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ORIGINAL RESEARCH

Bleached Palm Oil as a Bio-friendly Substitute for Xylene: A Comparative Study

[1]Rathy Ravindran, [2]Asari K Sruthi, [3]Mustafa Ameena, [4]Rajendran NKD Harish

ABSTRACT

Context: Xylene is known for its wide usage in tissue processing, staining, and coverslipping in the histology laboratory. The hazards of xylene are well documented, making it a potential occupational hazard for the histopathological technicians. There are many studies regarding toxicity of xylene and the pros and cons of using various substitutes that claim to be much safer, better, and faster.

Aim: The present study evaluates the efficacy of bleached palm oil as a bio-friendly substitute for xylene.

Objectives:
- Bleached palm oil as a clearing agent during tissue processing.
- The effects on the transparency and production of serial sections as deparaffinizing agent for hematoxylin and eosin (H&E) staining.
- To compare histological staining characteristics with that of xylene in H&E staining.

Settings and design: A total of 20 formalin-fixed specimens were obtained from the archives of the Department of Oral Pathology at our institution.

Materials and methods: Formalin-fixed specimen were obtained and cut into two; one tissue bit was processed with xylene as clearing agent in routine paraffin wax method (group I), while the other tissue bit was processed with bleached palm oil at 60°C (group II) as clearing agent and dewaxing agent. Sections in both groups were stained using H&E staining method.

Statistical analysis used: Chi-square test and Mann–Whitney U test were the tests used for statistical analysis.

Results: The H&E-stained sections were found to be adequate for diagnosis for 100% in group I and 95% in group II ($p > 0.05$). The H&E-stained slides, when reviewed after 1 year for staining characteristics, showed results to be comparable to that of xylene-processed tissue section.

Conclusion: This study concluded that substitution of xylene with bleached palm oil as clearing agent during tissue processing and as deparaffinizing agent during staining gives good tissue sections and is nontoxic, nonhazardous, nonflammable, biodegradable, economical, easy to handle, and readily available. The staining characteristics with H&E stain showed longevity without fading of the stain and hence can be archived.

Keywords: Bleached palm oil, Clearing agent, Deparaffinizing agent, Xylene.

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INTRODUCTION

Many sophisticated biological techniques have been introduced into pathological practice during past few decades that aid in precise diagnosis. However, the most widely used technique for routine diagnostic procedure is H&E-stained paraffin sections.[1] The H&E stain forms the backbone of daily pathological diagnostic work and the method of preparation of these sections remains unchanged for the last 150 years. Other components in the H&E staining procedure apart from hematoxylin and eosin are xylene and graded alcohols.[2] Xylene, an aromatic hydrocarbon, is known for its wide usage in tissue processing, staining, and coverslipping in the histopathology laboratory. The hazards of xylene are well documented, making it a potential occupational hazard for the histopathological technicians. The National Institute of Occupational Safety and Health recommended exposure limits for xylene, based on a time-weighted average, at 100 ppm for up to a 10-hour work shift in a 40-hour work week and 200 ppm for 10 minutes as a short-term limit.[3]

The main effects of inhaling xylene vapor are depression of the central nervous system with symptoms, such as headache, dizziness, nausea, and vomiting and long-term exposure that may lead to irritability, insomnia, extreme tiredness, tremors, impaired concentration, and short-term memory. Acute neurotoxicity, heart and kidney pathologies, some fatal blood dyscrasia, skin erythema, and drying and scaling of skin are other toxic effects of xylene. Because of the potential toxic and flammable nature of xylene, its substitutes like limonene reagents, aliphatic hydrocarbons, aromatic hydrocarbons, vegetable oils, and mineral oil substitutes have been utilized.[4] There are many studies regarding toxicity of

[1]Professor and Head, [2,3]Senior Lecturer, [4]Associate Professor

[1,3,4]Departmet of Oral and Maxillofacial Pathology, Azeezia College of Dental Sciences and Research, Meeyannoor, Kerala India

[2]Department of Oral Pathology & Microbiology, Sri Venkateshwaraa Medical College Hospital and Research Centre Ariyur, Puducherry, India

