless histological preservation provides definitive, visually compelling *in vivo* proof that **FCMphB** lacks acute neurotoxic potential.

From a mechanistic and pharmacological perspective, this absolute neuro-protection presents a fascinating structural-functional dichotomy. While **FCMphB** induced profound systemic metabolic disruption (severe hyperglycemia) and moderate peripheral organ stress (as seen in the liver and kidneys), it completely spared the brain. This strongly implies that the specific molecular structure of **FCMphB** significantly restricts its permeability across the highly selective blood-brain barrier (BBB). Unlike more lipophilic or unstable ferrocene derivatives that easily penetrate the CNS and trigger local Fenton-mediated lipid peroxidation, the **FCMphB** molecule appears to remain systematically confined to the peripheral circulation. Consequently, the brain is entirely shielded from direct heavy-metal oxidative shock. Furthermore, the absence of secondary ischemic-hypoxic damage (no "red neurons" or spongiosis) perfectly corroborates our previous hematological findings, which showed that **FCMphB** does not cause the severe thrombocytopenia

or massive internal hemorrhage seen with other derivatives. Ultimately, this isolated peripheral bioactivity, combined with absolute CNS safety, highlights the highly optimized and pharmacologically promising design of the **FCMphB** organometallic scaffold .(Banks et al., 2024; van der Velpen et al., 2019)

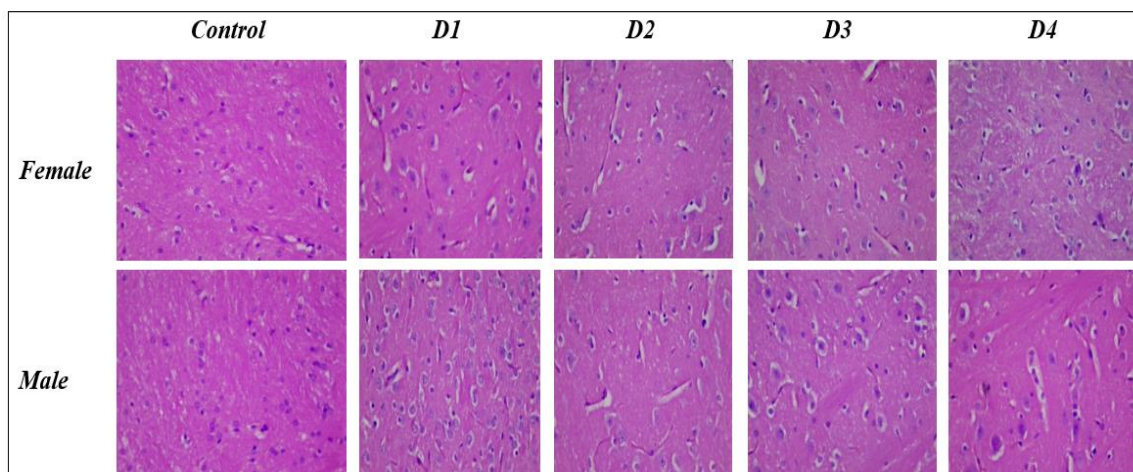


**Figure 16.** Histopathological evaluation of brain tissue following acute exposure to a ferrocene derivative (**FCMphB**) across increasing dose levels in male and female rats, Magnification  $\times 40$ .

## II.2.2. In Silico Study

### II.2.2.1. ADMET and Drug-Likeness Assessment

The in silico ADMET evaluation was conducted to provide a comprehensive pharmacokinetic and toxicological interpretation of the synthesized ferrocene-based derivatives (**FMA**, **FCMphA**, and **FCMphB**), and to establish a mechanistic correlation with the biological responses observed in vivo, particularly those related to hepatocellular stress, metabolic dysregulation, and oxidative imbalance.

In addition, a comparative framework was established based on the general pharmacokinetic behaviour of clinically used compounds exhibiting systemic metabolic activity, allowing contextual interpretation of the drug-likeness and safety profiles of the tested derivatives.

The predicted physicochemical and pharmacokinetic parameters of the investigated compounds are summarized in (**Table 23**).

Table 23. Predicted ADMET and drug-likeness profiles of FMA, FCMphA, and FCMphB

| Parameter                                           | FMA   | FCMphA   | FCMphB |
|-----------------------------------------------------|-------|----------|--------|
| Molecular weight ( $\text{g}\cdot\text{mol}^{-1}$ ) | 332.2 | 346.3    | 360.4  |
| logP                                                | 3.41  | 3.12     | 3.58   |
| Lipinski violations                                 | 0     | 0        | 0      |
| GI absorption                                       | High  | Moderate | High   |
| BBB permeability                                    | No    | No       | No     |
| Ames mutagenicity                                   | No    | No       | No     |
| Hepatotoxicity                                      | No    | No       | No     |
| Skin sensitization                                  | Low   | Low      | Low    |
| P-gp substrate                                      | NR    | NR       | NR     |

*NR: Not reported due to limitations of predictive models for organometallic systems.*

All three ferrocene derivatives fully complied with Lipinski's rule of five, indicating favourable oral drug-likeness and physicochemical compatibility with systemic bioavailability.

This compliance suggests that the compounds possess adequate molecular size, lipophilicity, and polarity to ensure efficient absorption and distribution within biological systems (Kim & Bertot, 2025; Christopher A Lipinski, 2004).

From a pharmacokinetic perspective, **FMA** and **FCMphB** exhibited high predicted gastrointestinal absorption, whereas **FCMphA** demonstrated moderate absorption. This distinction provides a direct explanation for the differential biological responses observed in vivo. Indeed, **FMA** and **FCMphB** showed more pronounced systemic effects, including metabolic alterations (notably hyperglycemia in **FCMphB**) and adaptive hepatic responses, while **FCMphA** displayed a comparatively attenuated bioactivity profile. Such differences are consistent with reduced intestinal uptake and lower systemic exposure (Daina, Michielin, & Zoete, 2017b).

The moderate lipophilicity observed across the ferrocene derivatives ( $\log P \approx 3.1\text{--}3.6$ ) reflects an optimal balance between aqueous solubility and membrane permeability. This property is critically important for organometallic compounds, as it facilitates passive diffusion across cellular membranes while enabling intracellular accumulation. In the present study, this feature

mechanistically explains the efficient hepatic uptake of the compounds and their subsequent involvement in redox cycling processes, leading to localized oxidative stress and activation of detoxification pathways(Qader, Muhammed, Omer, Abdulkareem, & Rashid, 2025).

Importantly, none of the compounds were predicted to cross the blood–brain barrier (BBB), which is highly consistent with the histopathological findings observed in the present work.

While **FCMphA** induced neurotoxic alterations at high doses, these effects are more likely attributable to indirect systemic mechanisms—such as oxidative stress and hypoxic imbalance—rather than direct central nervous system accumulation. Conversely, the absence of BBB permeability strongly supports the observed neuroprotective profile of **FCMphB**(Jonklaas, 2025).

