

To compare and evaluate the efficacy of the groundnut oil and coconut oil with xylene as a clearing agent.

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Abstract

Background: Xylene has always been the unmatched clearing medium known to be used tissue processing and staining. However, the toxic effects of “xylene” range from mild irritation to its severe form as a carcinogenic agent.

Aims & objective: This study purposes to evaluate the efficacy of “coconut oil” and “groundnut oil” as a substitute in contrast to “xylene” as a clearing agent.

Materials and Methods: Each of 40 specimens were fixed and then cut into three equal parts namely; Group A, Group B & Group C. Specimens were cleared in coconut oil, groundnut oil and xylene respectively and all the 3 groups were taken for Haematoxylin and eosin staining (H&E) and Immunohistochemical staining (IHC).

Results: Both the oils displayed better results than xylene whereas the coconut oil showed superior results than groundnut oil when checked for gross tissue features, staining quality and cytomorphometric analysis.

Conclusion: The results suggest that both coconut oil and groundnut oil has been shown to be an effective replacement for “xylene” as a clearing medium.

Key words: Clearing, coconut oil, groundnut oil, xylene

Introduction

Xylene is an aromatic hydrocarbon which is used as a clearing agent in the field of pathology. A histopathological laboratory pathologist and specialist are often exposed to xylene during procedures such as tissue processing, filtering, staining, cover slip and cleaning tissue processors. It is widely used as a dealcoholization agent of choice.⁽¹⁾ Despite of its toxicity to pathologists and laboratory personnel and the danger it possesses to the environment, xylene is still used in pathology laboratories as a clearing agent.⁽²⁾

The common symptoms that appear after exposure to xylene vapours are throat and nasal irritation, gastrointestinal symptoms i.e., gastric discomfort, nausea, vomiting as well as skin allergies like skin erythema, drying, scaling. It has been shown that laboratory workers exposed to xylene for 1.5 to 18 years have been diagnosed with an equal number of common toxic diseases including bone marrow toxicity and pancytopenia most commonly seen after xylene wound contamination.⁽³⁾ It was with this in mind that the current work

was released to reduce the use of xylene by introducing non-toxic, non-toxic biohazard and highly economical substances such as Groundnut oil and Coconut oil as a cleansing agent during tissue processing.⁽⁴⁾

Material And Methods

The present study was carried out over a period from Jan 2020 to April 2021. The material for our study was collected from patients referred to Santosh Dental College and Hospital during routine extractions and surgical procedures. Each of 40 specimens removed during surgical procedure were fixed with 10% buffered formalin for 48 hours and then sorted into three equal parts and arranged in experimental groups namely; Group A, Group B & Group C. In Group A Tissue processing was done with groundnut oil, in Group B processing was done with coconut oil and in Group C (Control) it was done by xylene as a clearing agent. Following processing with their respective clearing agent all the 3 groups were taken for H/E and IHC staining.

All the three groups were evaluated under the following parameters-

I) Gross tissue specimen evaluation

- a) Rigidity
- b) Translucency
- c) Change after impregnation
- d) Ease of sectioning

II) Hematoxylin and eosin-stained slide evaluation

- a) Nuclear staining.
- b) Cytoplasmic staining
- c) Clarity of staining

III) Cytomorphometric Analysis

- a) Mean area of individual cell
- b) Mean area of nucleus

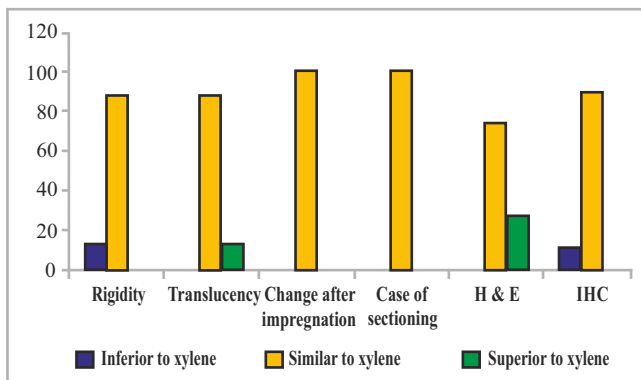
Results

1. Gross Tissue Specimen Evaluation

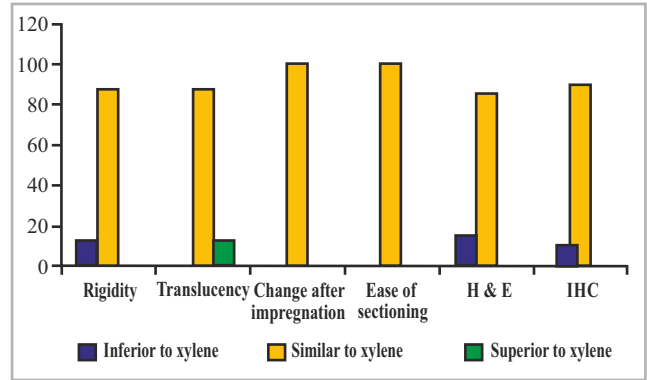
The rigidity of the tissues treated by coconut oil was similar to xylene in 87.5% of the samples. The translucency in 12.5 % of the tissues is superior to the xylene. The change after impregnation and ease of sectioning is 100% same as that of xylene (Graph-1). The rigidity and translucency of the tissues treated by groundnut oil was similar to xylene in 87.5% of the samples. There was no difference in change after impregnation and ease of sectioning while treating the samples with groundnut oil. The samples showed that the tissues were 100% same as that of xylene (Graph -2).

