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Exploring Coconut Oil as a Green Substitute for Xylene in Histology Clearing

A Thorough Literature Review on Enhanced Quality Descriptions

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ABSTRACT

Coconut oil, derived from tropical plants, contains steroid compounds, tocopherols, and tocotrienols that can replace alcohol in tissue preparations. These substances are then substituted with solutions that bind effectively to paraffin. The primary objective of this study is to evaluate the quality of histology preparations using natural ingredients, specifically coconut oil and xylene, as alternatives in the clearing process. Utilizing the literature review method, we accessed research journals from Google Scholar and Pubmed databases, focusing on PICO keywords within 2013-2022. The findings indicate that coconut oil can efficiently clear tissue preparations, allowing paraffin to permeate the tissue's pores. This, in turn, enhances the visibility of cellular structures such as the nucleus and cytoplasm when observed under a microscope. The study highlights coconut oil as a credible substitute for xylene, recognizing its ability to produce high-quality histology preparations. Its adoption as an alternative to xylene is attributed to its eco-friendly composition and non-toxic properties, positioning it as a promising choice for advancing histological techniques.

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Introduction

Histology, commonly known as histotechnics, is a scientific discipline dedicated to systematically exploring tissues. This intricate field initiates with a crucial step, namely the

precision sectioning of tissues, strategically designed to isolate and highlight specific organs of interest. The subsequent stages in histotechnics involve a highly meticulous process, transforming these segmented tissues into meticulously prepared specimens. This methodical preparation is undertaken with great care and precision, ensuring that the resulting specimens are optimally suited for comprehensive microscopic examination [1]. The significance of this process lies in its pivotal role in unraveling the intricacies of cellular structures and tissue compositions, ultimately contributing to a deeper understanding of physiological and pathological phenomena. The precision and attention to detail inherent in histotechnics underscore its crucial role in advancing our knowledge of biological tissues and their functions.

The practice of histology preparation is acknowledged as the gold standard in the diagnostic processing of tissue or biopsy specimens. This comprehensive approach encompasses several crucial stages, beginning with fixation, stabilizing tissues, then dehydration, clearing, embedding, and culminating in subsequent sectioning and staining procedures. Each stage is pivotal in ensuring the accurate and detailed analysis of tissue structures and cellular components [2]. This holistic methodological framework underscores the significance of histology preparation in elucidating pathological conditions and contributes to advancing medical diagnostics.

An essential phase within the histopathology preparation process is clearing, a pivotal step involving the removal of the dehydrating agent and substitution with a solution adept at binding to the infiltration media. Traditionally, xylene, an aromatic hydrocarbon possessing flammable characteristics and a distinctive odor, has been the conventional choice for this procedure. Despite its widespread use, concerns arise due to the inherent high toxicity and carcinogenic properties of xylene, posing potential long-term health risks for both individuals and pathologists exposed to it over extended durations [3]. In light of these health considerations, there is a growing exploration of alternative agents, such as coconut oil, which may offer a safer and more environmentally friendly option for the histopathological clearing process.

Coconut oil, derived from coconuts (Cocos nucifera L), offers a promising alternative. Rich in steroid compounds, tocopherols, and tocotrienols, coconut oil possesses non-polar solvents that dissolve alcohol in tissue preparations. This unique property allows it to substitute xylene in the clearing process [4]. Typically obtained from copra through heating and chemical purification, coconut oil's non-polar nature facilitates the dissolution of fat in tissues, opening tissue pores and allowing alcohol to be replaced by paraffin during the infiltration process. This enhances tissue transparency and compactness [5],[6].

Following the clearing stage, the final step in histology preparation involves staining the tissue. Hematoxylin and Eosin (HE) staining is a common technique, where Hematoxylin

imparts a blue color to cell nuclei, and Eosin imparts red hues to the cytoplasm and collagen. This staining process is indispensable for contrasting tissue elements and facilitating microscopic observation [7].

Material and Methods

This research adopts a literature review methodology to acquire a robust theoretical foundation to address the problem formulation inherent in the study. The focus is on implementing an effective search strategy through electronic journals, with keywords tailored to align with the research objectives.

The search strategy involves scouring Google Scholar and Pubmed databases, utilizing a framework pattern or search engine that aligns with the research context. The structured search is guided by the PICO model, with a clear definition of the Problem (clearing), Intervention (coconut oil), Comparison (xylene), and Outcome (tissue processing). This strategic approach ensures a targeted exploration of relevant literature to extract valuable insights for the current study.

