Original Article

Kerosene as an Alternative to Xylene in Histopathological Tissue Processing and Staining: An Experimental Study

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Abstract

Background: Conventional tissue processing is as old as 100 years and still remains the gold standard. Tissue processing involves many steps, of which one of the important steps is clearing. Xylene is one of the common clearing agents used in laboratory, but it is carcinogenic and teratogenic. **Aim:** The aim of this study was to substitute conventionally used xylene with kerosene in tissue processing and staining. **Materials and Methods:** Thirty bits of chicken tissue samples were collected; each was randomly separated into two groups: tissue processing and staining. Instead of conventional xylene, we used kerosene. The tissue blocks were subjected to sectioning and staining, and finally, they were observed under light microscope. **Results:** Tissue samples that were processed and cleared with kerosene showed equal clearing and staining without any alterations of the tissue morphology and cellular details with that of xylene. **Conclusion:** Kerosene can be used as a substitute to xylene without posing any health risk or compromising the cellular integrity.

Keywords: *Cellular Integrity, clearing agent, kerosene, xylene*

Introduction

Conventional tissue processing and staining forms the backbone of daily pathological diagnostic work. Tissue processing is a physical process that involves chemical solutions reacting with biological specimens.[1] The main aim of tissue processing is to embed the tissue in solid medium, so that it is firm enough to support the tissue and give it sufficient rigidity to enable thin sections to be cut, and yet, soft enough not to damage the knife or tissue. Next to tissue processing, staining plays a major role in laboratories.[2] Hematoxylin and eosin (H&E) stain is the most commonly used stain for the paraffin sections, and it remains to be a gold standard technique for routine diagnostic work.[3]

Both in tissue processing and in H&E staining, one of the important components used is xylene, which is used to carry out the intermediate steps of clearing, deparaffinization, rehydration, and dehydration of tissue sections during the processing and staining.[1] Xylene is an aromatic hydrocarbon compound that is widely used in industry and medical technology as a solvent. It is a colorless, pungent liquid or a gas occurring naturally in petroleum, coal, and wood tar. It is so named because it is found in crude wood spirit (Greek: *xýlon*, meaning wood). In dentistry, xylene is used in histological laboratories for tissue processing, staining, and cover slipping, and also in endodontic retreatment as a gutta-percha solvent. Its high solvency factor allows maximum displacement of alcohol and provides the tissue its transparency, which improves paraffin penetration.[4] In staining procedures, xylene poses excellent dewaxing and clearing capabilities so that it contributes to brilliantly stained slides.[5]

Inhalation of xylene vapor causes depression of the central nervous system with symptoms such as

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headache, dizziness, nausea, and vomiting.[3] Prolonged contact with xylene and its vapor may cause irritability, insomnia, agitation, extreme tiredness, tremors, impaired concentration, and deterioration in memory. Acute neurotoxicity, heart and kidney pathologies, some fatal blood dyscrasia, anemia, skin erythema, and drying and scaling of skin are caused by the inhalation of xylene.[4] Toxic effects of xylene are due to the generation of reactive oxygen species and ATP depletion.[6]

Considering the earlier discussed facts, it is clear that it is better to replace xylene completely from laboratory procedures. Substitution is finding of an alternate substance that can carry out same function and that may minimize the hazard. Kerosene is a flammable hydrocarbon liquid. The word "kerosene," derived from Greek *keros*, meaning wax, is usually called paraffin in the United Kingdom. It is a thin clear liquid formed from hydrocarbon, with a density of 0.78–0.81 g/cm^3. It is obtained from fractional distillation of petroleum between 150°C and 275°C.[7]

Thus, the present study was aimed to substitute kerosene for xylene as a clearing agent in tissue processing and staining and to observe its effect on the tissue morphology and staining characteristics during routine H&E staining.