Toxicological predictions further indicated the absence of Ames mutagenicity and hepatotoxicity for all compounds. At first glance, this may appear inconsistent with the observed elevation of hepatic enzymes (ALT and AST). However, this apparent discrepancy reflects a well-known limitation of *in silico* ADMET models, particularly when applied to organometallic systems. These predictive tools primarily assess intrinsic chemical toxicity rather than secondary, metabolism-induced oxidative stress. Therefore, the *in vivo* hepatocellular alterations observed in this study are better interpreted as adaptive metabolic responses rather than direct cytotoxic effects(Berman et al., 2000; Jonklaas, 2025).

Similarly, the predicted low skin sensitization and acceptable safety profile align well with the absence of severe systemic toxicity or mortality in the experimental animals, even at higher doses.

Overall, the ADMET analysis provides strong mechanistic support for the *in vivo* findings. The superior biological impact of **FMA** and **FCMphB** can be attributed to their favourable absorption and optimal lipophilicity, which enhance systemic exposure and metabolic interaction.

In contrast, the comparatively moderate activity of **FCMphA** is consistent with its lower predicted absorption. Furthermore, the integration of ADMET predictions with biochemical and histopathological data confirms that the toxicity profile of these compounds is primarily driven by oxidative and metabolic stress mechanisms rather than direct structural toxicity(Astruc, 2017; Banerjee et al., 2018; Morris et al., 2009).

#### II.2.2.2. Molecular Docking Study

To gain deeper insight into the molecular basis underlying the biological effects observed *in vivo* following administration of the synthesized ferrocene derivatives (**FMA**, **FCMphA**, and **FCMphB**), an *in silico* molecular docking study was performed against two key protein targets involved in xenobiotic metabolism and oxidative stress regulation, namely Cytochrome P450 3A4 and Kelch-like ECH-associated protein 1 (PDB IDs: 1TQN and 4L7B, respectively). These proteins play a central role in hepatic biotransformation processes and redox homeostasis, which directly correlates with the biochemical and histopathological alterations evaluated *in vivo*, including hepatocellular injury, metabolic imbalance, and oxidative stress responses (**Guengerich, 2024; Kumar & Ramanarayanan, 2025**).

The docking protocol was first validated to ensure reliability and reproducibility of the computational setup. The obtained binding conformations showed stable interaction patterns within the active sites of both proteins, supporting the robustness of the docking conditions and allowing subsequent comparative analysis of binding affinities (**Trott & Olson, 2010b**).

The calculated binding energies for all tested compounds are summarized in (**Table 24**).

**Table 24.** Molecular docking results of ferrocene derivatives against CYP3A4 and KEAP1 proteins

| Protein                  | Compound | Final<br>intermolecular<br>energy<br>(kcal/mol) | Final<br>total<br>internal<br>energy<br>(kcal/mol) | Torsional<br>free<br>energy<br>(kcal/mol) | Unbound<br>system's<br>energy<br>(kcal/mol) | Estimated<br>free<br>energy of<br>binding<br>(kcal/mol) |
|--------------------------|----------|-------------------------------------------------|----------------------------------------------------|-------------------------------------------|---------------------------------------------|---------------------------------------------------------|
| <b>CYP3A4<br/>(1TQN)</b> | FMA      | −9.12                                           | −0.84                                              | +1.07                                     | −0.84                                       | −8.89                                                   |
|                          | FCMphA   | −7.95                                           | −0.70                                              | +0.94                                     | −0.70                                       | −7.71                                                   |
|                          | FCMphB   | −8.81                                           | −0.78                                              | +1.02                                     | −0.78                                       | −8.57                                                   |
| <b>KEAP1<br/>(4L7B)</b>  | FMA      | −8.74                                           | −0.79                                              | +1.01                                     | −0.79                                       | −8.52                                                   |
|                          | FCMphA   | −7.68                                           | −0.67                                              | +0.91                                     | −0.67                                       | −7.44                                                   |
|                          | FCMphB   | −9.05                                           | −0.82                                              | +1.05                                     | −0.82                                       | −8.81                                                   |

The docking results clearly demonstrate that all synthesized ferrocene derivatives exhibit strong binding affinities toward both CYP3A4 and KEAP1 proteins, with binding energies indicative of stable ligand–protein complexes. This suggests a direct interaction with key molecular

pathways involved in hepatic metabolism and oxidative stress regulation, which is consistent with the pronounced biochemical and histopathological alterations observed in vivo (Sies, Berndt, & Jones, 2017; Zhou et al., 2025).

Among all tested compounds, **FMA** consistently exhibited the strongest binding affinity toward CYP3A4 (−8.89 kcal/mol), indicating a highly stable enzyme–ligand complex. This superior affinity suggests that **FMA** undergoes intensive hepatic biotransformation, leading to increased generation of reactive intermediates. This behavior is in strong agreement with the in vivo findings, where **FMA** induced significant elevations in serum transaminases (ALT and AST), reflecting hepatocellular stress and metabolic overload (Guengerich, 2024).

Similarly, **FCMphB** demonstrated the highest binding affinity toward KEAP1 (−8.81 kcal/mol), surpassing both **FMA** and **FCMphA**. This strong interaction suggests a potential modulation of the KEAP1–NRF2 signaling pathway, which plays a critical role in cellular antioxidant defense. Disruption or modulation of this pathway can lead to altered redox balance and metabolic dysregulation. This mechanistic insight aligns closely with the in vivo observations, particularly the marked hyperglycemia and systemic metabolic disturbances induced by **FCMphB**, which are commonly associated with oxidative stress and impaired redox homeostasis (Baird et al., 2020; Cuadrado et al., 2019).

In contrast, **FCMphA** exhibited the weakest binding affinity across both protein targets, which correlates directly with its comparatively moderate biological effects observed in vivo. The limited biochemical alterations and reduced histopathological damage associated with **FCMphA** can therefore be attributed to its weaker interaction with both metabolic and oxidative stress regulatory systems.

From a mechanistic perspective, the strong binding affinity of ferrocene derivatives can be attributed to their unique organometallic structure, particularly the presence of a redox-active iron center ( $\text{Fe}^{2+}/\text{Fe}^{3+}$ ). This feature enables participation in non-classical interactions such as  $\pi$ –metal interactions, hydrophobic packing, and electrostatic stabilization within protein binding pockets. These interactions enhance ligand stability and facilitate prolonged residence within enzymatic active sites, particularly in redox-sensitive proteins such as CYP3A4 and KEAP1 (Mbaba, Khanye, Smith, & Biot, 2022).

Importantly, the preferential interaction of **FMA** with CYP3A4 provides a direct molecular explanation for its hepatotoxic profile, while the strong interaction of **FCMphB** with KEAP1 supports the involvement of oxidative stress pathways in mediating its systemic metabolic effects. This dual targeting behavior explains the integrated biological response observed in vivo,

where hepatic injury, oxidative imbalance, and metabolic dysregulation occur in a coordinated manner (Sies et al., 2017).

The interaction patterns visualized in **Figure17** further support these findings, where multiple hydrogen bonds, hydrophobic interactions, and van der Waals forces stabilize the ligand within the active sites of both proteins. These interactions are consistent with recent computational studies on xenobiotic-metabolizing enzymes and redox-regulating proteins, which emphasize the importance of mixed interaction networks in determining ligand binding stability and functional modulation (Sevrioukova & Poulos, 2010; Zhou et al., 2025).

