Original Article

# Kerosene: Contributing agent to xylene as a clearing agent in tissue processing

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## Abstract

**Background:** Research methodology in oral and maxillofacial pathology has illimitable potential. The tissue processing involves many steps of which one of the most important step is "Clearing," which is a process of replacing dehydrant with a substance which is miscible with embedding medium or paraffin wax. Xylene is one of the common clearing agents used in laboratory, but it is also hazardous. The main aim of this study is to substitute conventionally used xylene by a mixture of kerosene and xylene in clearing steps without altering the morphology and staining characteristics of tissue sections. This will also minimize the toxic effects and tend to be more economical.

**Materials and Methods:** One hundred and twenty bits of tissue samples were collected, each randomly separated into 4 groups (A, B, C and D) and kept for routine tissue processing till the step of clearing; during the step of clearing instead of conventional xylene, we used mixture of xylene and kerosene in 4 ratios ([A-K:X – 50:50]; [B-K:X – 70:30]; [C – Ab. Kerosene]; [D – Ab. Xylene – as control]) and observed for the light microscopic study adopting H and E staining, IHC (D2-40), Special stains (periodic acid–Schiff and congo red) procedure. The result was subjected to statistical analysis by using Fisher's exact test.

**Results:** The results obtained from the present study were compared with control group, i.e., D and it was observed that Groups A and B were absolutely cleared without altering the morphology of tissue and cellular details; optimum embedding characteristics and better staining characteristics were also noted, whereas Group C presents poor staining characteristics with reduced cellular details. Embedded tissues in Group C presented with rough, irregular surface and also appeared shrunken.

**Conclusion:** Combined mixture of xylene and kerosene as a clearing agent in different ratio, i.e., Group A (K:X – 50:50) and B (K:X – 70:30) can be used without posing any health risk or compromising the cellular integrity.

**Keywords:** Kerosene, staining, tissue processing, xylene

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## INTRODUCTION

Conventional tissue processing is as old as 100 years and it still remains the gold standard against which all technologies and methods need to be assessed.[1] Tissue processing is a physical process that involves chemical solutions reacting with biological specimens.[2] The main purpose of tissue processing

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is to embed the tissue in solid medium, 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.[3]

For any tissue specimen to undergo diagnosis, it has to follow few procedural steps, as follows:

Fixation – It is defined as a process by which the constituents of cells and therefore, of the tissues are fixed in a chemical and partly also in physical state so that they will withstand subsequent treatment with various reagents with a minimum of loss, significant distortion or decomposition and keep the tissue in as lifelike manner as possible. There are many types of fixatives such as (a) simple fixative; (b) compound fixative; (c) microanatomical fixative; (d) cytological fixative; and (e) histochemical fixative; of which the most commonly used fixative is 10% neutral-buffered formalin.[4,5] Dehydration – It is the process by which the water content in the tissue is removed. This is usually done with increasing grades of alcohols such as 70%, 80%, 90% and absolute alcohol.[6] Other dehydrants which can be used are acetone and dioxane. Clearing – The use of clearing agent is necessary as the dehydrating agent is not miscible with the impregnating medium. The term clearing denotes the fact that they have similar refractive index as that of proteins.[2] The change in the appearance of tissue indicates effectiveness of clearing process.[7] Infiltration – After clearing, tissues are transferred to molten paraffin wax in an embedding oven from infiltration and impregnation. During this process, the clearing agent is eliminated from the tissue by diffusion and molten wax is infiltrated. The wax, which has diffused or infiltrated into the tissue after replacing the clearing agent, gets deposited in the tissues. Commonly used impregnating and embedding media is "Paraffin wax."[3]

## Clearing

Following dehydration, the tissues are saturated with alcohol, precluding infiltration with paraffin, which is immiscible with alcohol. At this point in the process, "clearing agents" are used because of their ability to mix with alcohols and to dissolve paraffin. Clearants are more properly called as "dealcoholization agents" or "ante medium."[6-8]

## Xylene

The most commonly used clearing agent is xylene. It is used for both tissue processing and staining. The historical use of xylene in histology laboratory is an example of failed substitution.[4,5]

Since 1950s, it is used as a safest alternative to dangerous chemicals such as aniline oil, benzene and chloroform. By the late 1970s, there was great concern about its safety with evidence that its acute neurotoxicity was greater than benzene and toluene. In addition to advantages of xylene as a clearing, it equally shares the drawbacks also, as shown in Table 1.[9-12]