Materials and Methods

The present study was performed in the Department of Oral Pathology and Microbiology, Vivekanandha Dental College for Women, Tiruchengode, Tamil Nadu, India, using 30 bits of chicken tissue samples, which were divided into two groups (group A and group B). Two sections of 4 µm thickness were made. One section of each sample was processed with kerosene and stained with H&E, and in the staining procedure, it was cleared with kerosene instead of xylene [Tables 1 and 2]. The other section was routinely processed with xylene and stained with H&E under the standard protocol and cleared with xylene. The stained slides were observed under light microscope for nuclear morphology, cytoplasmic morphology, uniformity of staining, and crispness of staining, and the parameters were graded as good/fair/poor. Two independent observers evaluated all the slides to reduce the subjective bias. The scores for each slide were totaled. A score of ≤2 was graded as inadequate for diagnosis. Slides with a score of 3–5 were assigned as adequate for diagnosis. Chi-square test was used for statistical analysis (SPSS software, version 16).

Table 1: Tissue processing procedure using kerosene

Procedure	Group A (kerosene)
Dehydration	
50% alcohol	1 hour
70% alcohol	1 hour
90% alcohol	1 hour
100% alcohol	Overnight
Clearing	
Kerosene I	2 hour
Kerosene II	2 hour

Results

After tissue processing, both the tissues of group A (kerosene) and group B (xylene) were in good shape without any form of shrinkage or depression in embedding paraffin wax. Almost both the groups were similar in their morphology without any alterations. These tissues were embedded in paraffin wax and subjected for sectioning. 4 micron thickness sections were made which were subjected for further staining procedures using H and E. The sections were observed in light microscope under ×10 and ×20 magnification. The results revealed that group A showed better nuclear and cytoplasmic morphology, clarity, uniformity, and crispness of staining, which was statistically significant with a *P* value of 0.001 [Figure 1 and Table 3], whereas group B produced good nuclear and cytoplasmic morphology, clarity, uniformity, and crispness of staining results when compared to kerosene with a *P* value of 0.393, which was statistically insignificant [Figure 2 and Table 4]. While comparing the parameters of the sections processed and cleared using xylene and kerosene better results were seen with xylene

Table 2: H&E staining procedure using kerosene as a clearing agent

Procedure	Group B (xylene)
Kerosene I	20 minutes
Kerosene II	20 minutes
100% alcohol	2 minutes
90% alcohol	2 minutes
70% alcohol	2 minutes
50% alcohol	2 minutes
Hematoxylin	3 minutes
50% alcohol	2 minutes
70% alcohol	2 minutes
90% alcohol	2 minutes
95% alcohol	2 minutes
95% alcohol	2 minutes
Eosin	2 minutes
95% alcohol	2 minutes
95% alcohol	2 minutes
100% alcohol	2 minutes
Kerosene I	4 minutes
Kerosene II	4 minutes

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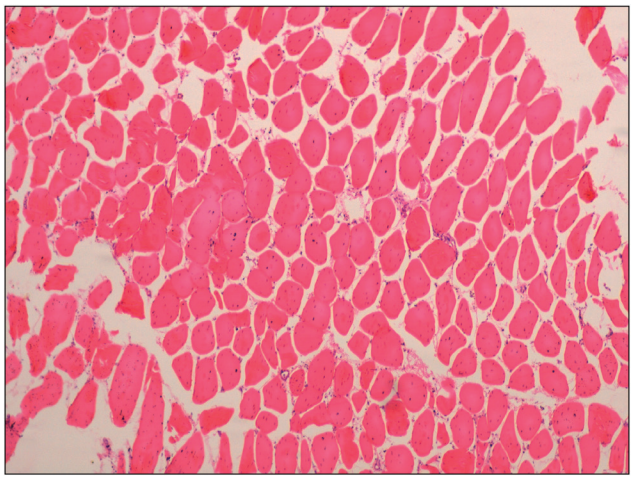


Figure 1: Photomicrograph of sections cleared with kerosene under x20 magnification

Table 3: Comparison of parameters under group A (kerosene)

Parameters	Good	Fair	Poor	Total	Chi-square	*P*
Block tissues after sectioning	10		5	15	30.14	0.001**
Nuclear morphology	8	7		15		
Cytoplasmic morphology	9		6	15		
Uniformity of staining	11		4	15		
Crispness of staining	8	7		15		
Total	46	14	13	75		

