International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2025: 7.089

Comparative Study of Efficiency of Coconut Oil and Xylene as Deparaffinization Agent in Histopathological Tissue Sections

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Abstract: ***Background: Xylene is the most commonly used deparaffinizing agent in routine histopathology; however, its toxic effects have prompted the search for safer alternatives. Coconut oil has been proposed as a biodegradable and eco-friendly substitute. Aim: To evaluate the efficacy of coconut oil as a deparaffinizing agent for routine hematoxylin and eosin (H&E) staining and compare its performance with xylene. Materials and Methods: This comparative study included 100 histopathological specimens. Two serial 4-µm sections were prepared from each specimen. One section was deparaffinized using xylene and the other using coconut oil. Stained sections were evaluated by a blinded pathologist for nuclear staining, cytoplasmic staining, staining uniformity, wax retention, and overall histological appearance. Results: Xylene demonstrated superior staining quality in all evaluated parameters. Good nuclear and cytoplasmic staining was observed in 98% of xylene-treated sections compared with 68% of coconut oil-treated sections. Uniform staining was achieved in 99% versus 66%, satisfactory wax removal in 100% versus 60%, and good overall histological appearance in 100% versus 30% of sections processed with xylene and coconut oil, respectively. Conclusion: Xylene produced superior staining quality compared with coconut oil for routine H&E staining. Although coconut oil is a safer and environmentally friendly alternative, it did not provide staining quality comparable to xylene under the conditions employed in this study. Further studies are required to optimize its use as a potential deparaffinizing agent.***

Keywords: Coconut oil; Xylene; Deparaffinization; Hematoxylin and eosin staining; Histopathology; Tissue processing

1. Introduction

Histopathological examination remains the gold standard for the diagnosis of a wide spectrum of diseases, and the quality of tissue processing plays a vital role in ensuring accurate microscopic interpretation. Hematoxylin and eosin (H&E) staining is the most commonly employed staining technique because it provides excellent visualization of tissue architecture, nuclear morphology, and cytoplasmic details. Effective deparaffinization is an essential prerequisite for successful H&E staining, as incomplete removal of paraffin wax adversely affects stain penetration and compromises histomorphological evaluation.[1,2]

Xylene has long been used as the standard clearing and deparaffinizing agent in histopathology because of its excellent ability to dissolve paraffin wax. However, prolonged occupational exposure to xylene is associated with several adverse health effects, including irritation of the skin, eyes, and respiratory tract, as well as neurological, hepatic, and renal toxicity. In addition, its volatility and environmental hazards have prompted increasing interest in identifying safer and eco-friendly alternatives for routine laboratory use.[3]

Several substitutes, including vegetable oils, cedarwood oil, olive oil, UltraClear™, liquid dishwashing solution, and diluted lemon water, have been investigated as alternatives to xylene. Although many of these agents have demonstrated promising results, none has consistently matched the effectiveness of xylene under routine laboratory conditions, and further validation is required before their widespread adoption.[4,5]

Coconut oil is a biodegradable, inexpensive, readily available, and relatively non-toxic natural product with favorable physicochemical properties. Previous studies have evaluated its use as a deparaffinizing agent and reported variable findings regarding staining quality and tissue morphology, highlighting the need for further investigation under standardized laboratory conditions.[6,7]

Therefore, the present study was undertaken to evaluate the efficacy of coconut oil as a clearing/deparaffinizing agent for routine H&E staining and compare its performance with xylene. The study also aimed to determine whether coconut oil could serve as a safer alternative to xylene by comparing nuclear staining, cytoplasmic staining, staining uniformity, wax retention, and overall histological appearance.

Aim

To evaluate the efficacy of coconut oil as a deparaffinizing/clearing agent for routine hematoxylin and eosin (H&E) staining and compare its performance with xylene.

Objectives

1) To compare the staining quality of tissue sections deparaffinized with coconut oil and xylene based on nuclear staining, cytoplasmic staining, staining uniformity, wax retention, and overall histological appearance.

