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Exploring the Viability of Kerosene and Xylene Blend as a Potential Substitute for Xylene in Histopathology Laboratory as a Clearing Agent: A Comparative Analysis

Running title: "Kerosene-Xylene Mixtures as Clearing Agents in Histopathology"

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Abstract: The use of xylene as a clearing agent in histopathology laboratories is limited by its hazardous nature, potential health risks, and environmental concerns. Kerosene is a potential alternative to xylene that is less toxic, more readily available, and cost-effective. This study investigated the clearing efficacy of kerosene and xylene mixtures in liver and fibroid tissues. The results showed that the clearing efficiency of the mixtures increased with decreasing kerosene concentration, with the best clearing observed in the mixture of 60% kerosene in xylene. These findings suggest that lower concentrations of kerosene in xylene may be used as an effective alternative to absolute xylene in histopathology laboratories.

Keywords: *Histopathology, Clearing Agent, Xylene, Kerosene, Tissue Processing.*

Introduction:

Histopathology is a crucial discipline in medical diagnostics, providing valuable insights into the microscopic characteristics of diseased tissues. In order to examine tissue samples under a microscope, histopathologists employ a series of techniques, including tissue processing, sectioning, staining, and mounting. Among these steps, tissue clearing plays a vital role in enhancing tissue transparency and facilitating accurate microscopic analysis.

Traditionally, xylene has been the most widely used clearing agent in histopathology laboratories (Shanmugam *et al.,* 2017). Xylene effectively removes lipid components from tissue sections, rendering them transparent and allowing light to pass through for improved visualization. However, the use of xylene presents significant challenges, including its hazardous nature, potential health risks to laboratory personnel, and environmental concerns associated with its disposal (Perez-Iratxeta *et al.,* 2018). Moreover, the availability of xylene can be limited in certain regions, leading to increased costs and logistical issues for laboratories.

To address these challenges, researchers and pathologists have been exploring alternative clearing agents that are safer, more readily available, and cost-effective. One such potential substitute is kerosene, a petroleum distillate that shares some chemical properties with xylene. Kerosene has been used in various scientific applications, including as a clearing agent in different fields (Hernandez-Tapia *et al.,* 2021). Its potential as an alternative to xylene in histopathology laboratories has garnered attention due to its lower toxicity, lower cost, and widespread availability.

This study aims to explore the viability of a kerosene and xylene blend as a potential substitute for xylene in histopathology laboratory procedures, specifically as a clearing agent. The research will focus on comparing the clearing efficacy, preservation of cellular morphology, immunohistochemical compatibility, time efficiency, and cost-effectiveness of the kerosene-xylene blend against traditional xylene usage.

Materials and Methods

Study Area

The study was conducted at the Department of Histopathology, University of Maiduguri Teaching Hospital.

Sample Collection and Laboratory Analysis

Samples from normal liver and fibroid tissues were obtained and grossed then fixed in 10% formal saline for 48 hours. The small representative fixed tissues were dehydrated using ascending grades of alcohol (70%, 80%, 90%, and absolute) for 1 hour 30 minutes each. The tissues were then cleared using different concentrations of xylene-kerosene mixture and pure xylene (A = 100ml xylene (Control), B = 80ml kerosene + 20ml xylene, C = 60ml kerosene + 40ml xylene, D = 40ml kerosene + 60ml xylene, E = 20ml kerosene + 80ml xylene, F = 100ml kerosene). The clearing process was carried out for 1 hour 30 minutes for each mixture. The tissues were impregnated in two changes of molten paraffin wax for 1 hour 30 minutes each. The tissues were then embedded in molten paraffin wax using tissue tech embedding molds. The embedded samples were trimmed, and thin sections (3-5 microns) were cut from the tissue blocks using a rotary microtome. The sections were floated in a preheated water bath at 45 degrees Celsius and then dried on a hot plate. The sections were dewaxed in two changes of xylene for 10 minutes each and hydrated in descending grades of alcohol (absolute, 90%, and 70%) briefly. The sections were stained using the Haematoxylin and Eosin staining technique, following the method described by Ochei (2008). After staining, the sections were dehydrated in ascending grades of alcohol (70%, 90%, and absolute) briefly and finally cleared in xylene (A = 100ml xylene (Control), B = 80ml kerosene + 20ml xylene, C = 60ml kerosene + 40ml xylene, D = 40ml kerosene + 60ml xylene, E = 20ml kerosene + 80ml xylene, F = 100ml kerosene respectively). The cleared sections were mounted in DPX mountant. The slides were viewed under a light microscope at 400x magnification. Photomicrographs were taken using a Best Scope camera. The results were compared and presented alongside the control (pure xylene).