The selected journals for review are those accessible in full text, with a preference for research journals that employ experimental methodologies and Hematoxylin Eosin staining techniques. Furthermore, the journal's inclusion criteria encompass a publication range spanning from 2013 to 2022. This deliberate selection process ensures that the literature under scrutiny is comprehensive and up-to-date, contributing to the relevance and reliability of the theoretical basis derived from the literature review.

Results

Following a systematic literature search using the PICO framework pattern, 150 journals were initially identified. After applying rigorous inclusion and exclusion criteria, a refined selection process yielded ten journals aligned with the study's objectives and criteria. The identified literature emphasizes the significance of the clearing stage in histology preparation. Traditionally, xylene is commonly employed in this process to ensure the removal of alcohol from the tissue, allowing subsequent infiltration by paraffin into the tissue pores. This clearing process assumes paramount importance in tissue preparation, as the presence of residual alcohol in the tissue can impede the entry of paraffin, potentially compromising the quality of the final tissue formation during the paraffin process.

The literature also underscores the importance of exploring natural materials for clearing. This shift towards natural materials is motivated not only by their ready availability but also by their environmentally friendly properties. Adopting natural substances in the clearing process aligns with a broader trend toward sustainable and eco-friendly

histopathological practices. These findings from the selected journals contribute to the enriched theoretical basis of the study, shedding light on the potential advantages and considerations associated with using natural materials in the crucial clearing stage of histology preparation.

A. Image quality of histology preparations using coconut oil instead of xylene

The image quality of histology preparations using coconut oil as an alternative to xylene has been explored in various research studies, offering valuable insights into the effectiveness of coconut oil in the clearing process. In the study by Ref. [8], the clarity achieved after the clearing process with coconut oil is comparable to that with xylene. Staining in quality preparations exhibited sufficient clarity, with the cell nucleus appearing blue and the cytoplasm distinctly red, garnering a score of 3, denoting good quality. Fig. 1 compares kidney tissue preparations using both xylene and coconut oil in the clearing stage.

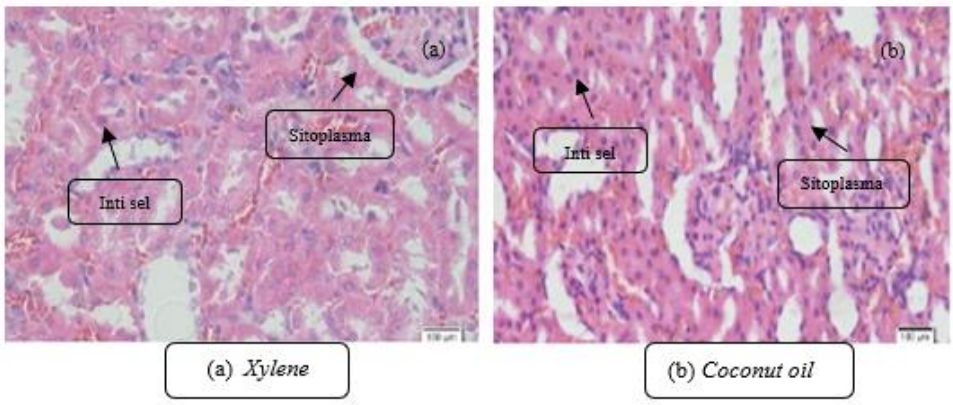


Fig. 1. Comparison of the quality of kidney tissue preparations in the clearing process using (a) xylene and (b) coconut oil [8]

Ref. [9] investigated the quality of the clearing process, reporting a score of 3 for xylene, indicating good clarity in the cell nucleus and cytoplasm. However, coconut oil received a score of two, reflecting less clear staining in the nucleus and cytoplasm, with reduced uniformity in the preparation. Fig. 2 showcases liver tissue preparations at the clearing stage using xylene and coconut oil.