Corresponding Author: Rathy Ravindran, Professor and Head Department of Oral and Maxillofacial Pathology, Azeezia College of Dental Sciences and Research, Meeyannoor, Kerala, India Phone: +919447363459, e-mail: rathyravindran27@gmail.com

xylene and the pros and cons of using various substitutes that claim to be much safer, better, and faster. Disposal of xylene is a major problem for the laboratories due to its potential environmental hazards.[5] This, along with its highly volatile nature, makes it a difficult compound for laboratories to handle. The aim of the present study is to utilize eco-friendly substitutes which are nontoxic, less biohazardous, and economical. Bleached palm oil promises to be a widely available and safe substitute. The present study evaluates the efficacy of bleached palm oil as a clearing agent during tissue processing, the effects on the transparency and production of serial sections, and as a deparaffinizing agent, and to compare staining characteristics with that of tissue processed and the sections deparaffinized using xylene.

Table 1: Conventional H&E staining procedure

Deparaffinization	Xylene	30 mts
Rehydration	80% alcohol	2 mts
	70% alcohol	2 mts
	60% alcohol	2mts
	Water wash	
Nuclear staining	Harris hematoxylin	10 mts
	Tap water wash	2 mts
Differentiation	Differentiation in 1% acid alcohol	1 dip
Bluing	Water wash	10 mts
Cytoplasmic stain	1% eosin	1.5 mts
Dehydration	70% alcohol	2 mts
	80% alcohol	2mts
	90% alcohol	2mts
	Xylene	5 mts
Mount	Total time 71 mts	

MATERIALS AND METHODS

A total of 20 formalin-fixed specimen were obtained and cut into two; one tissue bit was processed with xylene as a clearing agent in routine paraffin wax method (group I), while other tissue bit was processed with bleached palm oil (group II) as a clearing agent.

After fixation, group I tissues were dehydrated through three changes of alcohol (70, 80, and 95%) for ½ an hour in each and were cleared by using two changes of xylene (xylene I for ½ an hour and xylene II for 1 hour). The cleared tissues were infiltrated in two changes of molten paraffin wax for 1 hour 30 minutes each. Embedding was done in molten paraffin wax using cassettes and allowed to solidify before microtomy. Tissue blocks were all sectioned at 4 µm with a rotary microtome; sections were floated in a warm water bath and each picked in pairs on albuminized glass slides.

The slides were deparaffinized in xylene and stained with conventional H&E staining method (Table 1). The stained slides were viewed with a low-power (10×) and high-power (40×) magnification of a light microscope and photomicrographs were made (Figs 1 and 2).

After fixation, group II tissues were dehydrated through three changes of alcohol (70, 85, and 95%) for ½ an hour in each and were cleared by using two changes of bleached palm oil at 60°C for 1 hour each. The cleared tissues were infiltrated in two changes of molten paraffin wax (for 1 hour 30 minutes each). Embedding was done in molten paraffin wax using cassettes and allowed to solidify before microtomy. Tissue blocks were all sectioned at 4 µm with a rotary microtome; sections were floated in a warm water bath and each section picked in pairs on albuminized glass slides (Fig. 3).

The sections were deparaffinized in prewarmed bleached palm oil at 60°C and stained substituting bleached palm oil for xylene (Table 2 and Fig. 4). The stained slides were viewed with a low-power (10×) and high-power (40×) magnification of a light microscope and photomicrographs were made (Figs 5 and 6).

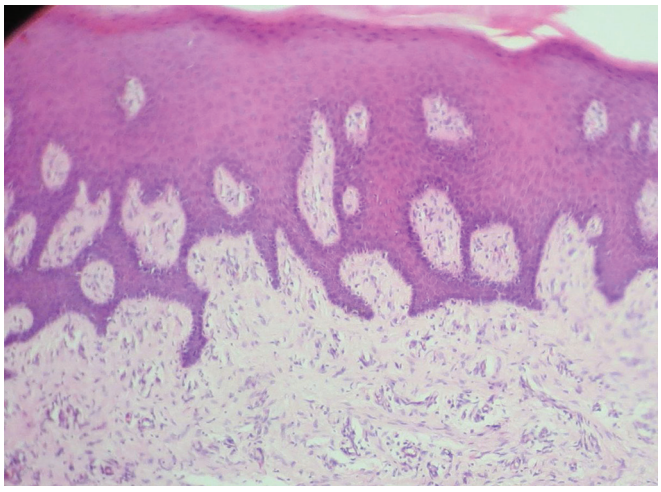


Fig. 1: Photomicrograph of section of tissue processed and stained using xylene as clearing and deparaffinizing agent (epithelium, H&E, 10×)