Results

The histological sections of two different organs, fibroid and liver tissues, were used in this research to assess their clearing capabilities when treated with Absolute Xylene and various concentrations of xylene-kerosene mixture. The results revealed distinct differences in the clearing efficiency of the two organs based on the concentration of the clearing agents used.

When both liver and fibroid tissues were cleared with 100% xylene, they exhibited a normal general tissue structure and were effectively cleared, serving as the control group (Figure 1). However, when cleared with 100% kerosene, both liver and fibroid tissues displayed unclear structures compared to the control group (Figure 2).

Similarly, when cleared with 80% kerosene in xylene, both liver and fibroid tissues showed unclear structures when compared to the control (Figure 3). However, when cleared with 60% kerosene in xylene, both liver and fibroid tissues exhibited a normal general tissue structure and were effectively cleared, similar to the control group (Figure 4).

Furthermore, when cleared with 40% kerosene in xylene, both liver and fibroid tissues demonstrated a normal general tissue structure and were effectively cleared, comparable to the control group (Figure 5).

Interestingly, when cleared with 20% kerosene in xylene, both liver and fibroid tissues exhibited well-cleared structures when compared to the control group (Figure 6).

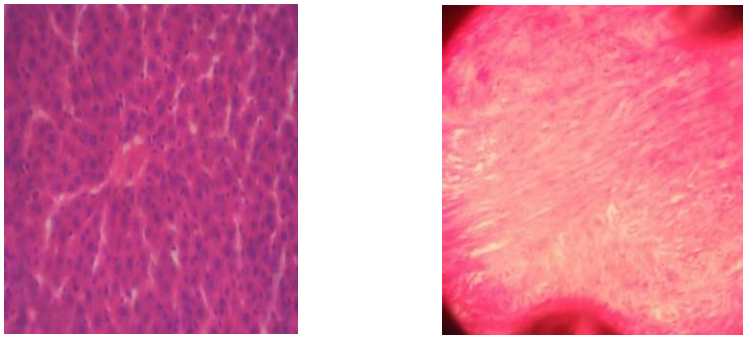

Figure 1: Liver (a) and Fibroid (b) Tissue Cleared with 100% Xylene

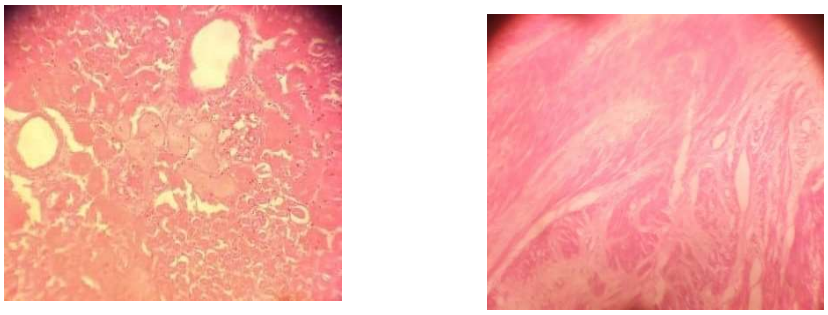

Figure 2: Liver (a) and Fibroid (b) Tissue Cleared with 100% Kerosine

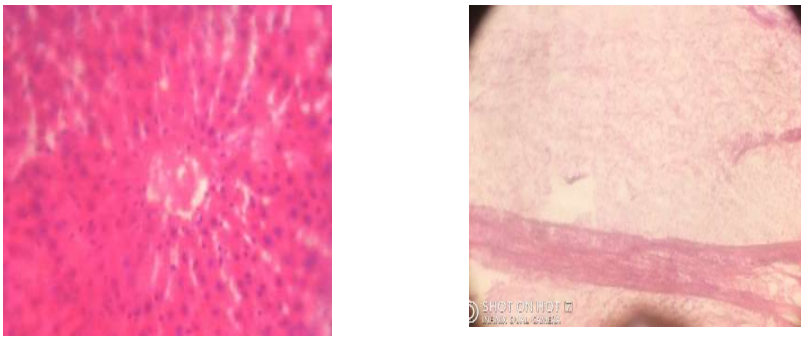

Figure 3: Liver (a) and Fibroid (b) Tissue Cleared with 80% Kerosene-Xylene Blend