Besides its drawbacks, still, xylene is used as one of the most commonly used clearing agents as it does not alter the tissue morphology and maintains the good staining quality. Advantages and disadvantages of xylene as a clearing agent are summarized in Table 2.[8-11]

## General applications

It is primarily used as a solvent in the printing, in rubber and leather industries and along with other solvents is widely used as a cleaning agent, thinner for paint and in varnishes, found in small amounts in airplane fuel and gasoline.[8-10]

## Medical applications

It can be used as a clearing agent both in tissue processing and staining.[9,10]

To minimize the toxic effects of xylene, the following preventive measures can be taken:[12,13]

**Table 1: The toxicity of xylene**

| Organs | Effects |
|---|---|
| Nervous system | Depression of CNS, headache, dizziness, nausea and vomiting |
| Eyes, nose and throat | Accidental splash in the eye may damage surface eye, produces effects on nose and throat |
| Lungs | Chest pain and shortness of breath, pulmonary edema (potentially life-threatening condition) |
| Liver and kidney | Injure the liver and kidney |
| Blood | Anemia due to contamination of xylene with benzene |
| GIT | Nausea, vomiting and gastric discomfort |
| Musculoskeletal system | Reduced grasping power, reduced muscle power in extremities |
| Skin | Irritation of skin, dermatitis, dryness, flaking and cracking |
| Cancer | Cancer may develop |
| Reproductive system | Teratogenic (Fetotoxic) effects |

GIT: Gastrointestinal tract, CNS: Central nervous system

**Table 2: An overview of advantages and disadvantages of xylene in histopathology laboratory**

| Advantages | Disadvantages |
|---|---|
| Rapid in action | Inflammable |
| Possible to determine the end point with some accuracy | Vapor is an irritant, may cause skin erythema, drying and scaling and secondary infections |
| | Acute neurotoxicity |
| | Fatal blood dyscrasias |
| | Teratogenic |
| | Long-term immersion of the tissue, results in tissue distortion |

- Substitution
- Local exhaust ventilation
- Proper protective equipment (PPE)
- Substitution is finding of alternate substance that can perform same function and which may lessen the hazard. The following alternatives for xylene are suggested:
    1. Toluene – works well, is more tolerant of small amounts of water left in the tissues, does not harden the tissue, but is 3 times more expensive than xylene; Methyl salicylates – rarely used as it is more expensive and has a nice smell; Propylene oxide – most common clearing agent in the EM technique; Chloroform – good tolerance rate, tissues can be left overnight without rendering them unduly brittle; less shrinkage of tissue, noninflammable but is slow in action[14-16]
    2. Kerosene – It is a combustible hydrocarbon liquid, name derived from Greek – Keros - wax. Is usually called paraffin in the UK. Is a thin clear liquid formed from hydrocarbon, with density of 0.78 – 0.81 g/cm.$^3$ Is obtained from fractional distillation of petroleum between 150 and 275°C.[17] Uses of kerosene are discussed as shown in Table 3.[2,17,18]

Despite its widespread use, it has certain advantages with a few disadvantages which are discussed as shown in Table 4.[17,18]

- Local exhaust ventilation

Workplace can be modified to reduce the inhalational hazards by installing local exhaust ventilation with a proper hood.[13] The local exhaust ventilation is very effective; it removes the contaminant rather than diluting it. It should be in a fixed position, located close to the source of the hazard and have five key components.[13]

- A fan or a blower that provides enough negative air pressure to drawn in contaminated air
- A hood that allows the effective capture of the contaminant
- A system of ducts that transport the contaminated air away from the workplace
- An air cleaning device that removes the contaminants from the air
- A source of makeup air that replaces the air removed from the workplace.

- PPE.

Personal hygiene practices and protective equipment reduce the amount of a substance that is absorbed by the worker's body after he/she has been exposed to it and also prevent hazardous chemicals from being carried home. They include thorough hand washing and removing outer protective clothing before entering clean areas; usage of impervious clothing; a face mask or full face organic respirator to reduce the inhalational hazards; safety goggles/face shields for eye protection and periodic medical examination and biologic monitoring to detect worker's exposure to xylene.[8,13]

Considering the above-discussed facts, it is clear that it is not justified to replace xylene completely as a clearing agent. To minimize the toxic effects of xylene, the present study was aimed for adding a contributing agent like kerosene to xylene as a clearing agent in different proportions and to observe its effect on the tissue morphology and staining characteristics.