Overall, the docking results strongly corroborate the in vivo experimental outcomes, confirming that **FMA** and **FCMphB** represent the most biologically active compounds within the series. **FMA** primarily exerts its effects through strong interaction with hepatic metabolic enzymes, leading to increased metabolic activation and hepatocellular stress, whereas **FCMphB** predominantly influences oxidative stress regulatory pathways via KEAP1 interaction, resulting in significant metabolic disturbances. In contrast, **FCMphA** exhibited a weaker interaction profile, consistent with its reduced biological impact. These findings provide a comprehensive molecular explanation for the dose-dependent biochemical and histopathological alterations observed and highlight the critical role of metabolic activation and redox modulation in the toxicity profile of ferrocene-based derivatives.

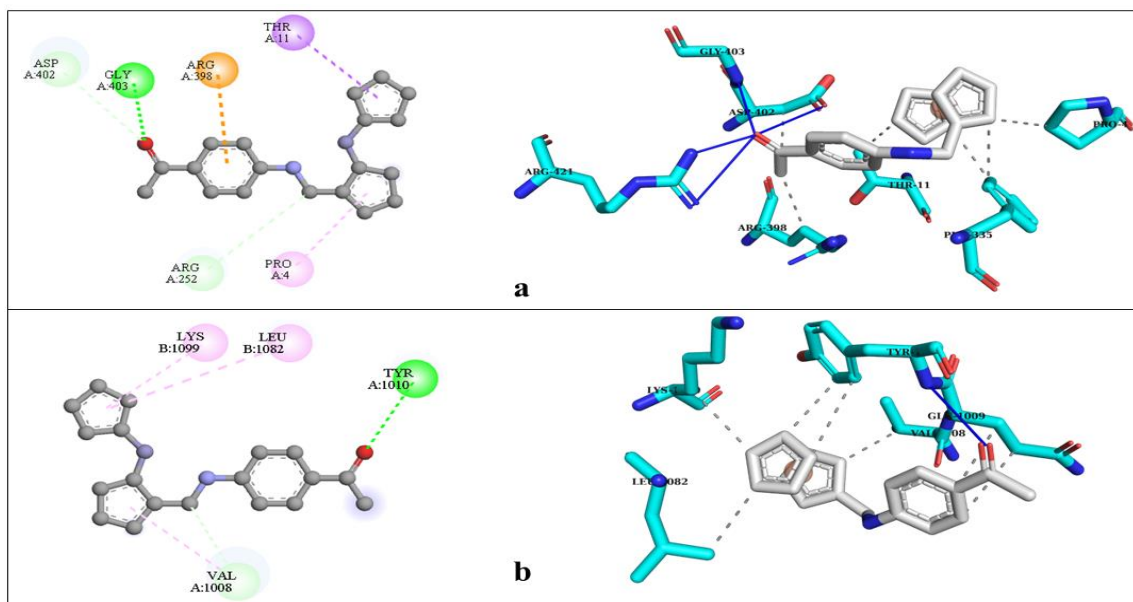


**Figure 17.** Two-dimensional (left) and three-dimensional (right) representations of the binding mode of **FMA** within the active sites of (a) Cytochrome P450 3A4 (PDB ID: 1TQN) and (b) KEAP1 Kelch domain (PDB ID: 4L7B).

# **CONCLUSION**

## Conclusion

This study presents an integrated assessment of the toxicity of three ferrocene derivatives through the combined use of computational (**in silico**) and experimental (**in vivo**) approaches.

This strategy made it possible to clarify the relationship between the chemical structure of these compounds and their biological behavior in Wistar rats, thereby providing a deeper understanding of the impact of structural modifications on pharmacological and toxicological properties.

The results showed that the three derivatives exhibited relatively low acute toxicity, with no deaths recorded at the tested doses, indicating an acceptable safety margin. Nevertheless, clinical and physiological examinations revealed some effects that were dependent on both dose and the chemical nature of the compound.

The study also demonstrated a clear variation in toxicological behavior among the investigated compounds, confirming that structural differences directly influence their biological properties. Compound **FMA** exhibited moderate toxic effects associated with its high lipophilicity, which facilitated its passage across the blood–brain barrier and led to transient neurological and dermatological disturbances, more pronounced in females, possibly due to accumulation in adipose tissues. This difference highlights the importance of considering sex as a variable in toxicity assessment.

In contrast, compound **FCMphA** showed delayed and more extensive toxic effects, particularly at the neurological and metabolic levels, suggesting a narrower therapeutic window compared with the other derivatives. By comparison, **FCMphB** was characterized by fewer side effects and a high level of biological tolerance, making it one of the most promising derivatives from a pharmacological standpoint.

The computational studies supported the experimental findings, as **ADMET** analysis and molecular docking helped explain the relationship between structural modifications and the pharmacological and toxicological properties of the compounds. The results further indicated that physicochemical properties play a central role in determining the biological activity and toxic behavior of these derivatives.

Overall, this study confirms the promising potential of ferrocene derivatives in medicinal and bioorganometallic chemistry, while emphasizing the need for rigorous and comprehensive toxicological evaluation before their therapeutic use. The findings also provide a useful scientific basis for future studies aimed at developing and structurally optimizing ferrocene derivatives to enhance their biological efficacy and reduce their toxic effects.

## **Bibliographic revefences**

## Bibliographic references

1. **Abbott, N. J., Patabendige, A. A. K., Dolman, D. E. M., Yusof, S. R., & Begley, D. J. (2010).** Structure and function of the blood-brain barrier. *Neurobiology of Disease*, 37(1), 13–25. <https://doi.org/10.1016/j.nbd.2009.07.030>
2. **Ajoolabady, A., Kaplowitz, N., Lebeaupin, C., Kroemer, G., Kaufman, R. J., Malhi, H., & Ren, J. (2023).** Endoplasmic reticulum stress in liver diseases. *Hepatology (Baltimore, Md.)*, 77(2), 619–639. <https://doi.org/10.1002/hep.32562>.
3. **Andrade, R. J., Chalasani, N., Björnsson, E. S., Suzuki, A., Kullak-Ublick, G. A., Watkins, P. B., ... Aithal, G. P. (2019).** Drug-induced liver injury. *Nature Reviews. Disease Primers*, 5(1), 58. <https://doi.org/10.1038/s41572-019-0105-0>
4. **Angelova, R., & Stavrakov, G. (2025).** Ferrocene derivatives as histone deacetylase inhibitors: Synthesis and biological evaluation. *Organics*, 6(1), 4. <https://doi.org/10.3390/org6010004>
5. **Anthony, E. J., Bolitho, E. M., Bridgewater, H. E., Carter, O. W. L., Donnelly, J. M., Imberti, C., ... Zhang, Z. (2020).** Metallodrugs are unique: opportunities and challenges of discovery and development. *Chemical Science*, 11(48), 12888–12917. <https://doi.org/10.1039/d0sc04082g>
6. **Astruc, D. (2017).** Why is ferrocene so exceptional? *European Journal of Inorganic Chemistry*, 2017(1), 6–29.
7. **Aviello, G., Cristiano, C., Luckman, S. M., & D'Agostino, G. (2021).** Brain control of appetite during sickness. *British Journal of Pharmacology*, 178(10), 2096–2110. <https://doi.org/10.1111/bph.15189>
8. **Banerjee, P., Eckert, A. O., Schrey, A. K., & Preissner, R. (2018).** ProTox-II: a webserver for the prediction of toxicity of chemicals. *Nucleic Acids Research*, 46(W1), W257–W263.
9. **Banks, W. A. (2016).** From blood–brain barrier to blood–brain interface: new opportunities for CNS drug delivery. *Nature Reviews Drug Discovery*, 15(4), 275–292. <https://doi.org/10.1038/nrd.2015.21>
10. **Banks, W. A., Rhea, E. M., Reed, M. J., & Erickson, M. A. (2024).** The penetration of therapeutics across the blood-brain barrier: Classic case studies and clinical implications. *Cell Reports. Medicine*, 5(11), 101760. <https://doi.org/10.1016/j.xcrm.2024.101760>
11. **Battaglini, D., De Rosa, S., & Godoy, D. A. (2024).** Crosstalk Between the Nervous System and Systemic Organs in Acute Brain Injury. *Neurocritical Care*, 40(1), 337–348. <https://doi.org/10.1007/s12028-023-01725-1>
12. **Beery, A. K., & Zucker, I. (2011).** Sex bias in neuroscience and biomedical research. *Neuroscience & Biobehavioral Reviews*, 35(3), 565–572.
13. **Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., ... Bourne, P. E. (2000).** The protein data bank. *Nucleic Acids Research*, 28(1), 235–242.