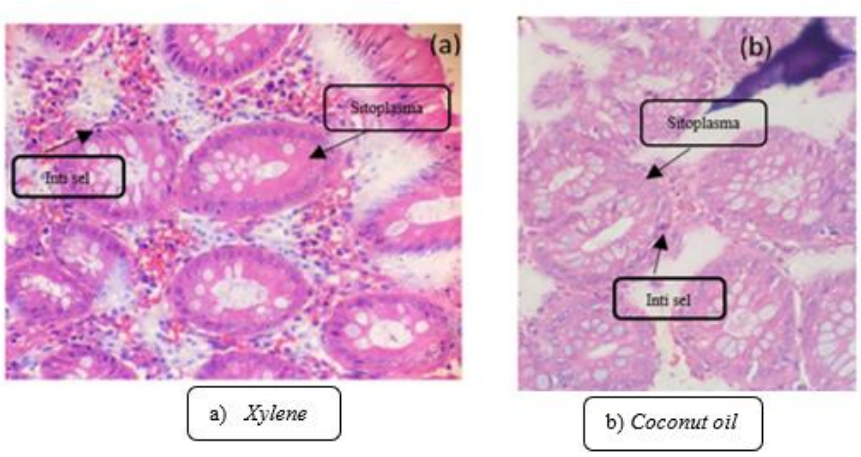


Fig. 2. Quality of liver tissue preparations at the clearing stage using (a) xylene and (b) coconut oil [9]

Ref. [10] highlighted that both xylene and coconut oil treatments resulted in clearly visible morphologies and cell structures, earning a score of 3 for good quality. Fig. 3 illustrates histopathological kidney tissue preparations using xylene and coconut oil in the clearing process.

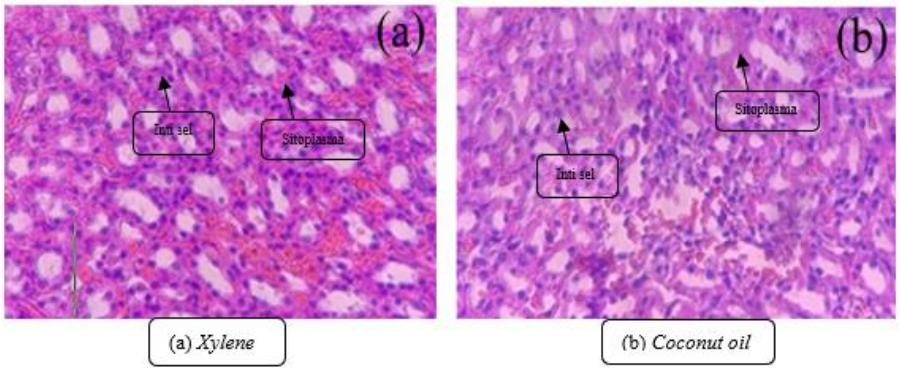


Fig. 3. Quality of histopathological kidney tissue preparations using (a) xylene and (b) coconut oil [10]

In Ref. [11], the image quality after the clearing stage using coconut oil received a score of 3, indicating good visibility of the blue cell nucleus and red cytoplasm, with clear color uniformity. Although xylene preparations displayed slight tissue shrinkage, both methods produced comparable results. Fig. 4 displays fallopian tube tissue preparations stained with HE dye using xylene and coconut oil during the clearing process. These research findings collectively contribute to a nuanced understanding of the image quality achieved by using coconut oil as an alternative to xylene in the clearing stage of histology preparations.

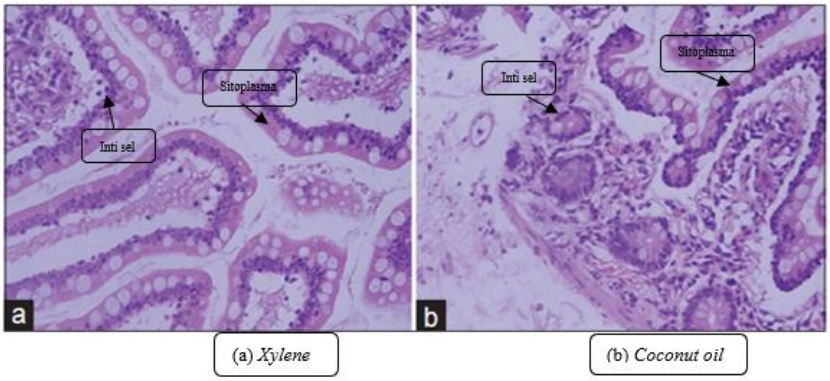


Fig. 4. Preparation of fallopian tube tissue stained with HE dye during the process of clearing (a) xylene and (b) coconut oil [11]

B. Image quality of histology preparations using coconut oil instead of xylene

As defined by Ref. [12], effectiveness refers to the successful accomplishment of goals through optimal use of natural resources. This achievement is evaluated based on the fulfillment of predetermined objectives. In the context of histopathology, assessing the effectiveness of natural materials as substitutes for xylene involves considerations such as the manufacturing procedures of coconut oil for tissue processing, price comparisons between coconut oil and xylene, and the potential risks or impacts on medical laboratory technologists and pathologists when exposed to these reagents over an extended period.