**Table 3: Applications of kerosene**

| General applications | Medical applications |
|---|---|
| As a cooking fuel | As a clearing agent in contribution with xylene |
| To prevent mosquitoes from breeding in pools of standing water | In X-ray crystallography, used to store crystals |
| Used as solvent, as a thread cutting and reaming lubricant | |

**Table 4: Overview of advantages and disadvantages of kerosene**

| Advantages | Disadvantages |
|---|---|
| Is considered as noncarcinogenic and nonteratogenic (Khattak *et al.*, 1999; Risher and Rhodes, 1995) | Most common health effect associated with chronic or repeated kerosene exposure is dermatitis (Ritchie *et al.*, 2003) |
| Acute toxic handling and using kerosene are minimal, provided that the products are used in accordance with the current safety practices (Henry, 1998) | |

## MATERIALS AND METHODS

### Preparation and mixture of solvents

- The two compounds, i. e., kerosene and xylene were mixed in the following proportion
- Kerosene (50%): Xylene (50%); Kerosene (70%): Xylene (30%); Absolute Kerosene (100%); Absolute Xylene (100%).

### Experimental design and setting

*Preparation and mixture of solvents*

- A total of 120 soft tissue samples were selected measuring approximately 1.0–1.5 cm in size. The samples were randomly divided into 4 different groups as – A, B, C and D consisting of 30 each. A combination of kerosene and xylene is used in different combinations as a clearing agent.

*Groups*

- Group A – Kerosene (50%): Xylene (50%); Group B – Kerosene (70%): Xylene (30%);

Group C – Absolute Kerosene (100%); Group D – Absolute Xylene (control group) [Figure 1 a-d].

### Modified tissue processing

Tissues were randomly separated into 4 groups. Each tissue samples were kept for routine tissue processing till 5 changes of alcohol (70, 80, 90 and 2 changes of abs. Alcohol). During the process of clearing (except for Group D), instead of conventional xylene, 2 changes of mixture of kerosene and xylene were used in the ratio of Group A – 50:50; Group B – 70:30; and Group C – 100 kerosene for 2 h.

### Histological procedure

All the above-mentioned groups [Group A, B, C and D] underwent steps of tissue processing, which included dehydration through graded ethanol, clearing [tissue sections were cleared in Group A (K-50: X-50), B (K-70: X-30) and C (Ab. K) while control tissue sections were cleared in Group D (Ab. X) for two hours] and infiltration in paraffin wax for about 2 h at 56°C and embedding of the tissues in paraffin wax was done. All the histological procedures were carried out under same laboratory conditions. Sections were obtained on a rotary microtome at 4 μm thickness and were subjected to Hematoxylin and Eosin (H and E) staining, IHC marker D2-40 and also few special stains such as periodic acid–Schiff (PAS) and congo red.

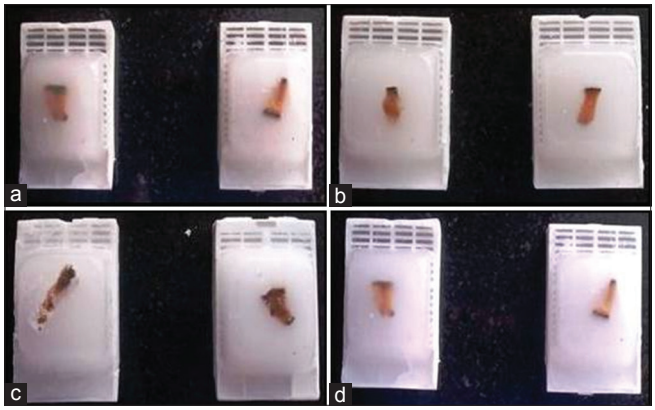



**Figure 1:** (a-d) – Photographs showing block tissue and sectioning (Group A – [K:X – 50:50]; Group B [K:X – 70:30]; Group C [Abs. Kerosene]; Group D [Abs. Xylene])

## RESULTS

### Block tissue and sectioning

Block tissue and sectioning – the embedded blocks of tissue in Group A, B, C and D were carefully observed and analyzed. The tissues with Group A, B and D were properly embedded and in good shape without any form of shrinkage or depression in embedding paraffin wax. However, in Group C, the tissues were opaque in appearance, shrinked or depressed in the embedding paraffin wax and which also affected the proper sectioning of tissues and also there is observation of wrinkling or folding of tissue sections while cutting with microtome [Figure 1a-d, Table 5 and Graph 1].

