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Original Research Paper

Pathology

EVALUATION OF COCONUT OIL AS A ECOFRIENDLY ALTERNATIVE TO XYLENE IN THE HISTOPATHOLOGY LABORATORY

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ABSTRACT

Aim : To compare the efficacy of coconut oil to xylene as a deparaffinising and de-alcoholising agent

Methods: Forty formalin fixed tissues were divided into groups A and B. Conventional tissue processing using xylene (Group A) and coconut oil (Group B) were run in parallel.. . Sections were stained using conventional Haematoxylin and Eosin . Slides were scored for Nuclear and Cytoplasmic staining, Clarity and Intensity

Results: Good nucleocytoplasmic staining was observed in 100% of sections in both the groups. Adequate uniformity, clarity and intensity of staining observed in 100% of group A sections and 98.3% in group B. Coconut oil cleared tissue sections was superior in translucency when compared to xylene. Staining quality was as good as xylene.

Conclusion: coconut oil showed excellent results in tissue processing and is a ecofriendly alternative to xylene.

KEYWORDS : Deparaffinising Agent, Clearing Agent, Coconut Oil, Xylene,

Introduction

Clearing is an important step in the preparation of histological sections, aiming to remove alcohol from tissues prior to infiltration of paraffin wax. The excellent compatibility of xylene with alcohol and paraffin wax governs its wide use in Histopathology Laboratories as a clearing and de-waxing agent. [1,2]. Many animal studies have demonstrated that excessive exposure to xylene can cause toxicity to multiple tissues such as the CNS, liver, skin, and lung [3-4].

Xylene causes an increase in the refractive index of tissue as the dehydrants is removed and when clearing is completed, the tissue becomes quite translucent. . Generally only three changes of xylene are required to reach this stage. If the tissue remains opaque it means that either water or ethanol remains in the tissue. (5)

Various xylene substitutes, such as, limonene reagents, aliphatic hydrocarbons, vegetable oils and mineral oils were tried in the past to avoid xylene in the laboratory.(6,7) However, these substitutes were found to be less effective and more expensive.

Xylene free environment in laboratories can be acheived by substituting xylene with ecofriendly materials which are non toxic, readily available, less expensive and safe.(7) Thus, the present study was designed to use the easily available coconut oil as an alternative substitute for xylene in routine tissue processing and dewaxing agent during staining procedure.

Materials and Methods

This single blinded study was carried out in the Department of Pathology at MIMS Calicut . Specimens were selected from different anatomical sites, such as skin, buccal mucosa, muscle and lymph node . . Forty different tissue sample pairs were selected and assigned identification numbers from 1-40 and divided into two experimental groups labeled A and B. The tissues were fixed in 10% buffered formalin for 48 hours. Group A tissues were processed with xylene as clearing agent in routine paraffin wax method while group B tissues were processed with coconut oil as clearing agent.

After fixation, group A and B tissues were both dehydrated through ascending grades of alcohol for 1 hour each . Group A tissues were dealcoholized (cleared) by using three changes of xylene, one hour in each change. Group B tissues were cleared using three changes of coconut oil at 60□C for 1 hour each . Both were then infiltrated with three changes of paraffin wax for 1 hour each . After embedding blocks were sectioned at 2- 4 µm with a rotary microtome.

Before staining sections from Group A and B were dewaxed at 60°C in a hot air oven for 1 hour. Group A tissues were deparaffinised in 3 changes of xylene 5 minutes each, hydrated through 2 changes of descending grades of alcohol and rinsed in water. Group B tissues meanwhile were deparaffinised in pre-warmed coconut oil at 60 C for 10 minutes. The slides were stood upright for 1 minute to drain off excess oil and rinsed in 2 changes of 1.7% dish washing solution pre-warmed at 60□ C for 10 minutes each to degrease the sections before rinsing in water.

Group A and B sections were stained in Harris's haematoxylin for 5minutes , rinsed in tap water for 5 minutes and differentiated in 1% acid alcohol for 10 seconds. After bluing with lithium carbonate sections were counterstained in 1% eosin solution for 2-3 minutes.