2) To assess whether coconut oil can serve as a safer alternative to xylene for routine histopathological deparaffinization

2. Review of Literature

Criteria for choosing good deparaffinizing agent: -

- Fast tissue penetration
- Minimal tissue injury
- Quick elimination of the dehydrating agent
- Minimal toxicity
- Low flammability
- Low cost

In the year of 2021 a study was conducted by Snega Thamilselvan et al to evaluate the efficacy of cedarwood oil as a substitute of xylene in clearing process of histopathology using tissue samples from oral squamous cell carcinoma resection specimens collected at the department of Oral Pathology and Microbiology from Saveetha Dental College and Hospitals. 50 tissue samples total were used in the investigation and they were split into two groups. Of these, 25 tissue specimens (Group A) underwent processing using cedarwood oil as a xylene substitute, while 25 tissue specimens (Group B) underwent regular xylene processing. Based on the various tissue types—such as skin, muscle, adipose tissue, glands and mucosa— each group was divided into five subgroups, each containing five tissue specimens. Initially, the chosen tissue samples were dehydrated for 30 minutes each using 1 change of 99.9% 2-isopropyl alcohol and 2 changes of acetone. Following dehydration, 100 millilitres of cedarwood oil solution were used to clean the tissues. The 99.9% pure cedarwood oil was bought from the neighbourhood commercial organic market. To prevent the production of crystalline cedrol, 95 millilitres of cedarwood oil were mixed with 5 millilitres of xylene. A little amount of thymol crystals, which are a bactericidal component of botanical thyme oil, were also added. The tissue specimens cleansed with xylene were exposed to 40°–45° for 30 minutes (2 changes), whereas the tissues were cleared in cedarwood oil solution for an entire night at room temperature (37°–39°). This study demonstrates that in routine histopathological tissue processing, cedarwood oil's cleaning capacity is comparable to that of xylene. This will assist us in keeping the histopathology laboratory atmosphere conducive to good health. Even though we have very encouraging results, more research is needed, ideally with a bigger sample size. [8]

A study was carried out by BIRGITTE BRUUN RASMUSSEN et al, on the topic of Vegetable oils instead of xylene in tissue processing in the year of 1992 at Department of Pathology, Roskilde County Hospital, Roskilde, Denmark. Two tissue blocks were processed in parallel in xylene and vegetable oil from each of the 232 specimens that were sent for histopathologic assessment. The samples showed a wide range of tissue types. To enable the assessment of histologic and cytologic details, Hematoxylin-eosin was applied to all sections. Additionally, a subset of tissue slices was stained using a variety of histochemical and immunohistochemical methods. The samples treated with vegetable oil were deemed equally suitable for histologic diagnosis in all cases. No changes were observed between the immunohistochemical and histochemical staining. Though it's unclear how stable vegetable oil-processed tissue will be in the long run.[9]

In the year of 2017 a project on Honey and olive oil was carried out by Keerthi muddana at el, the purpose of this investigation was to determine whether olive oil and honey might be used in place of xylene and formalin, respectively. A total of thirty 1-2 cm routine biopsy samples were obtained. The study group was divided into Group A and Group B. Group A received standard processing treatment. After being fixed in honey for twenty-four hours, Group B underwent standard processing and was thereafter submerged in olive oil rather than xylene. Hematoxylin and eosin staining will be used as the standard method for all the sections. After the experiments they conclude that honey preservation of tissue yields better results than formalin preservation. It was discovered that olive oil worked better as a clearing agent than xylene.[10]

A study was carried out by Viswanathan Prema et al in 2020 at Department of Oral and Maxillofacial Pathology, KSR Institute of Dental Science and Research, Tiruchengode, Tamil Nadu, India. Where 15 tissue blocks with paraffin embedded were chosen. Every block was divided into four pieces. Group A underwent standard H&E staining using xylene as the deparaffinizing agent, while Group B, C, and D had xylene-free H & E staining using 1.7% dishwashing solution, 95% lemon water and 100% coconut oil, respectively. One pathologist graded the slides in a blind manner, taking into account factors such wax retention, uniformity, crispness, and clarity of staining, as well as nuclear and cytoplasmic staining. It was concluded that 100% coconut oil, 95% diluted lemon water and 1.5% dishwashing solution can all be utilised as efficient substitutes for xylene in standard H&E staining procedures. They also came with the added benefits of being disposable, inexpensive, safe, easy to handle and non-hazardous. Our findings need to be confirmed by larger sample size investigations.[11]