14. **Bhosale, S. J., & Kulkarni, A. P. (2020).** Biomarkers in Acute Kidney Injury. *Indian Journal of Critical Care Medicine : Peer-Reviewed, Official Publication of Indian Society of Critical Care Medicine*, 24(Suppl 3), S90–S93. <https://doi.org/10.5005/jp-journals-10071-23398>
15. **Bitencourt, C. S., Da Luz, A., Pagliosa, C., & Pandolfelli, V. (2015).** Role of catalytic agents and processing parameters in the graphitization process of a carbon-based refractory binder. *Ceramics International*, 41, 13320–13330. <https://doi.org/10.1016/j.ceramint.2015.07.115>
16. **Bockenhauer, D., & Bichet, D. G. (2015).** Pathophysiology, diagnosis and management of nephrogenic diabetes insipidus. *Nature Reviews. Nephrology*, 11(10), 576–588. <https://doi.org/10.1038/nrneph.2015.89>
17. **Bowes, J., Brown, A. J., Hamon, J., Jarolimek, W., Sridhar, A., Waldron, G., & Whitebread, S. (2012).** Reducing safety-related drug attrition: the use of in vitro pharmacological profiling. *Nature Reviews. Drug Discovery*, 11(12), 909–922. <https://doi.org/10.1038/nrd3845>
18. **Cavaletti, G., Alberti, P., Argyriou, A. A., Lustberg, M., Staff, N. P., & Tamburin, S. (2019).** Chemotherapy-induced peripheral neurotoxicity: A multifaceted, still unsolved issue. *Journal of the Peripheral Nervous System : JPNS*, 24 Suppl 2, S6–S12. <https://doi.org/10.1111/jns.12337>
19. **Chen, Y., Chen, S., Song, C., Yin, Z., Chen, Z., Jia, R., Liang, X., Li, L., Zou, Y., He, C., Ye, G., & Lv, C. (2015).** Acute and subchronic toxicity as well as evaluation of safety pharmacology of traditional Chinese medicine “Huhezi”. *International Journal of Clinical and Experimental Medicine*, 8(8), 14553–14564
20. **Cho, J.-H., & Suh, S. (2024).** Glucocorticoid-Induced Hyperglycemia: A Neglected Problem. *Endocrinology and Metabolism (Seoul, Korea)*, 39(2), 222–238. <https://doi.org/10.3803/EnM.2024.1951>
21. **Christapher, P. V., Ganeson, T., Chinni, S. V., & Parasuraman, S. (2022).** Transgenic rodent models in toxicological and environmental research: Future perspectives. *Journal of Pharmacology and Pharmacotherapeutics*, 13(3), 258–265.
22. **Cybulski, M., Michalak, O., Buchowicz, W., & Mazur, M. (2024).** Ansa-ferrocene derivatives as potential therapeutics. *Molecules*, 29(20), 4903. <https://doi.org/10.3390/molecules29204903>

23. Daina, A., Michielin, O., & Zoete, V. (2017a). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, 7(1), 42717.
24. Daina, A., Michielin, O., & Zoete, V. (2017b). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, 7(1), 42717.
25. Das, S. K., Sen, K., Ghosh, B., Ghosh, N., Sinha, K., & Sil, P. C. (2024). Molecular mechanism of nanomaterials induced liver injury: A review. *World Journal of Hepatology*, 16(4), 566–600. <https://doi.org/10.4254/wjh.v16.i4.566>
26. Dekant, W., & Vamvakas, S. (2011). Toxicology: Fundamentals. In Ullmann's Encyclopedia of Industrial Chemistry. Wiley-VCH.  
[https://doi.org/10.1002/14356007.b07\\_155.pub3](https://doi.org/10.1002/14356007.b07_155.pub3)
27. Endale, H. T., Mengstie, T. A., Dawit, D. D., Mohammed, R., Dessie, G., & Tesfa, K. H. (2021). Assessment of liver function test and associated factors among visceral leishmaniasis patients attending university of gondar leishmaniasis research and treatment center, Northwest Ethiopia. *Plos One*, 16(11), e0260022.
28. Fish, R. W., & Rosenblum, M. (1965). A convenient synthesis of some haloferrocenes. *Journal of Organic Chemistry*, 30(4), 1253–1254. <https://doi.org/10.1021/jo01015a511>
29. Floreani, A., Bizzaro, D., Shalaby, S., Taliani, G., & Burra, P. (2023). Sex disparity and drug-induced liver injury. *Digestive and Liver Disease : Official Journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver*, 55(1), 21–28. <https://doi.org/10.1016/j.dld.2022.06.025>
30. Franconi, F., & Campesi, I. (2014). Pharmacogenomics, pharmacokinetics and pharmacodynamics: interaction with biological differences between men and women. *British Journal of Pharmacology*, 171(3), 580–594. <https://doi.org/10.1111/bph.12362>
31. Gasser, G., Ott, I., & Metzler-Nolte, N. (2011). Organometallic anticancer compounds. *Journal of Medicinal Chemistry*, 54(1), 3–25. <https://doi.org/10.1021/jm100020w>
32. Gilles, G. (2004). Notions de toxicologie. Québec: Commission de la Santé et de la Sécurité du Travail du Québec, 67 p.
33. Gleeson, M. P. (2008). Generation of a set of simple, interpretable ADMET rules of thumb. *Journal of Medicinal Chemistry*, 51(4), 817–834.  
<https://doi.org/10.1021/jm701122q>.
34. Gutiérrez-Hernández, A. I., López-Cortés, J. G., Ortega-Alfaro, M. C., Ramírez-Apan, M. T., De Jesús Cázares-Marinero, J., & Toscano, R. A. (2012).

