Open Access Library Journal
2025, Volume 12, e12315
ISSN Online: 2333-9721
ISSN Print: 2333-9705

Cellular and Immunohistochemical Profile Organs of the Mouse Mus Musculus after in Utero Exposure to Antimalarial Drugs (Manalaria® and Kilma® Syrup)

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How to cite this paper: Mbaya, J.K., Lambert, M.S.G., Nsolani, N.M., Kahunu, G.M., Massamba, B.L. and Nsonizau, D.M.P. (2025) Cellular and Immunohistochemical Profile Organs of the Mouse Mus Musculus after in Utero Exposure to Antimalarial Drugs (Manalaria® and Kilma® Syrup). *Open Access Library Journal*, **12**: e12315.
https://doi.org/10.4236/oalib.1112315

Received: September 17, 2024
Accepted: February 10, 2025
Published: February 13, 2025

Abstract

Context: Taking antimalarials during pregnancy, particularly during the first trimester, is regulated by guidelines recommended by the regulatory authority in the Democratic Republic of Congo. Notwithstanding this regulation, it is clear that other antimalarial molecules are used in our environment, such as Manalaria® and Syrup Kilma®, which are not recommended during this gestational period. No visible congenital malformations were detected in Mus musculus mice exposed to these two antimalarials at therapeutic doses during the gestational period. The absence of visible malformations, not constituting sufficient proof of the safety of these molecules, motivated the initiation of the present study, the aim of which is to search for possible cellular and/or molecular anomalies in exposed Mus musculus mice. **Methods**: This is a cross-sectional experimental study involving 6 Mus musculus mice divided into three groups of two, the first group of which was subjected to Manalaria®, the second to Kilma® Syrup and the third which served as a control. Noble organs from each of these mice were removed and histological and immunohistochemical analyses were performed. **Results**: Microscopic examination of specimens taken from exposed mice revealed lesions of hepatic congestion, tubulointerstitial necrosis as well as pulmonary fibrosis. Immunohistochemical analysis, for its part, noted the presence of clusters of inflammatory cells including CD68-positive histiocytes/macrophages, CD20-positive B lymphocytes and a

few small CD3-positive T lymphocytes. **Conclusion**: We have not observed any congenital cellular or molecular abnomalities in Mus musculus mice whose mothers were exposed to phytomedicines. Furthermore, we report the presence of inflammatory lesions not probably linked to these products.

Subject Areas

Gynecology & Obstetrics

Keywords

Embryotocycity, Manalaria®, Mus Musculus, Kilma® Syrup

1. Introduction

Apart from large molecules such as heparin and insulin, the administration of most drugs early in gestation in mammals is always likely to have immediate or distant consequences on the product of conception (embryo or fetus). Due to passage of the placental membrane [1] [2].

As an illustration, we will always remember the congenital malformations observed in the 1960s in thousands of children following the administration of thalidomide to women at the start of pregnancy [2] [3].

Several antimalarials are prescribed to pregnant women with adverse effects and restrictions. Quinine is recommended during pregnancy, although the available evidence is mainly historical (no 1st trimester trial data), poor tolerability (nausea and vomiting) and hypoglycemia are common side effects, particularly in pregnant women [4] [5]. Animal studies have shown that quinine affects brain and inner ear development in rabbits, chinchillas, and guinea pigs at close to or below the therapeutic dose. Quinine caused embryonic death in rabbits, mice, chinchia and dogs at a relatively low dose [6].

Artemisinin combination treatment has common side effects, including nausea, vomiting, and diarrhea, which are also symptoms of malaria itself. Concerns have been raised regarding teratogenicity in early pregnancy following the embryotoxicity of artemisinin (in rats, rabbits and monkeys) at low doses [7] [8].

Sulfadoxine pyrimetamine is an antifolate, contraindicated during the first trimester; lumefantrine, amodiaquine, and piperaquine are considered probably safe, and mefloquine is approved for use in the first trimester of pregnancy (US and UK) [6].

In the Democratic Republic of Congo (DRC), antimalarials are among the medications commonly prescribed and well regulated by the Ministry of Public Health through its regulatory body, the National Malaria Control Program (PNLP). These antimalarials are mostly contraindicated or not tolerated in women during early pregnancy [9]-[12].

In addition to the antimalarials recommended by the PNLP, there are others on the Congolese pharmaceutical market, including Manalaria® and Sirop Kilma®. The

latter obtained marketing authorization without having been subjected to embryotoxicity tests [3]. This made it possible to initiate a study on congenital malformations visible in the Mus musculus mouse experimentally exposed in utero to Manalaria® and Kilma® Syrup which, however, revealed no visible malformation [13].

The absence of visible malformations in these mice exposed in utero in no way excludes the existence of tissue (histological) or molecular abnormalities. Hence the initiation of the present study which aims to research possible cellular or molecular lesions of the noble organs of mice exposed in utero to Manalaria® and Kilma® Syrup.