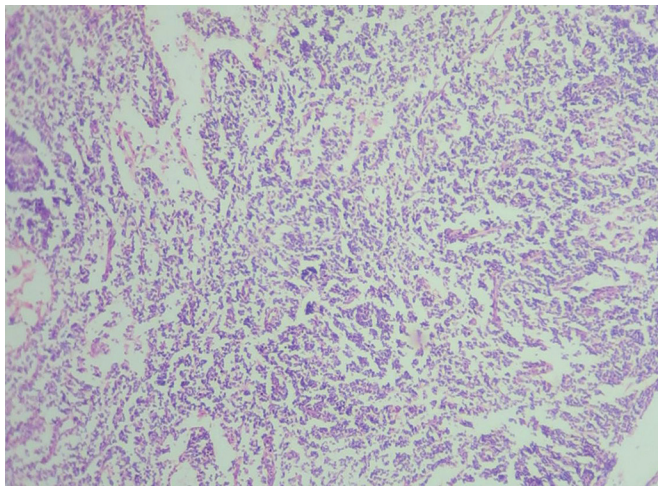


Fig. 2: Photomicrograph of section of tissue processed and stained using xylene as clearing and deparaffinizing agent (lymph node, H&E, 10×)

Table 2: Palm oil substitute H&E staining procedure

Deparaffinization	Prewarmed bleached palm oil at 60°C	15 mts
	Keep upright to drain off excess oil at 60°C	1 mt
	Diluted dishwashing soap 1.7% at 60°C	10 mts
	Wash slides in distilled water at 60°C	3 mts
	Wash slides in distilled water at room temp	2 mts
Nuclear staining	Harris hematoxylin at room temperature	10 mts
	Tap water wash	2 mts
Differentiation	Differentiation in 1% acid alcohol at room temperature	1 dip
Bluing	Water wash	10 mts
Cytoplasmic stain	1% eosin	1.5 mts
	Tap water wash	
	Wash slides in distilled water	
Dehydration	Over drying the sections at 60°C	5 mts
Mount	Total time 59 mts	