Ref. [13] underscores the importance of pathologists considering the cost and safety of reagents in a histopathology laboratory. The study suggests that the quality of coconut oil as an environmentally friendly clearing agent makes it more cost-efficient than xylene in the Hematoxylin and Eosin (HE) staining procedure. Not only is coconut oil relatively cheaper, but it is also derived from harmless materials, emphasizing the necessity for knowledge about coconut oil as a natural substitute for xylene in histopathology laboratories.

Ref. [14] reveals that coconut oil can replace alcohol and effectively clear tissue, presenting itself as a viable alternative to xylene. Despite the longer soaking time required in the clearing process, the advantages of using natural coconut oil, such as its environmental friendliness and non-toxic properties, outweigh the disadvantages. Tissues subjected to the clearing stage with coconut oil become more transparent, enhancing the visibility of tissue cells.

In the study by Ref. [15], the time required for soaking tissue blocks in the clearing process is compared between xylene and coconut oil. The research indicates that while coconut oil necessitates longer soaking times to achieve optimal results, the effort is directed toward maximizing absorption in the tissue preparation block. The goal is to obtain high-quality

results by effectively removing alcohol and clearing the tissue, making it comparable to tissue preparations using xylene.

A notable challenge in utilizing coconut oil in histological tissue processing is the need for standardization in its production, mainly through centrifugation. Despite the longer processing time compared to xylene, coconut oil is deemed effective as a natural substitute when it can enter tissue pores thoroughly and achieve tissue quality comparable to xylene. The emphasis remains on achieving effectiveness through carefully considering processing methods and the unique properties of natural substitutes like coconut oil.

Several comprehensive studies have consistently highlighted the efficacy of coconut oil as a compelling alternative to the traditional use of xylene in histology preparations. Incorporating natural ingredients, such as coconut oil, demonstrates its potential as an effective clearing agent and presents a promising avenue for minimizing exposure to toxic substances prevalent in xylene. This critical finding is particularly significant for laboratory technicians routinely exposed to these substances, as adopting coconut oil can significantly mitigate the risks associated with long-term exposure to toxic elements like xylene.

The analyses conducted on processed coconut oil further affirm its viability in histology preparations. The processed coconut oil consistently yields commendable results, showcasing its capability to produce high-quality outcomes in the clearing process. These findings underscore the effectiveness of coconut oil as a reliable substitute for xylene. By providing clear and well-defined histological preparations, coconut oil is an environmentally friendly choice and a safer option for laboratory personnel, addressing the imperative need for safer alternatives in histopathology laboratories.

In essence, the collective evidence from these studies reinforces that when appropriately processed and utilized, coconut oil stands out as an effective and safe alternative to xylene in the clearing process. Its ability to deliver optimal histological results and the potential to reduce exposure to harmful substances position coconut oil as a valuable and sustainable option that aligns with the growing emphasis on safety, health, and environmental considerations in laboratory practices.

Conclusion

Upon scrutinizing ten journal articles, the findings of this study affirm that coconut oil, assessed for preparation quality, exhibits remarkable clarity with a vivid blue cell nucleus and distinct red cytoplasm—this unique feature positions coconut oil as a promising natural clearing agent for histopathological examinations. The discernible advantage of using natural materials lies in their effectiveness as substitutes for xylene, which is attributed to their non-

toxic and environmentally friendly composition. Embracing such eco-friendly alternatives reflects a progressive shift towards sustainable practices within laboratory protocols.

The analysis of results in this study, focusing on the quality description of histology preparations employing coconut oil as a replacement for xylene in the clearing process, underscores the need for further experimental research. Specifically, exploring the clearing process with coconut oil in conjunction with other natural ingredients and employing different soaking times presents an avenue for comprehensive investigation. This experimental approach aims to unravel nuanced insights into natural substitutes' potential variations, optimizations, and broader applicability, thereby contributing to a more comprehensive understanding of their efficacy in histopathological procedures.

Conflict of Interest

The authors declare that there is no conflict of interest.

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