

AN ECO-FRIENDLY ALTERNATIVE TO XYLENE FOR DEPARAFFINIZATION IN THE HEMATOXYLIN AND EOSIN PROCEDURE

Dr PREETHI¹, Dr.SAI SUDHA.M², Dr ANBUMOZHI³

1.Assistant Professor, Department of pathology,SreeBalaji Medical College And Hospital

2.Assistant Professor, Department of pathology,SreeBalaji Medical College And Hospital

3.Associate Professor, Department of pathology,SreeBalaji Medical College And Hospital

Corresponding author :

Dr. Sai sudha.M, MD

4th main road, Dhandeeshwaramnagar

Velachery, Chennai , 600042

Tel : 9441147531

radha.5054@gmail.com

ABSTRACT

Introduction :

xylene is the widely used deparaffinizing agent in the Hematoxylin and Eosin procedure because of its high solvent property. It is biohazardous in nature and causes toxic effects to health. Hence there is need for the development of safer xylene alternatives in histopathological procedures.

Aim :

The present study aims to assess the efficacy of eco-friendly Sweet lime juice as a deparaffinizing agents in Hematoxylin & Eosin procedure and to compare it with routine Hematoxylin & Eosin procedure using xylene as a deparaffinizing agent.

Materials and methods:

The present study includes 50 paraffin embedded blocks, two sections were prepared from each block. One section(Group A) was stained with routine H & E stain with xylene and the other section(Group B) was stained with xylene -free H &E stain with sweet lime juice as deparaffinizing agent. Stained slides were examined by the pathologist and scores were given to the staining parameters like cytoplasmic and nuclear staining, clarity, crispness and uniformity of staining.

Results:

Staining parameters of all the group A and group B sections were examined and compared with each other. Group B sections with sweet lime juice shows 85 % adequacy of staining for the diagnosis.

Conclusion :

The results of the present study observed that the sweet lime juice can be used as safer and eco-friendly substitute to xylene for deparaffinization in Hematoxylin and Eosin staining procedure

Key words :

Sweet lime juice, Xylene, Deparaffinizing agent, Eco-friendly substitute

INTRODUCTION

Hematoxylin and Eosin staining procedure is the cornerstone in the histopathological diagnosis. In the Conventional Hematoxylin and eosin staining procedure deparaffinization and rehydration, nuclear staining, bluing, cytoplasmic staining and dehydration are the important steps. Xylene is the most commonly used deparaffinizing agent in the routine Hematoxylin and Eosin staining procedure because of its high solvency property. Its excellent clearing and dewaxing properties makes the tissues transparent and contributes to better stained slides^[1].

On the other side xylene was dangerous causing toxic effects on prolonged exposures in high concentrations. Not only xylene is inflammable it also causes toxic damage to many organs like skin, eyes, musculoskeletal system and nervous system^[2]. Xylene exposure occurs through inhalation, ingestion and skin or eye contact. In order to eliminate these adverse effects of xylene many alternatives like vegetable oils, limonene reagents, various aliphatic & aromatic hydrocarbons have been used during tissue processing.

The objective of our study was to test the efficacy of ecofriendly agent like sweet lime juice which is non toxic and economical as an alternative to xylene for deparaffinization in Hematoxylin and staining procedure.

MATERIALS AND METHODS

The cross-sectional study was conducted over a period of 3 months and it includes 45 paraffin embedded blocks from various histopathological tissues. Two sections were prepared from each block and each section was of 5 microns thickness. Two sections were considered in two different groups such as A and B. Group A Tissue sections were stained with routine Hematoxylin & Eosin procedure where Xylene was used as deparaffinizing agent. For the other Group B tissue sections, sweet lime juice was used as deparaffinizing agent in H& E Procedure. After staining procedure, both Group A & B slides were examined using microscope and their staining characteristics were compared and we gave a score at the end. The stained slides were given score based on the staining properties like cytoplasmic and nuclear staining, clarity, uniformity and crispness of staining. The final score with in a range of 3 to 5 was regarded as satisfactory for diagnosis and score less than 3 was considered as insufficient for diagnosis.

The images were captured using microscope in high power view (40X objective), and the magnification of the final image captured on the monitor was 400X. for each stained slide, individual cellular and nuclear outlines were observed to assess the uniformity, clarity and crispness of staining.

RESULTS

Staining characteristics like cytoplasm, nucleus, clarity and uniformity of staining, of all Group A and Group B sections were examined and compared with each other. (Figure 1 & 2) Staining properties of group B (Sweet lime juice as deparaffinize agent) were better when compared to Group A sections (Xylene as deparaffinize agent)

DISCUSSION:

Xylene is a synthetic aromatic hydrocarbon that is widely used in histopathology laboratories as clearing agent because of its excellent solvent properties. It is also biohazardous with many toxic side effects. The national institute of occupational health & safety has recommended xylene exposure limits as a Time weighted average^[3]. Because of these new regulations several substances have been introduced as xylene substitutes by conducting research studies in the recent period.

A study conducted by Ankle and Joshi^[4] and Ramulu et al^[3] concluded Dish wash soap is an effective xylene alternative as deparaffinizing agent in H&E staining procedure. Study conducted by Taneeru et al^[5] proved 60 % efficacy with limonene oil and 97.6% with sesame oil as deparaffinizing agent in H& E staining procedure.