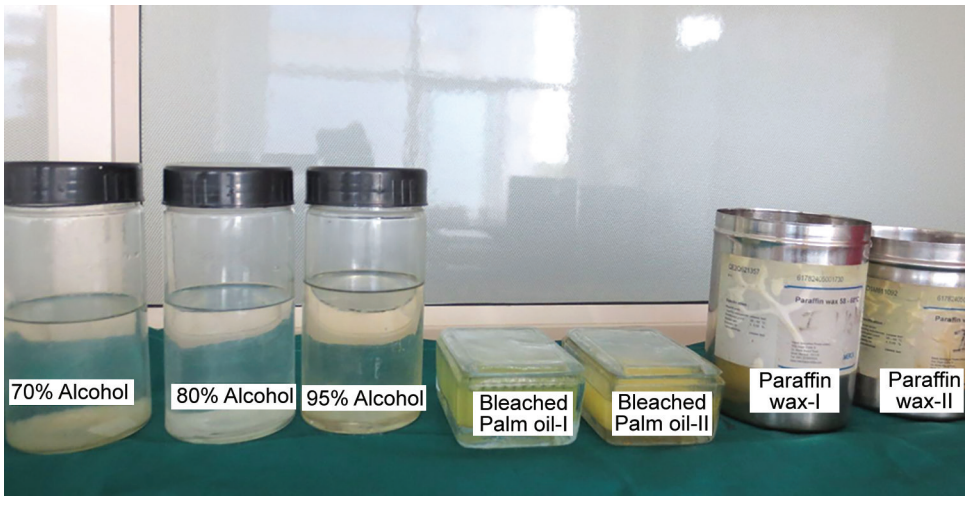


Fig. 3: Steps in tissue processing using bleached palm oil as xylene substitute

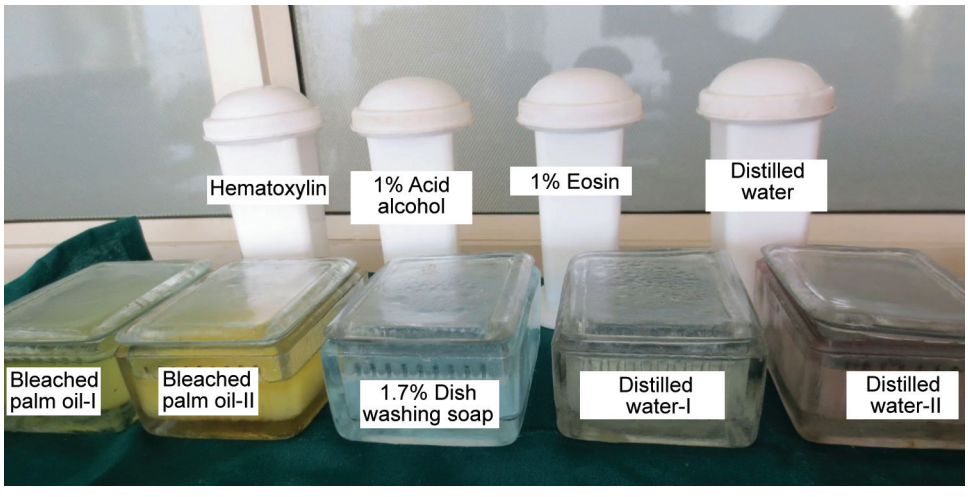


Fig. 4: Steps in staining using bleached palm oil as xylene substitute

During tissue processing in both groups, macroscopic observation based on transparency of tissues after clearing was done. Also, sectioning test was performed to assess the quality of sections produced during microtomy in both groups.

Sections were blinded and scoring was done by a pathologist for analyzing the staining characteristics (Table 3). Chi-square test and Mann–Whitney U test were the tests used for statistical analysis. Data were analyzed using Statistical Package for the Social Sciences

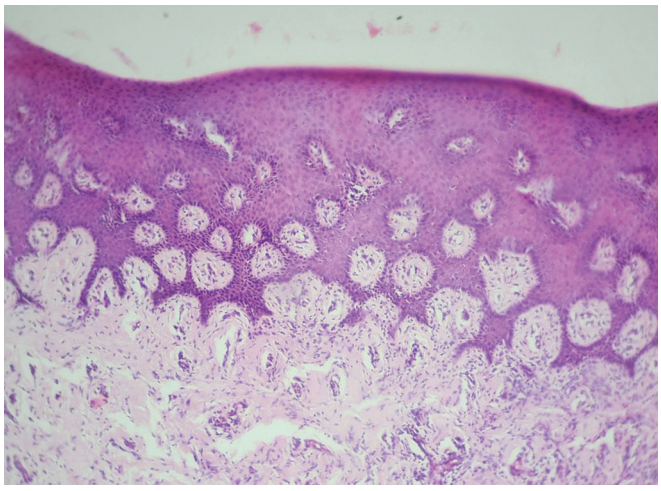


Fig. 5: Photomicrograph of section of tissue processed and stained using bleached palm oil as xylene substitute (epithelium, H&E, 10×)

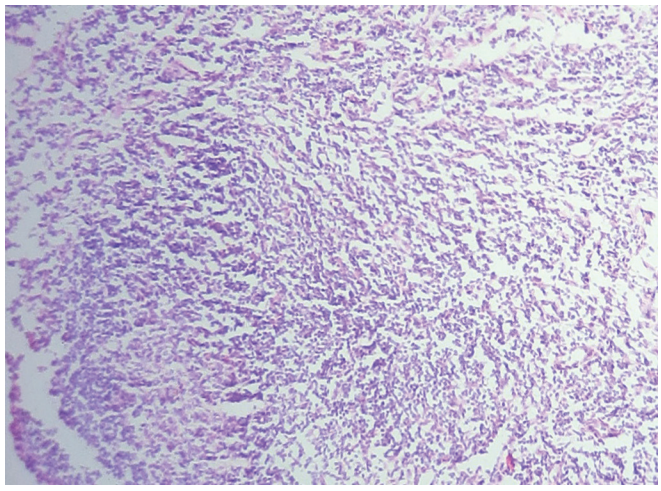


Fig. 6: Photomicrograph of section of tissue processed and stained using bleached palm oil as xylene substitute (lymph node, H&E, 10×)

Table 3: Scoring of H&E-stained slides

1	Nuclear staining	Adequate score 1 Inadequate score 0
2	Cytoplasmic staining	Adequate score 1 Inadequate score 0
3	Clarity of staining	Adequate score 1 Inadequate score 0
4	Uniformity of staining	Adequate score 1 Inadequate score 0
5	Intensity of staining	Adequate score 1 Inadequate score 0

Based on parameters, the maximum score a slide could obtain was 5. Score of ≥ 4 was considered as adequate for diagnosis

software. A p-value < 0.05 was considered statistically significant.