### Histological specimens

The sections were observed in light microscope under ×10 and ×40. The results revealed that Group A- Figure 2a-d and Group B- Figure 3a-d and Group D- Figure 4a-d showed good nuclear and cytoplasmic morphology, clarity, uniformity and crispness of staining [Tables 6-10 and Graphs 2-6] whereas for the Group C Figure 5a-d, i.e., Abs. kerosene failed to produce good results when compared to the rest of the groups. The sections of Group A were also subjected to IHC staining for D2-40 [Figure 6], and few special stains such as PAS and congo red [Figure 7] procedure which also presented clarity of staining. By these results, we would like to suggest that as

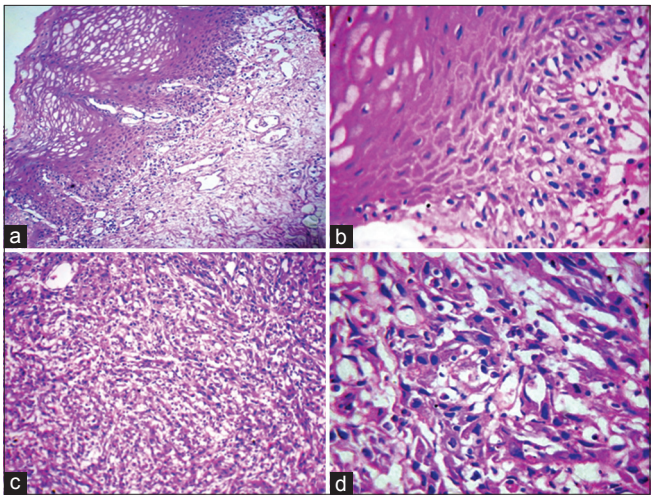



**Figure 2:** (a-d) – Photograph of Group A (cleared in 50 ml kerosene and 50 ml xylene) H&E, showing epithelium and connective tissue (A, C – ×10 and B, D – ×40)

**Table 5: Block tissues after sectioning in Groups A, B, C and D**

| Block tissues after sectioning | Group A K:X - 50:50 | Group B K:X - 70:30 | Group C Absolute kerosene | Group D Absolute xylene | Total | *P* | Significance | Test used |
|---|---|---|---|---|---|---|---|---|
| Good (%) | 28 (93.3) | 23 (76.6) | 6 (20) | 28 (93.3) | | <0.001 | Significant | Fisher's exact |
| Poor (%) | 2 (6.67) | 7 (23.) | 24 (80) | 2 (6.67) | | | | |
| Total (%) | 30 (100) | 30 (100) | 30 (100) | 30 (100) | 120 | | | |

**Table 6: Adequacy of nuclear morphology in Groups A, B, C and D**

| Nuclear morphology | Group A K:X - 50:50 | Group B K:X – 70:30 | Group C Absolute kerosene | Group D Absolute xylene | Total | *P* | Significance | Test used |
|---|---|---|---|---|---|---|---|---|
| Adequate (%) | 29 (96.7) | 20 (66.67) | 15 (50) | 29 (96.7) | | <0.001 | Significant | Fisher's exact |
| Inadequate (%) | 1 (3.3) | 10 (33.33) | 15 (50) | 1 (3.3) | | | | |
| Total (%) | 30 (100) | 30 (100) | 30 (100) | 30 (100) | 120 | | | |

**Table 7: Adequacy of cytoplasmic morphology in Groups A, B, C and D**

| Cytoplasmic morphology | Group A K:X - 50:50 | Group B K:X - 70:30 | Group C Absolute kerosene | Group D Absolute xylene | Total | *P* | Significance | Test used |
|---|---|---|---|---|---|---|---|---|
| Adequate (%) | 29 (96.7) | 19 (63.33) | 15 (50) | 29 (96.7) | | <0.001 | Significant | Fisher's exact |
| Inadequate (%) | 1 (3.3) | 11 (33.33) | 15 (50) | 1 (3.3) | | | | |
| Total (%) | 30 (100) | 30 (100) | 30 (100) | 30 (100) | 120 | | | |

**Table 8: Clarity of staining in Groups A, B, C and D**

| Clarity of staining | Group A K:X - 50:50 | Group B K:X - 70:30 | Group C Absolute kerosene | Group D Absolute xylene | Total | *P* | Significance | Test used |
|---|---|---|---|---|---|---|---|---|
| Present (%) | 29 (96.7) | 18 (60) | 12 (40.00) | 29 (96.7) | | <0.001 | Significant | Fisher's exact |
| Absent (%) | 1 (3.3) | 12 (40) | 18 (60.00) | 1 (3.3) | | | | |
| Total (%) | 30 (100) | 30 (100) | 30 (100) | 30 (100) | 120 | | | |