**Highly significant

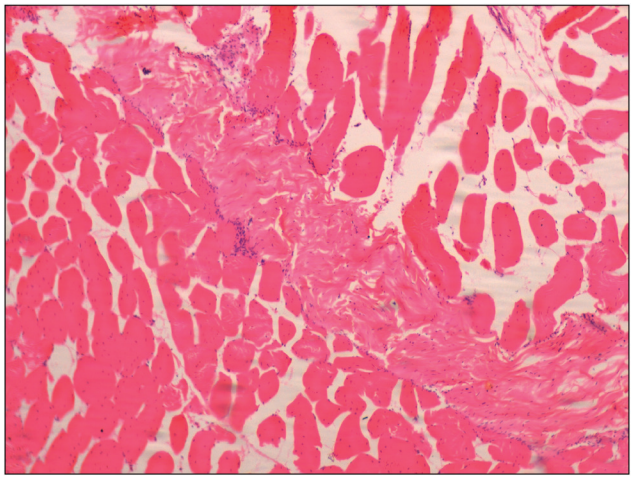


Figure 2: Photomicrograph of sections cleared with xylene under x20 magnification

(GROUP B) than kerosene (GROUP A) and they were statistically significant with a p value of 0.001 [Table 5]. But the morphology and the staining characteristics did not vary between both the groups. Thus, kerosene can be used as an alternative to xylene without modifying the tissue morphology and staining characteristics, with an added benefit of reduced toxicity.

Table 4: Comparison of parameters under group B (xylene)

Parameters	Good	Fair	Poor	Total	Chi-square	*P*
Block tissues after sectioning	14		1	15	4.10	0.393
Nuclear morphology	13		2	15		
Cytoplasmic morphology	14		1	15		
Uniformity of staining	12		3	15		
Crispness of staining	15		0	15		
Total	68	0	7	75		

Table 5: Comparison between kerosene and xylene

Group	Good	Fair	Poor	Total	Chi-square	*P*
Group A (kerosene)	46	14	15	75	170.88	0.001**
Group B (xylene)	68	0	7	75		
Total	114	14	22	150		

**Highly significant

Discussion

Regardless of the known toxicity of xylene, it is extensively used in histopathology laboratory devoid of any monitoring for the exposure and any standardized technique for disposal.[8] Various xylene substitutes were tried to shun xylene because of its toxic effects. However, during H&E staining procedure, xylene has been commonly used as a clearing agent of choice, in spite of its toxicity to people working in the laboratory and the risk it possesses to the atmosphere. Besides, xylene is also reported to have many flaws, such as being inflammable and volatile. Hence, xylene is considered to be a toxic substance as a clearing agent in histology.[9]

In this study, we used kerosene as a safe alternative to xylene in tissue processing and staining without altering the tissue morphology and staining characteristics. In a study conducted by Shah *et al.*,[7] mixtures of kerosene and xylene were used in different ratios (70:30) and (50:50) with a time period of 2 hours for tissue processing. But still the presence of xylene renders harmful side effects. In our study, we used 100% of kerosene for tissue processing. However, the results were contrary to those of Shah *et al.*[7] Our study showed no changes in the morphology without any shrinkage in both group A and group B. We observed that increasing the clearing time in kerosene improves the quality of sectioning and also clearing.

Similarly in case of staining, Shah *et al.*[7] observed that mixture of kerosene and xylene in the ratio of 50:50 and 70:30 showed good nuclear and cytoplasmic morphology, clarity, uniformity, and crispness of staining. On the contrary, in our study, kerosene showed better nuclear and cytoplasmic morphology, clarity, uniformity, and crispness of staining. However, xylene showed good results when compared with kerosene. But by increasing the staining time of kerosene, it produces better nuclear, cytoplasmic morphology, and staining characteristics.

It can be inferred by the aforementioned findings that kerosene can be used as a safe alternative to xylene without altering the morphology of the tissue and the staining characteristics.

Conclusion

Considering the toxicity of xylene, it is essential to reduce its use in histopathology laboratory without compromising the staining quality and consequently the proper diagnosis. Our study shows that kerosene is equivalent to xylene in routine tissue processing and H&E staining. Therefore, without losing the quality of the histological details, kerosene can be used in the histopathological laboratory as an alternative to xylene. This will help us in maintaining a healthy and nontoxic laboratory environment. Even though we have significant positive results in our hands, additional studies are mandatory including larger human samples and with multiple observers.

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Conflicts of interest

There are no conflicts of interest.

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