2. Methods

This is a cross-sectional experimental study on Mus musculus mice exposed in utero to two plant-based antimalarials: Manalaria® and Syrup Kilma®; not previously recommended by the NMCP as antimalarials in pregnant women, particularly at the start of pregnancy. This research was jointly carried out in the Human Embryology and Anatomical Pathology laboratories of the Faculty of Medicine of the University of Kinshasa, in the DRC, during the period from November 2023 to May 2024. We chose the mouse as experimental animal model following the mastery of the manipulation of this model by our work team [13]-[15], but also as an animal model living in the tropical environment where malaria is rife.

With reference to the International Ethical Regulations for Animal Experimentation in Biomedical Research (REIEARB) and standards for laboratory animals, we applied the 3R rule (replacement, reduction and refinement). The replacement was zero on a total of 18 mice from the first line, the reduction brought us to 6 mice and the refinement was done according to sex, only female mice were exposed [14]. Six female mice presenting no visible congenital malformation were thus selected and divided into three distinct groups including two mice exposed in utero to Manalaria®, two others to Kilma® syrup and two final mice not exposed, therefore considered as controls. The mice were immolated, in accordance with the ethical rules for use in laboratories handling animals, after narcosis by inhalation of chloroform vapors for 3 minutes. [15]-[20] After dissection, the following organs were removed from each of the sacrificed mice: lungs, kidneys, liver, stomach, heart and brain, for a total of 8 organs removed per mouse.

The collected specimens were, after identification and conservation in jars containing 10% buffered formalin, sent to the Anatomical Pathology laboratory for histopathological and immunohistochemical analyzes in search of cellular and immunohistochemical lesions. After a careful macroscopic examination (external and internal), each specimen underwent all the different stages of tissue treatment (dehydration, clarification, impregnation in paraffin, inclusion or embedding, microtome sections, staining with hematoxylin and eosin then assembly with Eukitt). The mounted slides were read using a Leica DMRB trinocular optical microscope for the search for histopathological lesions. Concerning the immunohistochemical analysis, sections were made and placed on silanated slides before immunostaining with peroxidase in three layers using the Benchmark GX brand

device. The antibodies listed in Table 1 below were used following the dilutions recommended by the manufacturers.

Table 1. Antibodies used and their dilutions.

Antibody	Firm	Initial form	Dilution
CD3	DAKO	Concentré 1 ml	1/200
CD2O	DAKO	Concentré 0.5 ml	1/100
CD68	Microm	Ready to use (RTU)	1/60

Anti-CD3: for T lymphocytes; Anti-CD20: for B lymphocytes; Anti-68: for histiocytes.

3. Results

3.1. Microscopic Analysis

48 samples were taken from 6 mice exposed in utero to antimalarial products or not. Histological examination of the specimens noted the following results.

In mice exposed to Manalaria®, congestion of the liver parenchyma was mainly observed in one mouse (Figure 1). In mice exposed to Kilma ® syrup, hemorrhagic necrosis of the liver as well as hemorrhagic necrosis of the liver parenchyma were observed in one mouse (Figure 2 and Figure 3). No lesions were observed in mice not exposed to drugs. Microscopic analysis of the brains, lungs and hearts of different mice in all groups did not detect any lesions.

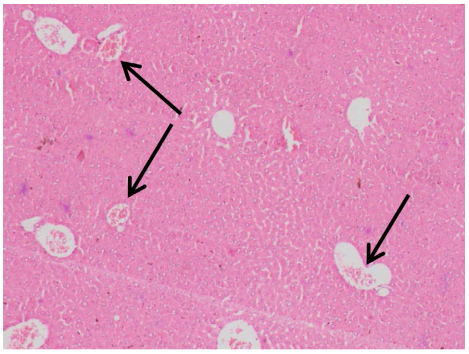

Figure 1. Liver parenchyma marked by passive congestion (black arrows), HE: X 10.

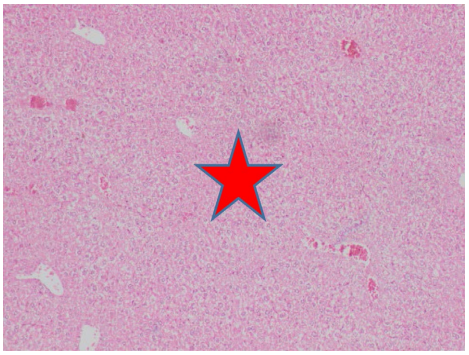

Figure 2. Liver parenchyma marked by hemorrhagic necrosis (red star), HE: X 20.

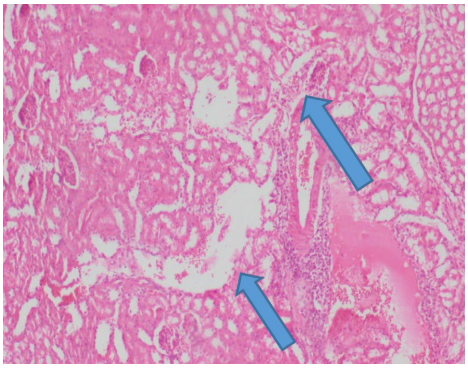

Figure 3. Focus of tubulointerstitial necrosis (blue arrows), HE: X 10.