**Table 9: Uniformity of staining in Groups A, B, C and D**

| Uniformity of staining | Group A K:X - 50:50 | Group B K:X - 70:30 | Group C Absolute kerosene | Group D Absolute xylene | Total | *P* | Significance | Test used |
|---|---|---|---|---|---|---|---|---|
| Present (%) | 29 (96.7) | 18 (60.0) | 8 (26.66) | 29 (96.7) | | <0.001 | Significant | Fisher's exact |
| Absent (%) | 1 (3.3) | 12 (40.0) | 22 (73.3) | 1 (3.3) | | | | |
| Total (%) | 30 (100) | 30 (100) | 30 (100) | 30 (100) | 120 | | | |

**Table 10: Crispness of staining in Groups A, B, C and D**

| Crispness of staining | Group A K:X - 50:50 | Group B K:X - 70:30 | Group C Absolute kerosene | Group D Absolute xylene | Total | *P* | Significance | Test used |
|---|---|---|---|---|---|---|---|---|
| Present (%) | 29 (96.7) | 16 (53.3) | 7 (23.3) | 29 (96.7) | | <0.01 | Significant | Fisher's exact |
| Absent (%) | 1 (3.3) | 14 (46.6) | 23 (76.6) | 1 (3.3) | | | | |
| Total (%) | 30 (100) | 30 (100) | 30 (100) | 30 (100) | 120 | | | |

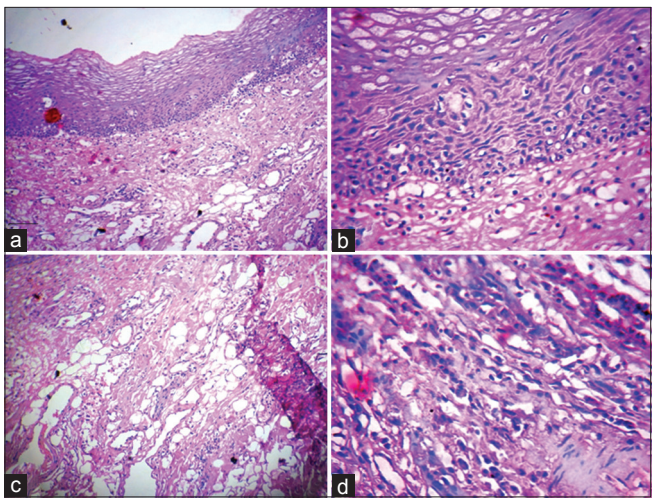



**Figure 3:** (a-d) – Photograph of Group B (cleared in 70 ml kerosene and 30 ml xylene) H&E, showing epithelium and connective tissue (A, C – ×10 and B, D – ×40)

the Groups A, B and D have almost similar or better results when compared to Group C, combination of kerosene and xylene can be used without altering the tissue morphology and staining characteristics with additional advantage of reduced toxicity when xylene was used alone.

## DISCUSSION

Xylene became the clearing agent of choice when chloroform and other clearing agents were declared as carcinogen and a safer alternative was needed, but when xylene was also identified as a health hazard (carcinogenic and teratogenic), replacing it with safer chemicals became a major objective for researchers and manufacturers.[3]

The proposed substitutes included vegetable oils, alkanes, methyl salicylates and propylene oxide, but each one has its own drawbacks as a clearing agent. In the present study, we used kerosene as a safest alternative to xylene in different proportions without altering the tissue morphology and staining characteristics.

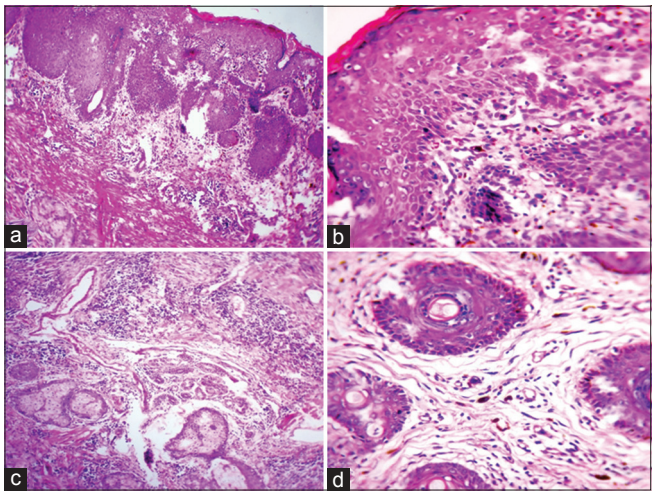



**Figure 4:** (a-d) – Photograph of Group D (cleared in Abs. Xylene) H&E, showing epithelium and connective tissue (A, C – ×10 and B, D – ×40)