2. H&E and IHC-stained slide evaluation

On the H&E-stained slides evaluation, it showed that in 26.7% of cases the staining is superior than xylene while in IHC 10.8% of samples staining inferior to xylene (Graph-1 & Fig-2). On the H&E-stained slides evaluation, it shows that in 15% of cases the staining is inferior than xylene. In IHC 10.8% of samples staining inferior to xylene (Graph-2 & Fig-3)



Graph -1 Distribution of Different Variables in Coconut Oil



Graph-2 Distribution of Different Variables in Groundnut Oil

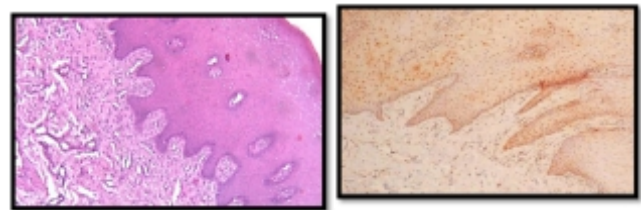


Fig 2-H&E and IHC-stained sections treated with Coconut oil under magnification

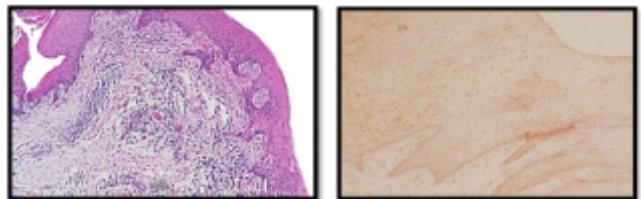
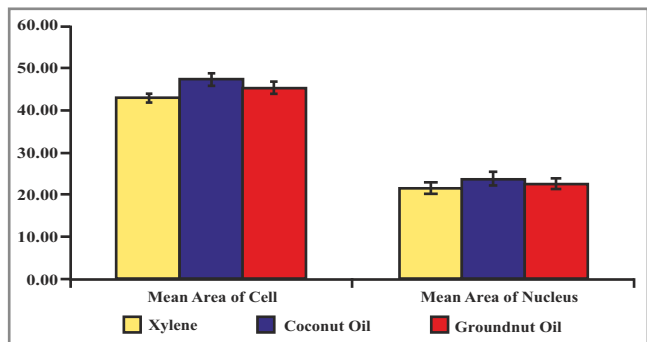


Fig 3-H&E and IHC-stained sections treated with Groundnut oil under magnification

3. Cytomorphometric Analysis

Cytomorphometrically significant higher mean area of cell was observed among coconut oil when compared to Xylene (Mean difference = -4.29; P=0.001) and Groundnut oil (Mean difference = 1.94; P=0.001). Similarly, statistically significant higher mean area of nucleus among coconut oil when compared to Xylene (Mean difference = -2.14; P=0.001) and Groundnut oil (Mean difference = 1.03; P=0.001) (Graph-4).



Graph-4: Comparison of mean areas of cell and nucleus among Xylene, Coconut Oil and Groundnut oil.

Discussion

The results of the present study presented that both coconut oil and groundnut oil showed better results than xylene when gross tissue evaluation and H&E slide assessment was done. Whereas the groundnut oil showed inferior results with the IHC staining of the samples.⁵ Still the role of groundnut oil and coconut oil as clearing agents need to be explored in the staining with special stains. In cytomorphometric analysis there was significant higher mean area of cell noted in Coconut oil followed by Groundnut oil and Xylene respectively. In addition, these oils are economical and readily available in tropical countries, such as India and other nations. By this study we also evaluated that coconut oil as a clearing agent during tissue processing and as a dewaxing agent during staining procedure has better effect on transparency, rigidity and quality of staining as compared to groundnut oil treated counterparts.⁶

Moreover, coconut oil also evaluated less shrinkage both macroscopically and microscopically when compared to groundnut oil. The only limitation of the study is related with coconut oil is its solidification temperature, it gets easily solidified.⁷ However, this can be easily fixed by maintaining its liquid phase during clearing procedure by keeping it in a temperature-controlled chamber.

Conclusion

The results of the present study revealed that these mineral oils can be used as an alternative to xylene in histopathology laboratories. The result was statistically substantial. These mineral oils show similar results to xylene in change after impregnation and ease of sectioning. Whereas in some aspects like rigidity, transparency and quality of staining they were even better than xylene. Both the oils gave equivalent results to xylene but the coconut oil showed better

characteristic features than groundnut oil specimen with respect to the quality of staining. The present study concludes that substituting xylene with these oils is highly desirable from the points of view of both the quality of work and safety.

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Nil.

Conflicts Of Interest

There are no conflicts of interest

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