RESULTS

The embedded blocks of groups I and II were carefully observed. Sectioning was smooth and it was easy to obtain a section of 4 µm. Sections were comparable to that of conventional type. After clearing, 100% of tissues using xylene were transparent compared with 95% using bleached palm oil as substitute. Tissues were easily sectioned in 100% tissues cleared using xylene and in 90% tissues cleared with palm oil (Table 4).

Table 4: Comparison of transparency of tissue between groups I and II

Transparency	*Group I (Xylene) n = 20*	*Group II (Bleached palm oil) n = 20*
Adequate	20 (100%)	19 (95%)
Inadequate	0	1(5%)
Sectioning		
Easy	20 (100%)	18 (90%)
Difficult	0	2 (10%)

The quantity of histological staining yielded is given in Table 5. Adequate nuclear staining and cytoplasmic staining were 95% in both groups I and II, and statistically no significant differences were noted (p > 0.05). The intensity of staining and clarity of staining were 95% in group I and 100% in group II (p > 0.05) and uniformity of staining was 95% in both groups I and II (p > 0.05). No statistically significant differences were noted between the groups. The staining was found to be 100% adequate for diagnosis in group I and it was 95% in group II. The scores for the adequacy for diagnosis are given in Table 6. Kappa statistics were used to find out interobserver and intraobserver variability for the characteristics between the two groups. It has been found that there is no interobserver and intraobserver variability

Table 5: Comparison of groups I and II for the staining characteristics

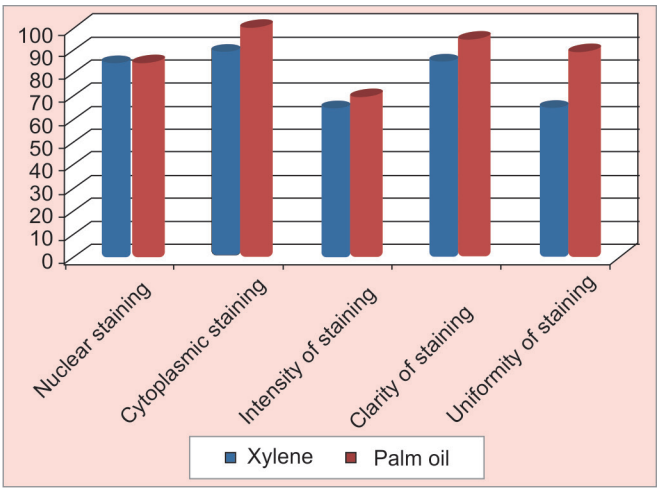
		Nuclear staining	*Cytoplasmic staining*	*Intensity of staining*	*Clarity of staining*	*Uniformity of staining*
Group I (xylene) n = 20	Adequate	19 (95%)	19 (95%)	19 (95%)	19 (95%)	19 (95%)
	Inadequate	1 (5%)	1 (5%)	1 (5%)	1 (5%)	1 (5%)
Group II (bleached palm oil) n = 20	Adequate	19 (95%)	19 (95%)	20 (100%)	20 (100%)	19 (95%)
	Inadequate	1 (5%)	1 (5%)	0	0	1 (5%)
Mann–Whitney U test	200	200	190.000	190.000	200	
Z-value	0.000	0.000	−1.000	−1.000	0.000	
p	1.00	1.00	0.799	0.799	1.00	

Table 6: Scores for the adequacy for diagnosis of the stained sections

Score (total score 5)	*Group I (xylene) n = 20*	*Group II (bleached palm oil) n = 20*
Adequate (score ≥ 4)	20 (100%)	19 (95%)
Inadequate (score < 4)	0	1

Table 7: Comparison of quality of staining between conventional and palm oil substitute H&E staining after a period of 1 year