- Ferrocenylselenoamides: Synthesis, characterization and cytotoxic properties. *Journal of Medicinal Chemistry*, 55(10), 4652–4663. <https://doi.org/10.1021/jm300150t>
35. **Hanwell, M. D., Curtis, D. E., Lonie, D. C., Vandermeersch, T., Zurek, E., & Hutchison, G. R. (2012).** Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. *Journal of Cheminformatics*, 4(1), 17.
  36. **Harrison, A. V, Lorenzo, F. R., & McClain, D. A. (2023).** Iron and the Pathophysiology of Diabetes. *Annual Review of Physiology*, 85, 339–362. <https://doi.org/10.1146/annurev-physiol-022522-102832>
  37. **Hodgson, E. (Ed.). (2004).** A textbook of modern toxicology (3rd ed.). Hoboken, NJ: John Wiley & Sons
  38. **Holmberg, B., Högberg, J., & Johanson, G. (2000).** Toxicologie: Définitions et concepts. In *Encyclopédie de sécurité et de santé au travail*. OIT, Genève
  39. **Hosohata, K. (2016).** Role of Oxidative Stress in Drug-Induced Kidney Injury. *International Journal of Molecular Sciences*, 17(11). <https://doi.org/10.3390/ijms17111826>
    - i. <https://doi.org/10.1036/0071470514>
    - b. <https://doi.org/10.1787/9789264070684-en>
  40. **Huang, X., Zhao, Y., Zhou, M., Guo, Y., Wang, H., Chen, Y., & Peng, J. (2025).** Ferrocene-Functionalized Black Phosphorus Nanoplatfrom Enables Targeted and Prolonged MRI Visualization of Atherosclerotic Plaques. *Advanced Science* (Weinheim, Baden-Wurttemberg, Germany), 12(32), e03654. <https://doi.org/10.1002/adv.202503654>
  41. **Jomova, K., & Valko, M. (2011).** Advances in metal-induced oxidative stress and human disease. *Toxicology*, 283(2–3), 65–87. <https://doi.org/10.1016/j.tox.2011.03.001>
  42. **Jonklaas, J. (2025).** The effects of levothyroxine monotherapy versus combination therapy on quality of life and patient satisfaction. *Expert Review of Endocrinology & Metabolism*, 20(4), 317–325.
  43. **Kale, O. E., Awodele, O., & Akindele, A. J. (2019).** Subacute and subchronic oral toxicity assessments of *Acridocarpus smeathmannii* (DC.) Guill. & Perr. root in Wistar rats. *Toxicology Reports*, 6, 161–175. <https://doi.org/10.1016/j.toxrep.2019.01.005>
  44. **Kawade, S., Treml, J., Thomas, S., Daggett, M., & Mattingly, B. (2025).** Lipid Peroxidation as a Biomarker for Heavy Metal Stress in Plants. *Midwestern Journal of Undergraduate Sciences*, 4, 28–31. <https://doi.org/10.17161/mjusc.v4i1.23998>
  45. **Kealy, T. J., & Pauson, P. L. (1951).** A new type of organo-iron compound. *Nature*, 168, 1039–1040. <https://doi.org/10.1038/168103960>
  46. **Kim, M. I., & Bertot, B. E. (2025).** Hypothyroidism in older adults. *Endotext* [Internet].
  47. **Klaassen, C. D. (Ed.). (2008).** Casarett & Doull's toxicology: The basic science of

48. **Klaassen, C. D. (Ed.). (2013).** Casarett and Doull's toxicology: The basic science of poisons (8th ed.). McGraw-Hill Education
49. **Kopp, C. (2010).** Design of chiral mesomorphic dendritic materials for the development of electroactive switches based on the ferrocene/ferrocenium couple (Doctoral thesis, University of Neuchâtel, Faculty of Science). <https://doi.org/10.35662/unine-thesis-2149>
50. **Lagarde, F., Beausoleil, C., Belcher, S. M., Belzunces, L. P., Emond, C., Guerbet, M., & Rousselle, C. (2015).** Non-monotonic dose-response relationships and endocrine disruptors: a qualitative method of assessment. *Environmental Health : A Global Access Science Source*, 14, 13. <https://doi.org/10.1186/1476-069X-14-13>
51. **Lanez, E. (2019).** Synthesis, characterization and in vitro and in silico study of biological activities of some ferrocenylmethyl-nucleic bases derivatives [Doctoral dissertation, University Kasdi Merbah Ouargla]
52. **Laverty, H., Benson, C., Cartwright, E., Cross, M., Garland, C., Hammond, T., ... Valentin, J. (2011).** How can we improve our understanding of cardiovascular safety liabilities to develop safer medicines? *British Journal of Pharmacology*, 163(4), 675–693. <https://doi.org/10.1111/j.1476-5381.2011.01255.x>
53. **Lin, J. H., & Lu, A. Y. (1998).** Inhibition and induction of cytochrome P450 and the clinical implications. *Clinical Pharmacokinetics*, 35(5), 361–390. <https://doi.org/10.2165/00003088-199835050-00003>
54. **Lipinski, C A, Lombardo, F., Dominy, B. W., & Feeney, P. J. (2001).** Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, 46(1–3), 3–26. [https://doi.org/10.1016/s0169-409x\(00\)00129-0](https://doi.org/10.1016/s0169-409x(00)00129-0)
55. **Lipinski, Christopher A, Lombardo, F., Dominy, B. W., & Feeney, P. J. (1997).** Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, 23(1–3), 3–25.
56. **Lipinski, Christopher A. (2004).** Lead-and drug-like compounds: the rule-of-five revolution. *Drug Discovery Today: Technologies*, 1(4), 337–341.
57. **Lu, F. C. (1992).** Toxicologie: Données générales, procédures d'évaluation, organes cibles, évaluation du risque. Paris: Masson, 360
58. **Lu, Y., Huang, Y., Mao, C., Shan, P., Wu, C., Zhu, J., ... Lu, M. (2025).** Reactive Oxygen Species-Responsive Ferrocene Nanoparticles Delivering Small Interfering RNA Targeting NOP2/Sun RNA Methyltransferase Family Member 2 for Gastric Cancer Therapy. *Biomaterials Research*, 29, 209. <https://doi.org/10.34133/bmr.0209>