Other studies conducted by Ananthaneni A et al^[6], Vishwanathanprema et al^[7] and Sermadi et al^[8] used eco- friendly substances like 95% lemon water, sesame oil, 100% coconut oil and dishwash soap as xylene substitutes and concluded to be effective as deparaffinizing agents in routine H and E staining procedure.

The present study aims to develop sweet lime juice as an alternative to xylene as deparaffinizing agent in the Hematoxylin and Eosin staining procedure. Sweet lime juice is used to dissolve the wax in the staining procedure because of its solvent property. In the present study cytoplasmic staining is same in the both Group A and Group B, but the nuclear staining is found to be superior with Group A. Other parameters like uniformity, crispness and clarity of the staining were better in Group B sections when compared to Group A. Results of the present study shows that sweet lime juice has 85 % adequacy and found to be used as an alternative to xylene in H and E staining procedure.

CONCLUSION:

The present study results found that the ecofriendly and cost effective Sweet lime juice can be used as a xylene substitute for deparaffinization in the routine Hematoxylin and Eosin staining procedure. However further studies are necessary with large samples to prove that these eco friendly xylene substitutes are better and safe.

REFERENCES:

1. Kandyala R, Raghvendra SP, Rajasekharan ST. Xylene: an overview of its health hazards and preventive measures. *J Oral Maxillofac Pathol.* 2001;4:1–5. [[PMC free article](#)] [[PubMed](#)] [[Google Scholar](#)]
2. Rai R, Yadav R, Bhardwaj A. Biosafe substitutes to xylene: A Review *International Journal of Information Research and Review.* 2016; 3(6): 2529-2532.
3. Ramulu S, Koneru A, Ravikumar S, Sharma P, Ramesh D, Patil R. Liquid dish washing soap: an excellent substitute for xylene and alcohol in hematoxylin and eosin procedure. *J Orofac Sci.* 2012;4:37–42. [[Google Scholar](#)]
4. 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. [[PMC free article](#)] [[PubMed](#)] [[Google Scholar](#)]
5. Ananthaneni A, Namala S, Guduru VS, Ramprasad VV, Ramisetty SD, Udayashankar U, et al. Efficacy of 1.5% dish washing solution and 95% lemon water in substituting perilous

- xylene as a deparaffinizing agent for routine H and E staining procedure: a short study. *Scientifica (Cairo)* 2014;2014:707310. [[PMC free article](#)] [[PubMed](#)] [[Google Scholar](#)]
6. Taneeru S, Guttikonda VR, Vanajakshi CN, Korlepara R. Xylene free method for tissue processing: A pilot study. *Health Sci.* 2013;2:JS004.
 7. Prema V, Prasad H, Srichinthu KK, Kumar SS, Rajkumar K, Marudhamani C. Biofriendly substitutes for xylene in deparaffinization. *J Pharm Bioallied Sci.* 2020 Aug;12:S623-S630. [[PubMed](#)]
 8. Sermadi W, Prabhu S, Acharya S, Javali S. Comparing the efficacy of coconut oil and xylene as a clearing agent in the histopathology laboratory. *J Oral MaxillofacPathol.* 2014;18:S49–53. - [PMC](#) - [PubMed](#)

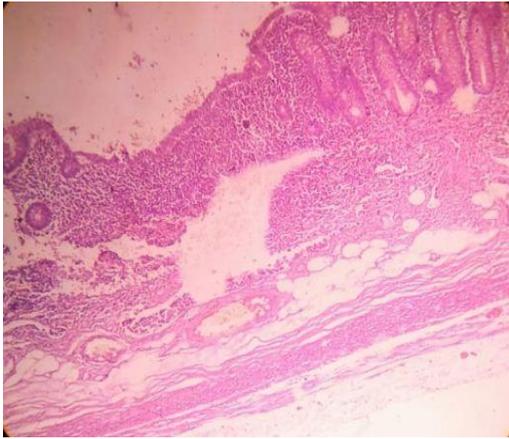


Figure 1 :Section from the appendix shows ulceration of the mucosa and transmurular inflammatory cell infiltrate (conventional H & E section with xylene)

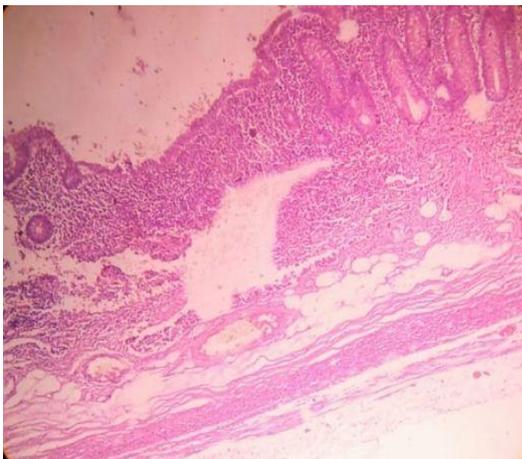


Figure 2 :Section from the appendix shows ulceration of the mucosa and transmurular inflammatory cell infiltrate(xylene free H&E section with sweet lime iuice)