		Nuclear staining	*Cytoplasmic staining*	*Intensity of staining*	*Clarity of staining*	*Uniformity of staining*
Group I (xylene) n = 20	Adequate	17 (85%)	18 (90%)	13 (65%)	17 (85%)	13 (65%)
	Inadequate	3 (15%)	2 (10%)	7 (35%)	3 (5%)	7 (35%)
Group II (bleached palm oil) n = 20	Adequate	17 (85%)	20 (100%)	14 (70%)	19 (95%)	18 (90%)
	Inadequate	3 (15%)	0	6 (30%)	1 (5%)	2 (10%)
Mann–Whitney U test		200.00	180.00	190.00	180.00	150.00
Z-value		0.000	−1.433	−0.333	−1.041	−1.869
p		1.000	0.152	0.739	0.298	0.062



Graph 1: Comparison of diagnostic adequacy of staining parameters between xylene and palm oil

between the two groups (p-value < 0.05). The limitation of the study is the subjective nature for assessing the quality of scores.

The slides were reanalyzed for the staining characteristics after 1 year by the same two pathologists. Adequate nuclear staining in 85% in both groups I and II and no statistically significant differences were noted (p-value > 0.05). The cytoplasmic staining was 100% in group II compared with 90% in group I. The intensity of staining was 65% in group I and 70% in group II (p-value > 0.05). The uniformity of staining was 65% in group I and 90% in group II (p-value > 0.05). The clarity of staining was 85% in group I and 95% in group II (p-value > –0.05) (Table 7). The staining was found to be 70% adequate for diagnosis in group I and 85% in group II (Graph 1).

DISCUSSION

Histology and histopathology laboratories are hazardous work places due to the abundance of chemicals that can pose major safety and health concerns. Xylene, the most common aromatic solvent used for paraffin embedding and deparaffinizing agent in histopathology laboratories, is highly toxic.[6] Considering the toxicity of xylene and its hazards, various substitutes, including vegetable oils and mineral oils, have been tried in the past.[7] In the present study, the tissues were processed with bleached palm oil at 60°C as clearing agent and dewaxing agent, as it is easily available, less expensive, and nonhazardous.

Macroscopic observation showed that 95% of palm oil-processed tissues were transparent after clearing as compared with 100% xylene-processed tissues. Block quality was "good" for all blocks except for one processed with palm oil at 60°C. During clearing, bleached palm oil having a refractive index of 1.455 at 50°C, closer to that of tissue proteins (which is between 1.33 and 1.4) infiltrates the intercellular spaces of tissues. This leads to reduction in the light scattering properties and increase in optical clearance of the tissue, making them appear transparent.[8] Clearing is enhanced when the tissue fats are dissolved. The bleached palm oil does not dissolve tissue fat like xylene does, and so, the increase in temperature to 60°C aided the tissue fats to dissolve before being displaced by the bleached palm oil, ensuring the clearing of tissues.[9] The increase in temperature increased the kinetic energy of the molecules and rate of diffusion, with a corresponding decrease in viscosity and thus alcohol diffuses out of the tissues allowing penetration of the bleached palm oil.[8] The clearing agent employed during tissue processing has effect on the ease of section cutting and on the final quality of the sections produced.[7] Easy microtomy with good serial sections was observed in 90% of the bleached palm oil-processed tissues as compared with 100% in the xylene-processed tissues.

Ofusori et al,[10] Kunhua et al,[11] and Stockert et al[6] substituted kerosene, SBO, and *n*-heptane respectively, for xylene. Although their studies found that the efficacy

of these xylene substitutes was comparable to xylene, these substances were still hazardous. Buesa[9] used a mixture of ethanol, isopropyl alcohol, and mineral oil as an alternative for xylene and found the mixture to be as efficient as xylene in de-alcoholization. Langman[12] used d-Limonene as a substitute for xylene and it appears to be a safe and effective replacement for xylene. A mixture of coconut oil and olive oil was used by Rasmussen et al[13] and they noted incomplete impregnation, leading to problems in the cutting sections and, therefore, they concluded that this mixture was ineffective as a clearing agent. Andre et al[14] used a mixture of peanut oil, soya bean oil, coconut oil, and cotton oil as a substitute for xylene. They concluded that the mixture was a poor alternative when compared with that of xylene. The study by Sermadi et al[7] inferred that coconut oil is an efficient substitute for xylene. Indu et al[5] concluded in their study that cedarwood oil can be an effective, eco-friendly, and safe alternative to xylene as a clearing agent in the histopathological laboratory. This study substituting palm oil is nonhazardous and less expensive compared with other mineral oils, easily available, and is a good substitute for xylene as clearing agent.