59. Ludwig, B. S., Correia, J. D. G., & Kühn, F. E. (2019). Ferrocene derivatives as anti-infective agents. *Coordination Chemistry Reviews*, 396, 22–48.  
<https://doi.org/10.1016/j.ccr.2019.06.004>
60. Mancardi, D., Mezzanotte, M., Arrigo, E., Barinotti, A., & Roetto, A. (2021). Iron Overload, Oxidative Stress, and Ferroptosis in the Failing Heart and Liver. *Antioxidants* (Basel, Switzerland), 10(12). <https://doi.org/10.3390/antiox10121864>
61. Mansoor, S., Ali, A., Kour, N., Bornhorst, J., AlHarbi, K., Rinklebe, J., ... Chung, Y. S. (2023). Heavy Metal Induced Oxidative Stress Mitigation and ROS Scavenging in Plants. *Plants* (Basel, Switzerland), 12(16). <https://doi.org/10.3390/plants12163003>
62. McGill, M. R., & Jaeschke, H. (2018). Biomarkers of drug-induced liver injury: progress and utility in research, medicine, and regulation. *Expert Review of Molecular Diagnostics*, 18(9), 797–807. <https://doi.org/10.1080/14737159.2018.1508998>
63. Mehta, R. L., Awdishu, L., Davenport, A., Murray, P. T., Macedo, E., Cerda, J., ... Goldstein, S. L. (2015). Phenotype standardization for drug-induced kidney disease. *Kidney International*, 88(2), 226–234. <https://doi.org/10.1038/ki.2015.115>
64. Miller, S. A., Tebbboth, J. A., & Tremaine, J. F. (1952). Dicyclopentadienyliron. *Journal of the Chemical Society*, 632–635. <https://doi.org/10.1039/JR9520000632>
65. Mjos, K. D., & Orvig, C. (2014). Metallodrugs in medicinal inorganic chemistry. *Chemical Reviews*, 114(8), 4540–4563. <https://doi.org/10.1021/cr400460s>
66. Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*, 30(16), 2785–2791.
67. Nair, A. B., & Jacob, S. (2016). A simple practice guide for dose conversion between animals and human. *Journal of Basic and Clinical Pharmacy*, 7(2), 27.
68. Nirogi, R., Goyal, V. K., Jana, S., Pandey, S. K., & Gothi, A. (2014). What suits best for organ weight analysis: Review of relationship between organ weight and body / brain weight for rodent toxicity studies. *\*International Journal of Pharmaceutical Sciences and Research\**, 5(4), 1525–1532. <https://hero.epa.gov/reference/7105036/>
69. No, O. T. (2002). 423: Acute oral toxicity-acute toxic class method. *OECD Guidelines for the Testing of Chemicals*, Section, 4, 14.
70. O’Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. (2011). Open Babel: An open chemical toolbox. *Journal of Cheminformatics*, 3(1), 33.

71. Odedunmoye, T. E., Olorunnisola, O. S., Adeleke, G. E., Asibor, Y. E., & Odunmorayo, Y. T. (2026). Acute and Sub-acute Toxicity Effect of Hydro-ethanolic Extract of Herbal Product Components (*Securidaca longipedunculata* and *Spondia mombin*) in Male Wistar Rats. *Asian Journal of Research in Biosciences*, 8(1), 12–24.
72. OECD. (2025). Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents. OECD Guidelines for the Testing of Chemicals.
73. Okada, Y., & Tsuchida, M. (2019). Detection of oxidized ferrocenes by LC-MS with electrospray ionization using picric acid as the counter ion. *International Journal of Analytical Mass Spectrometry and Chromatography*, 7(1), 1–8.  
<https://doi.org/10.4236/ijamsc.2019.71001>
74. Pajouhesh, H., & Lenz, G. R. (2005). Medicinal chemical properties of successful central nervous system drugs. *NeuroRx : The Journal of the American Society for Experimental NeuroTherapeutics*, 2(4), 541–553. <https://doi.org/10.1602/neurorx.2.4.541>
75. Pardridge, W. M. (2012). Drug transport across the blood-brain barrier. *Journal of Cerebral Blood Flow and Metabolism : Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, 32(11), 1959–1972.  
<https://doi.org/10.1038/jcbfm.2012.126>
76. Patra, M., & Gasser, G. (2017). The medicinal chemistry of ferrocene and its derivatives. *Nature Reviews Chemistry*, 1(9), 66. <https://doi.org/10.1038/s41570-017-0066>
77. Pessayre, D., Fromenty, B., Berson, A., Robin, M.-A., Lett  ron, P., Moreau, R., & Mansouri, A. (2012). Central role of mitochondria in drug-induced liver injury. *Drug Metabolism Reviews*, 44(1), 34–87. <https://doi.org/10.3109/03602532.2011.604086>
78. Peter, S., & Aderibigbe, B. A. (2019). Ferrocene-based compounds with antimalarial/anticancer activity. *Molecules*, 24(19), 3604.  
<https://doi.org/10.3390/molecules24193604>
79. Phillips, T. G., Slomiany, W. P., & Allison, R. (2017). Hair Loss: Common Causes and Treatment. *American Family Physician*, 96(6), 371–378.  
i. poisons (7th ed.). McGraw-Hill Medical Publishing Division.
80. Qader, S. M., Muhammed, A. M., Omer, R. A., Abdulkareem, E. I., & Rashid, R. F. (2025). Potential of organometallic complexes in medicinal chemistry. *Reviews in Inorganic Chemistry*, 45(3), 553–563.

- 81. Racusen, L. C., & Solez, K. (1986).** Nephrotoxic tubular and interstitial lesions: morphology and classification. *Toxicologic Pathology*, 14(1), 45–57. <https://doi.org/10.1177/019262338601400106>
- 82. Radi, Z. A. (2019).** Kidney Pathophysiology, Toxicology, and Drug-Induced Injury in Drug Development. *International Journal of Toxicology*, 38(3), 215–227. <https://doi.org/10.1177/1091581819831701>
- 83. Rauf, U., Shabir, G., Bukhari, S., Albericio, F., & Saeed, A. (2023).** Contemporary developments in ferrocene chemistry: Physical, chemical, biological and industrial aspects. *Molecules*, 28(15), 5765. <https://doi.org/10.3390/molecules28155765>
- 84. Ruggieri, A., Anticoli, S., D'Ambrosio, A., Giordani, L., & Viora, M. (2016).** The influence of sex and gender on immunity, infection and vaccination. *Annali Dell'Istituto Superiore Di Sanita*, 52(2), 198–204. [https://doi.org/10.4415/ANN\\_16\\_02\\_11](https://doi.org/10.4415/ANN_16_02_11)
- 85. Russmann, S., Kullak-Ublick, G. A., & Grattagliano, I. (2009).** Current concepts of mechanisms in drug-induced hepatotoxicity. *Current Medicinal Chemistry*, 16(23), 3041–3053. <https://doi.org/10.2174/092986709788803097>
- 86. Seidle, T., Robinson, S., Holmes, T., Creton, S., Prieto, P., Scheel, J., & Chlebus, M. (2010).** Cross-sector review of drivers and available 3Rs approaches for acute systemic toxicity testing. *Toxicological Sciences*, 116(2), 382–396. <https://doi.org/10.1093/toxsci/kfq143>
- 87. Sevrioukova, I. F., & Poulos, T. L. (2010).** Structure and mechanism of the complex between cytochrome P4503A4 and ritonavir. *Proceedings of the National Academy of Sciences*, 107(43), 18422–18427.
- 88. Silva, A. V., Norinder, U., Liiv, E., Platzack, B., Öberg, M., & Törnqvist, E. (2021).** Associations between clinical signs and pathological findings in toxicity testing. *ALTEX*, 38(2), 198–214. <https://doi.org/10.14573/altex.2003311>
- 89. Skat-Rørdam, J., Lykkesfeldt, J., Gluud, L. L., & Tveden-Nyborg, P. (2025).** Mechanisms of drug induced liver injury. *Cellular and Molecular Life Sciences : CMLS*, 82(1), 213. <https://doi.org/10.1007/s00018-025-05744-3>
- 90. Srinivasan, V. (2012).** Stress hyperglycemia in pediatric critical illness: the intensive care unit adds to the stress! *Journal of Diabetes Science and Technology*, 6(1), 37–47. <https://doi.org/10.1177/193229681200600106>
- 91. Stenn, K. S., & Paus, R. (2001).** Controls of hair follicle cycling. *Physiological Reviews*, 81(1), 449–494. <https://doi.org/10.1152/physrev.2001.81.1.449>