Another study was conducted by Wajid Sermadi et al, at Department of Dentistry, Chamrajnagar Institute of Medical Sciences, Chamrajnagar, India in 2014, For this 20 investigation, a total of 60 tissue specimens were taken. Every specimen was divided into two equal parts. The tissue bit was processed in two parts: one half at a time in xylene and the other half consecutively in coconut oil. For both solutions, the clearing time remained consistent at one hour each (two alterations). To assess for shrinking, the tissue pieces were measured both before and after cleaning. Additionally, the specimens were examined for noticeable alterations during cleaning and de-alcoholization. Hematoxylin and eosin (H and E) was applied to each section to enable examination of the histological features. A small number of the sections (salivary gland specimens) were additionally exposed to periodic acid Schiff (PAS) to determine if coconut oil was causing interference with this often used special staining technique. Utilising the advanced method of computer assisted morphometry, morphological characteristics such as mean cell area were observed to determine whether there were any consistent changes between the study groups. And their study's findings suggest that coconut oil is a good replacement for xylene because it is less expensive, less dangerous and causes less tissue shrinking. Without sacrificing the quality of the histological details, it can be

utilised as a de-paraffinizing agent in the histopathology laboratory. To determine which xylene substitute is superior, all of the alternatives must be carefully considered. To determine whether coconut oil is a more effective and secure alternative to xylene, more research in this field is anticipated. The specimen that has been treated with coconut oil will likely be exposed to various stains and sophisticated histology techniques, such as immunohistochemistry.[12]

Problem Statement

This study was design to evaluate the efficacy of coconut oil as deparaffinizing agent, as xylene are widely used chemicals with highly toxic properties. Some studies indicate that dishwashing solutions work best, others suggest that coconut oil works best and yet others indicate more research is necessary to validate the findings. Furthermore, no research has been done on this topic in the Bathinda sector, and the conclusions of any studies that have been done are unclear. Thus, the goal of my investigation is to obtain the most accurate data and insights possible.

3. Materials and Methods

Study design and setting: This comparative observational study was conducted in the Department of Pathology, Adesh Institute of Medical Sciences and Research, Bathinda, India. A total of 100 histopathological tissue specimens received for routine diagnostic evaluation were included in the study.

Study population: After routine tissue processing, two serial sections of 4 µm thickness were obtained from each paraffin-embedded tissue block. One section from each specimen was processed using xylene as the deparaffinizing agent (control group), while the corresponding serial section was processed using coconut oil (study group). Thus, a total of 200 tissue sections derived from 100 specimens were evaluated.

Inclusion and exclusion criteria: Viable histopathological tissue specimens measuring ≥5 mm were included in the study. Small biopsy specimens measuring <5 mm and tissues with inadequate morphology or processing artifacts were excluded.

Deparaffinization and H&E staining: Following sectioning, tissue sections were mounted on adhesive-coated glass slides and placed on a hot plate to facilitate melting of paraffin wax.

For the xylene group, slides were deparaffinized in two changes of xylene for 15 minutes each at room temperature. The sections were subsequently rehydrated through descending grades of alcohol (100%, 70%, and 50%), rinsed in distilled water, stained with Harris hematoxylin for 8 minutes, differentiated with 1% hydrochloric acid, blued under running tap water for 15 minutes, counterstained with 1% eosin for 2 minutes, dehydrated through ascending grades of alcohol, cleared in xylene, and mounted using DPX with a coverslip.

For the coconut oil group, slides were deparaffinized in two changes of coconut oil maintained at 90°C for 15 minutes each. The remaining staining procedure, including rehydration, hematoxylin and eosin staining, differentiation, dehydration, and mounting, was performed using the same protocol as the xylene group, except that the slides were air-dried before mounting without xylene clearing.

Evaluation of staining quality

All stained slides were examined under light microscopy by a pathologist blinded to the deparaffinization method. The staining quality was assessed using the following five parameters:

1) Nuclear staining
2) Cytoplasmic staining
3) Uniformity of staining
4) Wax retention
5) Overall histological appearance

Each parameter was graded using a binary scoring system, where 0 indicated poor staining quality and 1 indicated good staining quality.

4. Results

A total of 100 histopathological specimens were included in the study. Two serial sections were prepared from each specimen, with one section processed using xylene and the other using coconut oil as the deparaffinizing agent. All stained sections were evaluated independently by a blinded pathologist using five histological parameters: nuclear staining, cytoplasmic staining, staining uniformity, wax retention, and overall histological appearance.

Table 1: Comparison of nuclear staining between xylene and coconut oil

Nuclear staining	Xylene, n (%)	Coconut oil, n (%)
Good	98 (98.0)	68 (68.0)
Poor	2 (2.0)	32 (32.0)
Total	100 (100.0)	100 (100.0)

Good nuclear staining was observed in 98% of tissue sections processed with xylene compared to 68% of those processed with coconut oil.