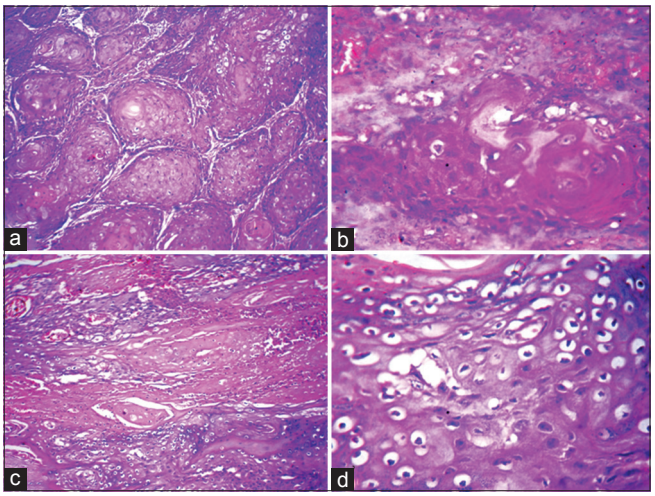
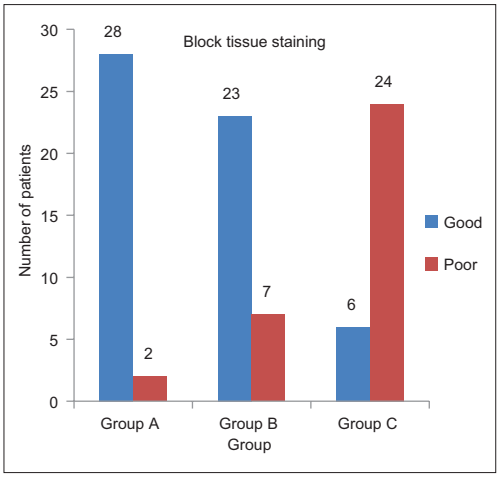
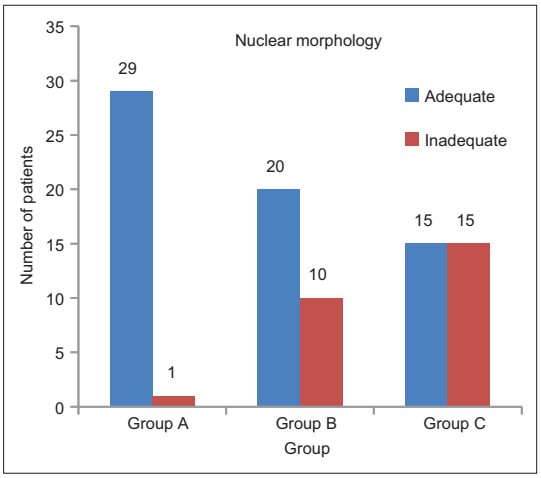


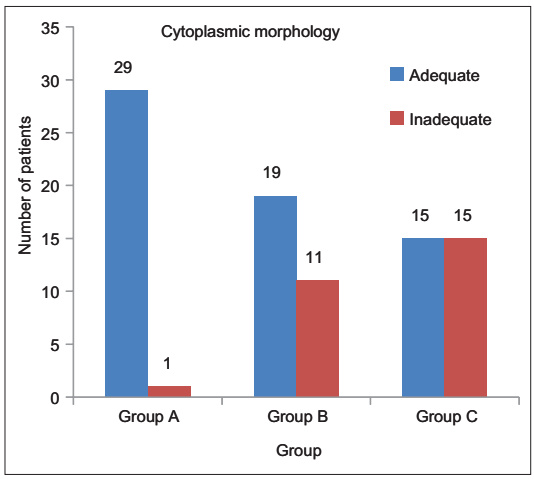

**Figure 5:** (a-d) – Photograph of Group C (cleared in Abs. kerosene) H&E, showing epithelium and connective tissue (A, C – ×10 and B, D – ×40)