- 92. Suvarna, K. S., Layton, C., & Bancroft, J. D. (2018).** Bancroft's theory and practice of histological techniques E-Book. Elsevier health sciences.
- 93. Tauheed, A. M., Mamman, M., Ahmed, A., et al. (2021).** Acute, sub-acute, sub-chronic and chronic toxicity studies of four important Nigerian ethnomedicinal plants in rats. *Clinical Phytoscience*, 7, 1. <https://doi.org/10.1186/s40816-020-00244-2>
- 94. Tiarti, S. A. R., & Riahi, M. (2021).** Toxicity evaluation: Study of the dose–effect relationship in a professional environment [Master's thesis, University of Oran 2 Mohamed Ben Ahmed]
- 95. Tiwari, V., Shandily, S., Albert, J., Mishra, V., Dikkatwar, M., Singh, R., ... Chand, S. (2025).** Insights into medication-induced liver injury: Understanding and management strategies. *Toxicology Reports*, 14, 101976. <https://doi.org/10.1016/j.toxrep.2025.101976>
- 96. Tron, I., Piquet, O., Baert, A., & Mouton, C. (2002).** Toxon: Manuel de toxicologie. ADEME, Angers .128p.
- 97. Trott, O., & Olson, A. J. (2010).** AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, 31(2), 455–461.
- 98. Vaidya, V. S., Ferguson, M. A., & Bonventre, J. V. (2008).** Biomarkers of acute kidney injury. *Annual Review of Pharmacology and Toxicology*, 48, 463–493. <https://doi.org/10.1146/annurev.pharmtox.48.113006.094615>
- 99. Van der Velpen, V., Teav, T., Gallart-Ayala, H., Mehl, F., Konz, I., Clark, C., ... Popp, J. (2019).** Systemic and central nervous system metabolic alterations in Alzheimer's disease. *Alzheimer's Research & Therapy*, 11(1), 93. <https://doi.org/10.1186/s13195-019-0551-7>
- 100. Van Ravenzwaay, B., Lewis, R. W., Sewell, F., Marty, S., & Botham, P. A. (2026).** Considerations Related to Dose Selection for Regulatory Animal Testing. *Current Opinion in Toxicology*, 100570.
- 101. Vandenberg, L. N., Colborn, T., Hayes, T. B., Heindel, J. J., Jacobs, D. R. J., Lee, D.-H., ... Myers, J. P. (2012).** Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocrine Reviews*, 33(3), 378–455. <https://doi.org/10.1210/er.2011-1050>
- 102. Veber, D. F., Johnson, S. R., Cheng, H.-Y., Smith, B. R., Ward, K. W., & Kopple, K. D. (2002).** Molecular properties that influence the oral bioavailability of drug candidates. *Journal of Medicinal Chemistry*, 45(12), 2615–2623.

- 103. Viau, C., & Tardif, R. (2003).** Toxicologie. In *Environnement et santé publique: Fondements et pratiques* (pp. 119–143)
- 104. Visconti, P., Primiceri, P., Longo, D., et al. (2017).** Photo-ignition process of multiwall carbon nanotubes and ferrocene by continuous wave Xe lamp illumination. *Beilstein Journal of Nanotechnology*, 8, 134–144. <https://doi.org/10.3762/bjnano.8.14>
- 105. Wang, R., Chen, H., Yan, W., Zhang, M., Zhang, T., & Zhang, Y. (2020).** Ferrocene-containing hybrids as potential anticancer agents: Current developments, mechanisms of action and structure-activity relationships. *European Journal of Medicinal Chemistry*, 190, 112109. <https://doi.org/10.1016/j.ejmech.2020.112109>
- 106. Wang, W., Chen, J., Zhan, L., Zou, H., Wang, L., Guo, M., ... Wu, W. (2025).** Iron and ferroptosis in kidney disease: molecular and metabolic mechanisms. *Frontiers in Immunology*, 16, 1531577. <https://doi.org/10.3389/fimmu.2025.1531577>
- 107. Waxman, D. J., & Holloway, M. G. (2009).** Sex differences in the expression of hepatic drug metabolizing enzymes. *Molecular Pharmacology*, 76(2), 215–228. <https://doi.org/10.1124/mol.109.056705>
- 108. Weaver, R. J., Blomme, E. A., Chadwick, A. E., Copple, I. M., Gerets, H. H. J., Goldring, C. E., ... Park, B. K. (2020).** Managing the challenge of drug-induced liver injury: a roadmap for the development and deployment of preclinical predictive models. *Nature Reviews. Drug Discovery*, 19(2), 131–148. <https://doi.org/10.1038/s41573-019-0048-x>
- 109. Woolbright, B. L., & Jaeschke, H. (2018).** Mechanisms of Inflammatory Liver Injury and Drug-Induced Hepatotoxicity. *Current Pharmacology Reports*, 4(5), 346–357. <https://doi.org/10.1007/s40495-018-0147-0>
- 110. Wu, G.-L., Tan, S., Tan, X., Chen, G., & Yang, Q. (2025).** Recent advances in ferrocene-based nanomedicines for enhanced chemodynamic therapy. *Theranostics*, 15(2), 384–407. <https://doi.org/10.7150/thno.101697>
- 111. Zucker, I., & Prendergast, B. J. (2020).** Sex differences in pharmacokinetics predict adverse drug reactions in women. *Biology of Sex Differences*, 11(1), 32. <https://doi.org/10.1186/s13293-020-00308-5>