Table 2: Comparison of cytoplasmic staining between xylene and coconut oil

Cytoplasmic staining	Xylene, n (%)	Coconut oil, n (%)
Good	98 (98.0)	68 (68.0)
Poor	2 (2.0)	32 (32.0)
Total	100 (100.0)	100 (100.0)

Xylene demonstrated superior cytoplasmic staining, with good staining observed in 98% of sections, whereas only 68% of coconut oil-treated sections showed good cytoplasmic staining.

Table 3: Comparison of staining uniformity between xylene and coconut oil

Uniformity of staining	Xylene, n (%)	Coconut oil, n (%)
Good	99 (99.0)	66 (66.0)
Poor	1 (1.0)	34 (34.0)
Total	100 (100.0)	100 (100.0)

Uniform staining was observed in 99% of xylene-treated sections compared with 66% of coconut oil-treated sections.

Table 4: Comparison of wax retention between xylene and coconut oil

Wax retention	Xylene, n (%)	Coconut oil, n (%)
Good	100 (100.0)	60 (60.0)
Poor	0 (0.0)	40 (40.0)
Total	100 (100.0)	100 (100.0)

Complete wax removal was achieved in all sections processed with xylene, whereas satisfactory wax removal was observed in only 60% of sections processed with coconut oil.

Table 5: Comparison of overall histological appearance between xylene and coconut oil

Overall histological appearance	Xylene, n (%)	Coconut oil, n (%)
Good	100 (100.0)	30 (30.0)
Poor	0 (0.0)	70 (70.0)
Total	100 (100.0)	100 (100.0)

All tissue sections processed with xylene demonstrated good overall histological appearance, whereas only 30% of sections processed with coconut oil achieved good overall histological quality.

5. Discussion

Xylene has long been the standard deparaffinizing agent in histopathology because of its excellent wax-dissolving properties and compatibility with routine hematoxylin and eosin (H&E) staining. However, its toxic effects and environmental hazards have prompted the search for safer alternatives.[2,4] Coconut oil has gained attention as a biodegradable, inexpensive, and non-toxic substitute with potential application in histopathology.[6,7]

In the present study, xylene demonstrated superior staining quality compared with coconut oil for all evaluated parameters. Good nuclear and cytoplasmic staining was observed in 98% of xylene-treated sections compared with 68% of coconut oil-treated sections. Similarly, xylene showed better staining uniformity (99% vs. 66%), wax retention (100% vs. 60%), and overall histological appearance (100% vs. 30%). These findings indicate that coconut oil was less effective as a deparaffinizing agent under the conditions employed in this study. The inferior performance of coconut oil may be attributed to incomplete paraffin removal, leading to residual wax that interfered with stain penetration and adversely affected tissue morphology. Efficient deparaffinization is essential for obtaining optimal nuclear and cytoplasmic staining and preserving histological details required for accurate diagnosis.[1,2]

Our findings differ from those reported by Sermadi et al., who observed staining quality comparable to xylene using coconut oil as a clearing agent.[7] Similarly, Prema et al. reported that coconut oil could be used as a biofriendly substitute for xylene during routine H&E staining.[6] The differences between these studies and the present findings may be due to variations in sample size, tissue type, deparaffinization temperature, processing protocols, and evaluation criteria.

Although coconut oil offers several advantages, including low toxicity, affordability, and environmental safety, these benefits cannot outweigh compromised staining quality in routine diagnostic practice. Accurate histopathological diagnosis depends on complete deparaffinization and optimal preservation of tissue morphology. Therefore, based on the findings of the present study, coconut oil cannot be recommended as a routine substitute for xylene under the protocol employed. Nevertheless, further studies using modified deparaffinization protocols, different grades of coconut oil, and larger multicentric samples are warranted to explore its potential application.[8]

6. Conclusion

The present study demonstrated that xylene produced superior staining quality compared with coconut oil for all evaluated parameters, including nuclear staining, cytoplasmic staining, staining uniformity, wax retention, and overall histological appearance. Although coconut oil is a biodegradable, inexpensive, and safer alternative with minimal health and environmental hazards, it did not provide staining quality comparable to xylene under the conditions employed in this study. Therefore, coconut oil cannot be recommended as a routine substitute for xylene for deparaffinization in H&E staining. Further studies with optimized deparaffinization protocols, different grades of coconut oil, and larger multicentric samples are warranted to explore its potential as an alternative deparaffinizing agent in histopathology laboratories.