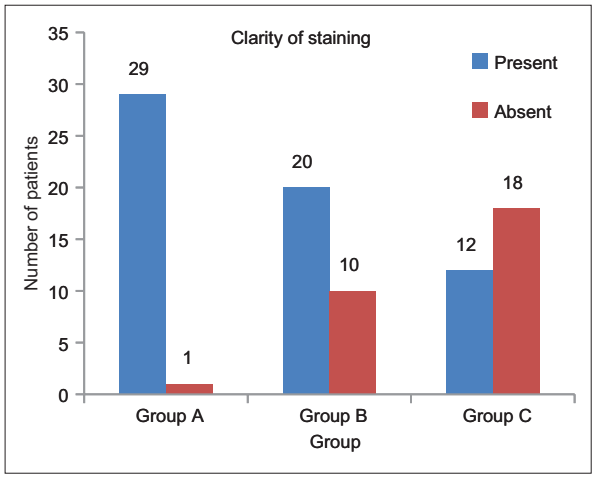

**Graph 1:** Comparison of adequacy of block tissue in Group A, B, C




**Graph 2:** Comparison staining of adequacy of nuclear staining in Group A, B, C



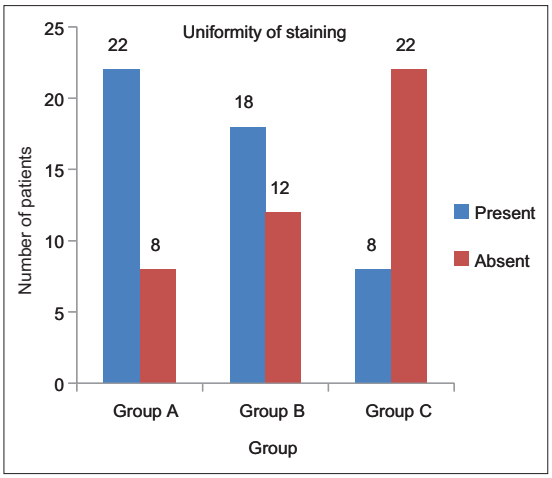

**Graph 3:** Comparison staining of adequacy of cytoplasmic staining in Group A, B, C



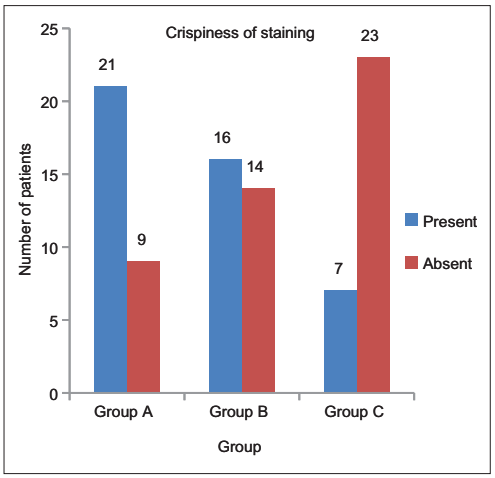

**Graph 4:** Comparison of adequacy of clarity of staining in Group A, B, C

## Blocks of tissue and sectioning

We observed that during embedding, the tissues showed significant shrinkage, appeared depressed after embedding and were hard at the time of cutting for Group D; also, opaque appearance was observed in contrast to translucent appearance of the tissue in Group A, B and C which indicates improper




**Graph 5:** Comparison of adequacy of uniformity of staining in Group A, B, C




**Graph 6:** Comparison of adequacy of crispness of staining in Group A, B, C

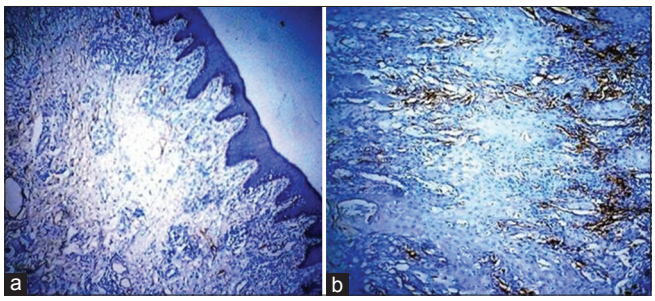



**Figure 6:** (a and b) – IHC stain D2-40 (cleared in 50:50 – K:X) (×10)

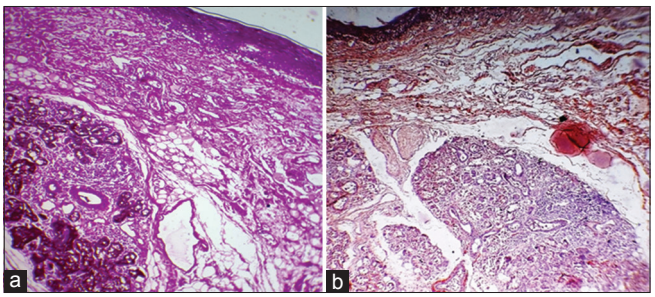



**Figure 7:** (a and b) – Special stain periodic acid–Schiff and congo red (cleared in 50:50 – K:X) (×10)

clearing of tissues. Difficulty in sectioning was observed with Group D due to hardness of the tissues imposed by high concentration of kerosene. Based on these findings, we would like to suggest that increased concentration of kerosene not only affects complete clearing but also increases the hardness of the tissues and thereby affecting their sectioning. By increasing the clearing time in kerosene, we can improve quality of sectioning and also clearing.