# **Annexes**

## Annexes01



**Figure01 :** Experimental Rats Used in the Study(Original photo,2026).

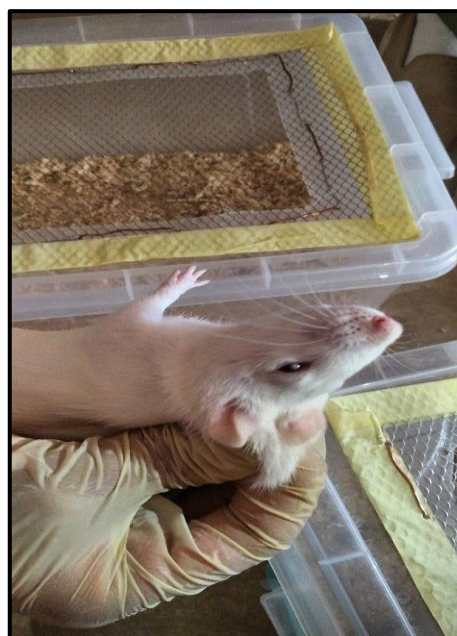


**Figure02:** Early Signs of Symptoms  
Observed During the Monitoring Period  
(Original photo,2026).



**Figure03 :** Housing Cage for  
Experimental Rats  
(Original photo,2026).

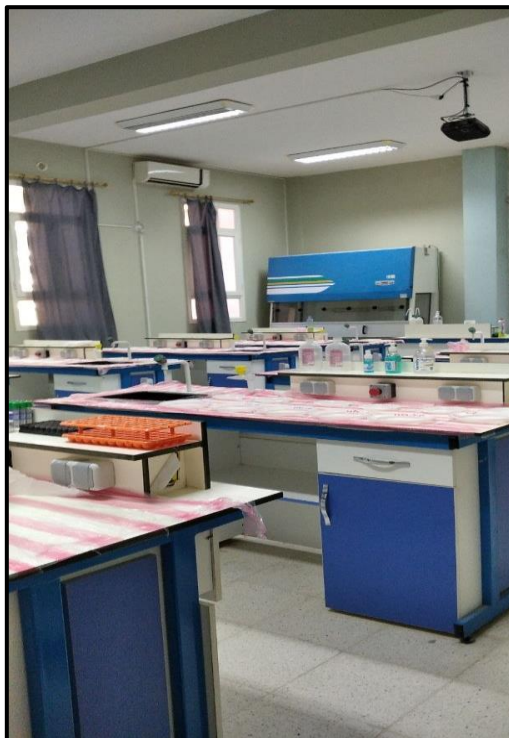


**Figure 04:** Preparation Phase for the Sacrifice Day(Original photo,2026).



**Figure 05:** Post-Sacrifice Experimental Rats(Original photo,2026).



**Figure 06:** Dissection and Organ Collection of Experimental Rats(Original photo,2026).

## Annexes 02

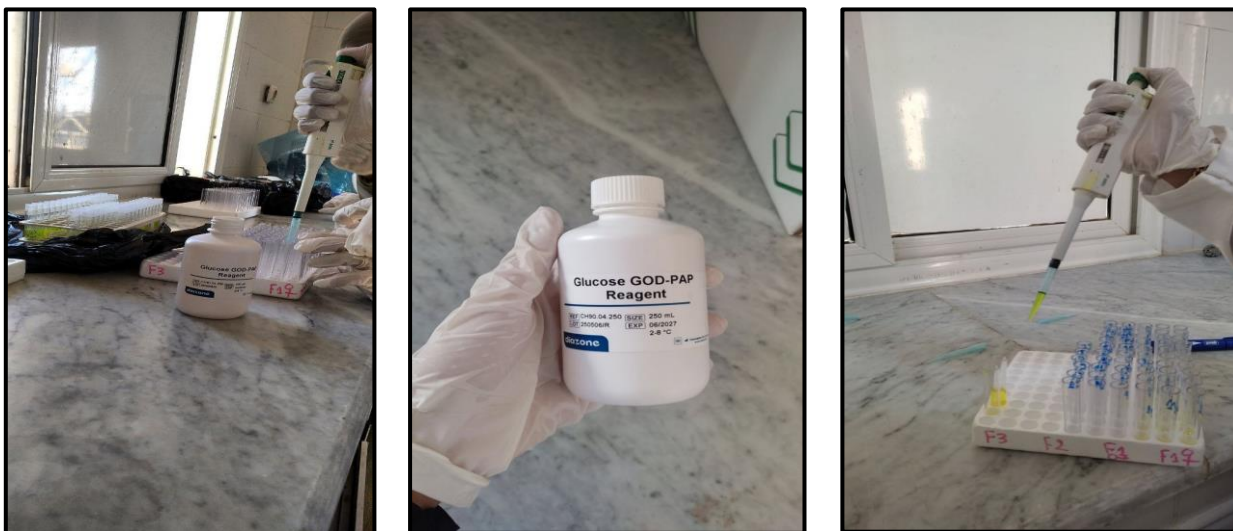


**Figure 07 :**Experimental Approach for Tracking Metabolic Shifts in Toxicological Research(Original photo,2026).

## Annexes 03



**Figure 08 :**Technical Stages for Tissue Processing and Embedding in Histological Preparation(Original photo,2026).

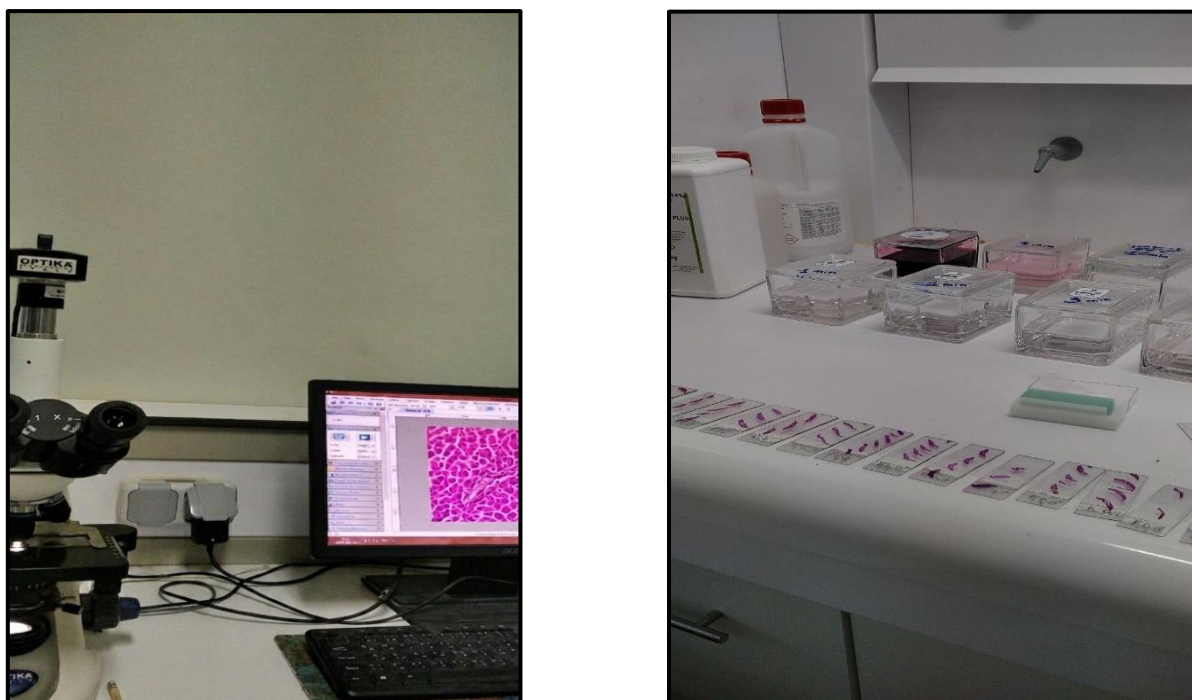


**Figure 09 :**Histopathological Evaluation and Microscopic Examination of Tissue Slides(Original photo,2026).