In testing the staining quality of the sections, five different criteria were used, namely nuclear staining, cytoplasmic staining, intensity of staining, clarity of staining, and uniformity of staining. For histological processing and staining, a minimally viscous material is required. Using bleached palm oil at 60°C along with 1.7% dishwashing soap solution at 60°C after dewaxing in bleached palm oil contributed to clarity of staining. At 60°C, the viscosity of bleached palm oil is reduced to 16.93 mPas from 77.19 mPas at 25°C. This increased the fluidity of the bleached palm oil and allowed its easy emulsification and the soap forms an interface (micelle) between the water and oil, resulting in gradual dissolution of the oil into water. The quality of staining of tissues is affected only if the dishwashing solution has been used as a dewaxing agent at 90°C and no observable changes attributed to 1.7% dishwashing soap solution at 60°C on the staining results.[8] In the present study also, 1.7% dishwashing soap solution at 60°C is used as a degreasing agent.

Bleached palm oil at 60°C deparaffinizes the sections, allowing the penetration of stains during staining and at 60°C, paraffin wax becomes molten and is displaced by the bleached palm oil through diffusion in line with Fick's Law which states that the rate of solution diffusion through tissues is proportional to the concentration gradient (the difference between the concentrations of the fluids inside and outside the tissue). Also, the use of 1.7% dishwashing soap solution at 60°C for degreasing the sections after dewaxing in bleached palm oil contributed to the clarity of staining.[8]

When all scores from all the parameters assessed were summed, 95% of the bleached palm oil-processed sections were found to be adequately processed and stained as compared with 100% of the xylene-processed sections. The nuclear staining and cytoplasmic staining and uniformity of staining were comparable between both groups (95%) and between groups, there is no statistically significant difference. The clarity of staining and intensity of staining were 100% in bleached palm oil-substituted group compared with 95% in xylene group and there were no statistically significant differences between the groups.

Ananthaneni et al[15] assessed the efficacy of dishwashing solution and diluted lemon water at 90°C and they found that diluted dishwashing solution showed comparatively superior uniform staining and less retention of wax. Ankle et al,[16] Ramulu et al,[17] and Negi et al[1] substituted 1.7% dishwashing soap solution, but it was done only as a substitute as deparaffinizing agent. Taneeru et al[18] substituted limonene oil and sesame oil for xylene to deparaffinize tissue sections during H&E staining and got better results with sesame oil. Premalatha et al[3] substituted refined mineral oil as a substitute for deparaffinizing agent and found it as a bio-friendly alternative to xylene.

In the present study, the stained slides were air dried before coverslipping, thereby eliminating the need for dehydration in alcohol and clearing in xylene before mounting. The disposal of palm oil is also easy compared with volatile compound xylene.

The advantages of substituting palm oil in H&E staining procedure are listed in Table 8. The alcohol-mediated rehydration and dehydration were not needed in staining procedure and thus is more cost-effective. The disadvantage of this procedure is that it is temperature-sensitive and requires electricity. The procedure cannot be done in the absence of power supply and also a slight drop or elevation in temperature can result in improper results. The study by Udonkang et al[8] substituting bleached palm oil suggested difficulty in fibrous tissues and glands in glandular tissues. This study was done in oral tissues and fibrous tissues are not much encountered in the present study.

On reanalyzing the slides after 1 year, it was found that there were no statistically significant difference in the quality of staining between groups I and II. This is first of the kind of study in which the slides processed

Table 8: Advantages of palm oil over xylene

	Xylene	*Palm oil substitute*
Cost	55 Rs/100 mL	19.2 Rs/100 mL
Time	71 mts (Staining)	59 mts (Staining)
Toxicity	Present	Absent
Biohazardous	Yes	No
Inflammability	Present	No
Quality of staining	Good	Good
Disposal	Difficult	Easy

with palm oil were reevaluated for assessing the keeping of H&E stain without fading and hence can be archived.