By this study, it is shown that mixture of xylene and kerosene at the ratio 50:50 (preferably) and 70 (K):30 (X) is an alternative or good substitute to absolute xylene when used alone in histological and cytological preparations. Kerosene is safer, cheaper and more preferable alternative when compared to xylene as a clearing agent without altering the tissue morphology and staining characteristics.

As mentioned earlier, xylene is carcinogenic and teratogenic, and kerosene poses skin dermatitis after prolonged exposure; thus, a mixture of kerosene and xylene can reduce the negative effects when used alone on humans and acts as an efficient clearing agent.

Histologically, sections of Groups A, B and D provided good morphology of nucleus, cytoplasm, uniform staining, crispness and clarity when compared to Group C where there is a loss in the morphology of nucleus, cytoplasm, crispness, clarity and also loss of striations of muscles. It can be inferred by the above-mentioned findings that using mixture of kerosene and xylene in the ratio 50:50 and 70:30 can act as a safest alternative to xylene without altering the staining characteristics.

## CONCLUSION

By the present study, it can be suggested that kerosene and xylene in the ratio of 50:50 is a safest alternative to xylene when used alone without altering the tissue morphology and also the staining characteristic and most importantly without posing any health risk.

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Nil.

### Conflicts of interest
There are no conflicts of interest.

## REFERENCES

1. Kango PG, Deshmukh R. Microwave processing: A boon for oral pathologists. J Oral Maxillofac Pathol 2011;15:6-13.

2. Ofusori DA, Ayoka AO, Adeeyo OA, Adewole SO. Mixture of kerosene and xylene: A contribution to clearing agents. Int J Morphol 2009;27:211-8.
3. Agrawal U. Histologic Procedure. Available from: http://www.scribd.com. [Last accessed on 2015 Aug 10].
4. Bancroft JD, Gamble M. Theory and Practice of Histological Techniques. London: Churchill Livingstone; 1982.
5. Culling CF. Handbook of Histological and Histochemical Techniques. London: Butterworths and Co. Ltd.; 1974.
6. Talukder SI. Histopathology Techniques, Tissue Processing and Staining. Updated October, 2007. p. 4-11.
7. Drury RA, Wallington EA. Carleton's: Histological Technique. 4th ed. New York: Oxford University Press; 1967.
8. Buesa RJ, Peshkov MV. Histology without xylene. Ann Diagn Pathol 2009;13:246-56.
9. Fay M, Eisenmann C, Diwan S. Toxicological Profile for Xylene. August, 1995. p. 1-267.
10. Khattak S, K-Moghtader G, McMartin K, Barrera M, Kennedy D, Koren G. Pregnancy outcome following gestational exposure to organic solvents: A prospective controlled study. JAMA 1999;281:1106-9.
11. Relevance to Public Health: Background and Environmental Exposure to Xylene in United States. 2007. p. 11-26.
12. Kandyala R, Raghavendra SP, Rajasekharan ST. Xylene: An overview of its health hazards and preventive measures. J Oral Maxillofac Pathol 2010;14:1-5.
13. Reinherdt PA, Leonard KL, Ashbrook PC. Xylene substitutes. In: Pollution Prevention and Waste Minimization in Laboratories. Vol. 3. Florida: CRC Press Levis publishers; 1996. p. 346.
14. Ankle MR, Joshi PS. A study to evaluate the efficacy of xylene-free hematoxylin and eosin staining procedure as compared to the conventional hematoxylin and eosin staining: An experimental study. J Oral Maxillofac Pathol 2011;15:161-7.
15. Smith J. Xylene Free-Tissue Processing – An Evaluation of Routine Use. Leica Microsystems GmbH HRB 5187 95.8025 Rev A; 2008.
16. Buesa RJ. Mineral Oil: The best xylene substitute for tissue processing yet? J Histotechnol 2000;23:143-9.
17. Henry JA. Composition and toxicity of petroleum products and their additives. Hum Exp Toxicol 1998;17:111-23.
18. Ritchie G, Still K, Rossi J 3rd, Bekkedal M, Bobb A, Arfsten D. Biological and health effects of exposure to kerosene-based jet fuels and performance additives. J Toxicol Environ Health B Crit Rev 2003;6:357-451.