Conflict of Interest: NIL

Funding: NIL

Prior publication: Nil

Support: Nil

Conflicts of interest: Nil

Encl: Research approval and Ethical approval

Contributor’s form signed by all the contributors

Source of support: Nil

Presentation at meeting, Conflicting Interest: Nil

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Proof of ethical and research aprovals:

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Office of Dean Academic Affairs

Ref. No. : AU/DAA/10/2023/FA/1324 **Dated : 16-10-2023**

To

The Principal
Adesh Institute of Medical Sciences & Research
Adesh University, Bathinda

Sub: Final approval of Research Protocols.

1. The Research Protocols of the M.Sc. Students has been approved as per the recommendations of the AIMSR Research Committee (ARC), Adesh Institute of Medical Sciences & Research, Adesh University and Ethics Committee of Biomedical & Health Research, Adesh University Bathinda, duly endorsed by Dean Post Graduate Studies & Research, vide letter no. AU/DPGS&R/10/2023/465 dated 10th October 2023 as per the following details:

S. No	Title of the Research Protocol	Principal Investigator	Supervisor	Co-supervisor
1.	Evaluation of Diagnostic Efficacy of Anti-CCP Antibody in comparison to ESR and CRP in diagnosis of Rheumatoid Arthritis	Japjot Singh M.Sc. Student Medical Biochemistry AIMSR	Dr. Kapil Gupta Professor Biochemistry, AIMSR	Dr. Nitin Bansal Prof & Head Orthopaedics, AIMSR
2.	Prevalence of HCV in Intravenous Drug Users in a tertiary care hospital	Kunal Arora M.Sc. Student Medical Microbiology AIMSR	Dr. Aditi Goyal Associate Professor Microbiology, AIMSR	Dr. Gurmeet Kaur Prof & Head Psychiatry, AIMSR
3.	Comparative study of efficacy of coconut oil and Xylene as Deparaffinizing agent in histopathological tissue sections	Prakash Chakraborty M.Sc. Student Histopathology AIMSR	Dr Arun Puri Prof & Head Pathology, AIMSR	Dr Navtej Singh Professor Pathology, AIMSR

2. They are advised to follow Standard Operating Procedures (SOPs) of Adesh University for conducting research and its publications and instructions issued by AIMSR Research Committee (ARC), Adesh Institute of Medical Sciences & Research, Adesh University and Ethics Committee of Biomedical & Health Research, Adesh University while approving their research protocol.
3. One approved copy of above mentioned Research Protocols is enclosed herewith.
4. The Principal Investigator and Supervisor of Principal Investigator may please be informed accordingly.

for Adesh University

16/10/23

Dean Academic Affairs

Encl: As above (03 approved copies of Research Protocols)

Copy to:

- Dean Post Graduate Studies & Research, Adesh University : for office record
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ETHICS COMMITTEE FOR BIOMEDICAL & HEALTH RESEARCH(EC_BHR)

Ref No: AU/EC_BHR/2K23/491 Date:- 30/9/23

Name of the Ethics Committee: Ethics Committee for Biomedical & Health Research, Adesh University, Bathinda

Title of the Research Protocol: Comparative Study of efficacy of Coconut oil and Xylene as Deparaffinizing agent in histopathological tissue sections

Principal Investigator Prakash Chakraborty, M.Sc. Histopathology, Adesh Institute of Medical Sciences & Research (AIMSR) Bathinda

Supervisor: Dr. Arun Puri, Prof. & Head Pathology, AIMSR Bathinda

Co- Supervisor Dr. Navtej Singh, Professor Pathology, AIMSR Bathinda

1. The Research Protocol was received through Member Secretary, AIMSR Research Committee for Ethical Approval vide Ref No: AIMSR/MC/Estt/08/2K23/995 Dated : 16. 08. 2023
2. Proposal was reviewed in a meeting held on 28th August, 2023
3. Hard copies of protocol was received and approved against the respective department letter vide AIMSR/Path/2023/9/906 on 26. 09. 2023
4. **Resolution:** Research Protocol was approved for carrying out the study. However, Principal Investigator will inform the Committee about the progress report of study every six months and when the study is completed, whichever is earlier.

Date of Approval: 30th September 2023

Dr. Mandeep Kaur
Member Secretary
Ethics Committee for Biomedical & Health Research
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