CONCLUSION

The present study shows that substitution of xylene with bleached palm oil as clearing agent during tissue processing and as deparaffinizing agent during staining gives good tissue sections and stained slides with good clarity and uniformity of staining. In addition, bleached palm oil is nontoxic, nonhazardous, nonflammable, biodegradable, economical, easy to handle, and readily available. Further studies using dense fibrous tissue and special stain are recommended.

REFERENCES

1. Negi A, Puri A, Gupta R, Chauhan I, Nangia R, Sachdeva A. Biosafe alternative to xylene: a comparative study. J Oral Maxillofac Pathol 2013 Sep;17(3):363-366.
2. Pandey P, Dixit A, Tanwar A, Sharma A, Mittal S. A comparative study to evaluate liquid dish washing soap as an alternative to xylene and alcohol in deparaffinization and hematoxylin and eosin staining. J Lab Physicians 2014 Jul;6(2):84-90.
3. Premalatha BR, Patil S, Rao RS, Indu M. Mineral oil–a bio friendly substitute for xylene in deparaffinization: a novel method. J Contemp Dent Pract 2013 Mar;14(2):281-286.
4. Kandyala R, Raghavendra SP, Rajasekharan ST. Xylene: an overview of its health hazards and preventive measures. J Oral Maxillofac Pathol 2010 Jan;14(1):1-5.
5. Indu S, Ramesh V, Indu PC, Prashad KV, Premalatha B, Ramadoss K. Comparative efficacy of cedarwood oil and xylene in hematoxylin and eosin staining procedures: an experimental study. J Nat Sci Biol Med 2014 Jul;5(2): 284-287.
6. Stockert JC, López-Arias B, Del Castillo P, Romero A, Blázquez-Castro A. Replacing xylene with n-heptane for paraffin embedding. Biotech Histochem 2012 Oct;87(7):464-467.
7. Sermadi W, Prabhu S, Acharya S, Javali SB. Comparing the efficacy of coconut oil and xylene as a clearing agent in the histopathology laboratory. J Oral Maxillofac Pathol 2014 Sep;18(Suppl 1):S49-S53.
8. Udonkang M, Eluwa M, Ekanem TB, Asuquo OR, Akpantah AO. Bleached palm oil as substitute for xylene in histology. JPCS 2014 Jan-Mar;8(2):8-14.
9. Buesa RJ. Mineral oil: the best xylene substitute for tissue processing yet. J Histotechnol 2000 Jun;23(2):143-149.
10. Ofusori DA, Ayoka AO, Adeeyo OA, Adewole SO. Mixture of kerosene and xylene: a contribution to clearing agents. Int J Morphol 2009 Mar;27(1):211-218.
11. Kunhua W, Chuming F, Tao L, Yanmei Y, Xin Y, Xiaoming Z, Xuezhong G, Xun L. A novel non-toxic xylene substitute (SBO) for histology. Afr J Tradit Complement Altern Med 2011 Oct;9(1):43-49
12. Langman JM. d-Limonene: is it a safe, effective alternative to xylene? J Histotechnol 2013 Jul;18(2):131-137.
13. Brunn Rasmussen B, Norring Hjort K, Mellerup I, Sether G, Christensen N. Vegetable oils instead of xylene in tissue processing. APMIS 1992 Sep;100(9):827-831.
14. Andre GG, Wenger JB, Rebolloso D, Arrington JB, Mehm WJ. Evaluation of clearing and infiltration mixtures (CIMs) as xylene substitutes for tissue processing. J Histotechnol 1994 Jun;17(2):137-142.
15. Ananthaneni A, Namala S, Guduru VS, Ramprasad VV, Ramisetty SD, Udayashankar U, Naik KK. 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 2014 Mar;2014:707310.
16. Ankle MR, Joshi PS. A study to evaluate the efficacy of xylene-free haematoxylin and eosin staining procedure as compared to the conventional haematoxylin and eosin staining: an experimental study. J Oral Maxillofac Pathol 2011 May-Aug;15(2):161-167.
17. Ramulu S, Koneru A, Ravikumar S, Sharma P, Ramesh DN, Patil R. Liquid dish washing soap: an excellent substitute for xylene and alcohol in hematoxylin and eosin staining procedure. J Orofac Sci 2012 Sep;4(1):37-42.
18. Taneeru S, Guttikonda VR, Vanajakshi CN, Korlepara R. Xylene free method for tissue processing: a pilot study. Health Sci 2013 Jan;2(3):JS004.