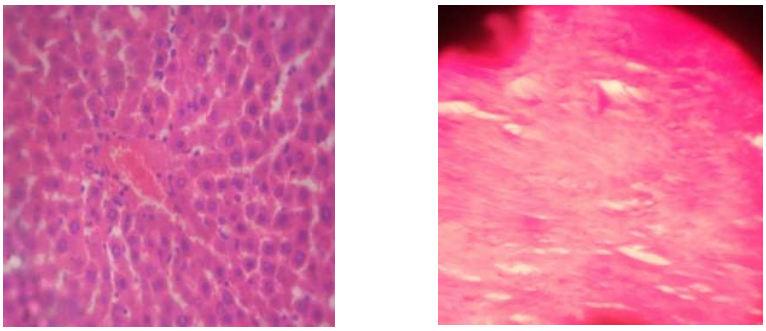

Figure 4: Liver (a) and Fibroid (b) Tissue Cleared with 60% Kerosene-Xylene Blend

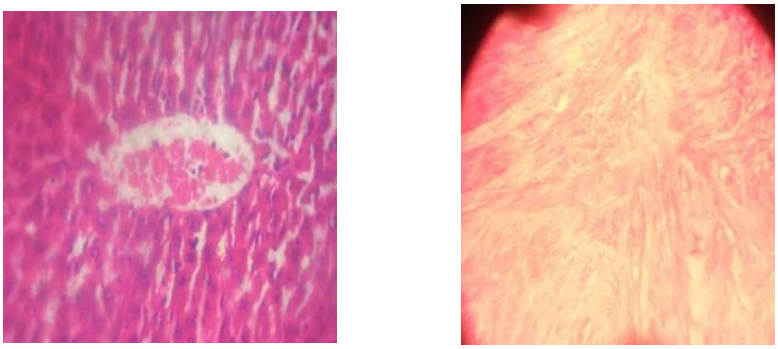

Figure 5: Liver (a) and Fibroid (b) Tissue Cleared with 40% Kerosene-Xylene Blend

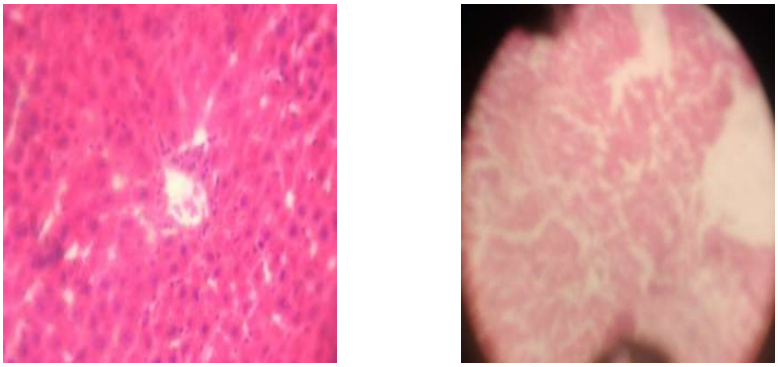

Figure 6: Liver (a) and Fibroid (b) Tissue Cleared with 20% Kerosene-Xylene Blend

Discussion

The histological sections of fibroid and liver tissues were used in this research to assess their clearing capabilities when treated with Absolute Xylene and various concentrations of xylene-kerosene mixture. The results revealed distinct differences in the clearing efficiency of the two organs based on the concentration of the clearing agents used.

From this study, it was observed that better tissue structure demonstration was observed with a decrease in kerosene concentration in xylene, and the best clearing was observed in the mixture of 60% kerosene in xylene. The findings are in line with previous research that reported a similar trend of improved clearing efficiency with lower kerosene concentrations (Amisha *et al.,* 2017).

These results have important implications for the use of xylene-kerosene mixtures as alternative clearing agents in histopathology. Lower concentrations of kerosene in the mixture appear to enhance the tissue clearing process, enabling better visualization of tissue structures. This suggests the potential for developing cost-effective and accessible alternatives to absolute xylene.

Further investigations are warranted to optimize the use of xylene-kerosene mixtures as clearing agents. It is important to assess their compatibility with downstream staining procedures and evaluate their effects on a broader range of tissue types. Additionally, studies comparing the performance of xylene-kerosene mixtures to other commercially available clearing agents would provide valuable insights into their overall efficacy.

In conclusion, this study demonstrates the differential clearing capabilities of liver and fibroid tissues when treated with xylene and various concentrations of xylene-kerosene mixtures. The findings highlight the potential of using lower concentrations of kerosene in xylene as an effective alternative to absolute xylene in histopathology laboratories. Further research and validation are

necessary to establish standardized protocols and ensure the compatibility of these mixtures with routine histopathological procedures.

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