3.2. Immunohistochemical Analysis

Samples from mice exposed "in utero" to Manalaria ® showed hepatic lesions containing numerous macrophages marked by the anti-CD68 antibody in a single mouse (**Figure 4**). Also, in mice exposed "in utero" to Kilma® Syrup, liver lesions presenting CD20 positive foci were in turn described in a single mouse (**Figure 5**).

In control mice, the presence of numerous small CD3-positive T cells was described in the liver parenchyma and foci of liver damage with CD20-positive lymphoid clusters in one of the mice (**Figure 6**).

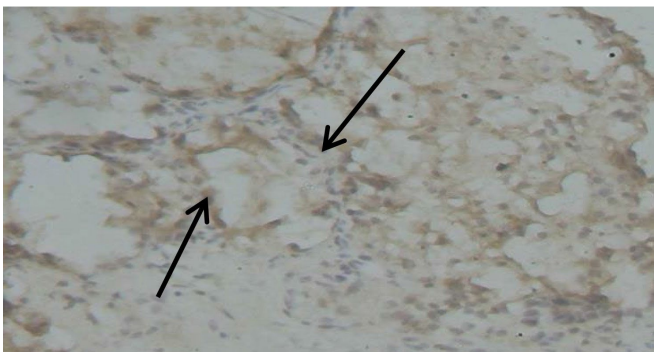

Figure 4. Liver lesions containing numerous CD68-positive macrophages (arrows), IHC: X 20.

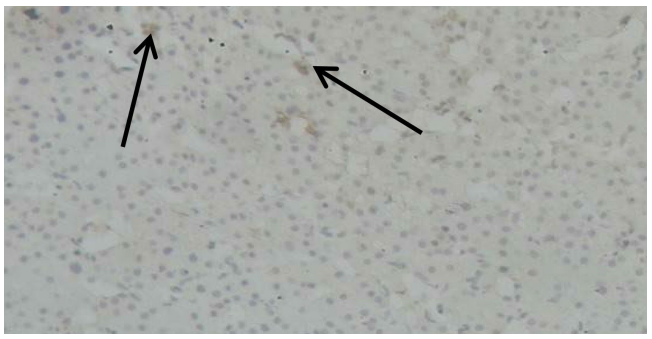

Figure 5. Numerous small CD3-positive T lymphocytes within the liver parenchyma, IHC: X 10.

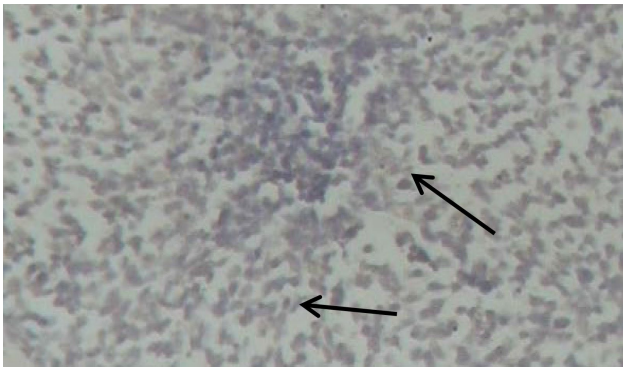

Figure 6. Hepatic lesion foci with CD20 positive lymphoid clusters, IHC: X 10.

4. Discussion

4.1. Microscopic Lesions

A mouse whose mother was exposed to Manalaria® mainly presented passive congestion lesions in the liver parenchyma. The lesion observed in mice whose mothers were exposed to Kilma® Syrup is essentially hemorrhagic necrosis of the liver. Traumatic manipulation of these organs during harvesting could explain the acute inflammatory lesions because these lesions are not present in all mice exposed to the same product [21] [22]. All in all, the lesions observed in our series would not be attributable to this exposure to drugs but are probably linked to the handling of the organs during harvesting.

4.2. Immunohistochemical Analysis

Foci rich in CD20 B lymphocytes in the liver in a mouse exposed to Manalaria® and CD68 positive in a mouse exposed to Kilma® Syrup in the renal interstitium justify the stimulation of B lymphocytes by CD3 positive T lymphocytes. This is indeed the classic pathway for activating humoral immunity which results from the inflammatory phenomenon linked to the handling of mice. Some authors have also described this reaction in mice [23]-[26]. The immunostaining observed on the lesions in our series therefore does not seem to be attributable to the drugs tested.

The results of the present study should be rightly considered; the fact that the mice used were non-transgenic constitutes the limitation. However, this study certainly has the strength of being the first in our field to address the search for possible cellular and molecular lesions after in utero exposure to Manalaria® and Kilma® Syrup.

5. Conclusion

The cellular and molecular lesions observed in our series do not appear to be linked to exposure to Manalaria® and Kilma® Syrup.

Acknowledgements

Merdy Mabiala: For technical support.

Conflicts of Interest

The authors declare no conflicts of interest.

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