Group A sections were dehydrated through ascending grades of alcohol ,cleared in xylene and mounted in DPX.

Group B sections were rinsed in water, dried in hot air oven at 60□C for 10 minutes and then mounted in DPX.

Criteria for evaluation

The gross tissue features such as translucency, rigidity after wax impregnation and ease in section cutting were noted down for each specimen. . The criteria used for evaluation of quality of staining was given by Sermadi Wajjid et al.(7)

Poor indicated that the tissue failed to take up the stain adequately/ unevenly (score = 0). 'Satisfactory' pointed toward details not visualized up to the mark (score = 1). 'Good' designated good contrast between the nucleus and cytoplasm and visibility of details, along with brilliance of staining (score = 2).

Intensity of staining evaluated as present (Score 2) or absent (Score 1). A score of 2 was considered adequate for diagnosis.

Observations and Results

Table 1 showed good nuclear and cytoplasmic staining in 100% of sections in both the groups, (p>0.05).

Table 1. Nuclear and Cytoplasmic staining in group A and group B

Nuclear staining	Group A	%	Group B	%
Poor (0)	0	0	0	0
Satisfactory (1)	0	0	0	0
Good (2)	60	100%	60	100%
Cytoplasmic staining				
Poor (0)	0	0	0	0

Satisfactory (1)	0	0	0	0
Good (2)	60	100%	60	100%

Adequate uniformity of staining noted in 100% of group A sections and 98.3% in group B (p=0.36,p>0.05). Clarity of staining of histological details was observed in 100% of sections in group A and 98.3% in group B, (p=0.36, p<0.05). Adequate intensity of staining observed in 100 % of sections of group A and 98.3 % in group B, (p=0.36, p>0.05), (Table 2).

Table 2. Summary of uniformity, clarity and intensity of staining in study groups

Uniformity of staining	Group A	%	Group B	%
Adequate	60	100%	59	98.30%
Inadequate	0	0	1	1.70%
Clarity of staining				
Satisfactory (1)	0	0	1	1.70%
Good (2)	60	100%	59	98.30%
Intensity of staining				
Present	60	100%	59	98.30%
Absent	0	0	1	1.70%

Coconut oil is more viscous than xylene but when subjected to heat it showed equal penetration to that of xylene and the sections were superior in translucency when compared with xylene. The overall staining quality was equivalent with xylene. Coconut oil is economical when compared to xylene the cost being only one tenth.

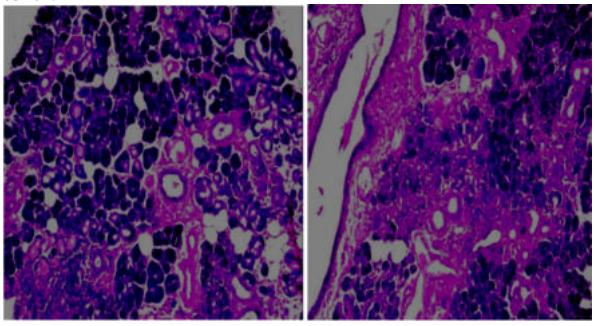

Figure -1: Comparison of tissue sections stained with H and E after clearing with A)coconut oil and B) xylene

Discussion

A wide range of chemicals which are potentially dangerous are employed in the pathology laboratory. . According to OSHA,Occupational Safety and Health Administration regulations, the permissible exposure limit of xylene is 100ppm as an 8-hour time-weighted average (TWA) concentration.(6) Under Resource Conservation and Recovery Act, it is considered to be a hazardous waste. . For years, xylene has been widely used as the deparaffinising and clearing agent of choice, despite its toxicity to personnel and environment. After the hazardous effects of xylene become indisputable in the 1970's, many potential substitutes became available. . Considering the hazardous effects of xylene, this study was done in search of a safe alternative to xylene using coconut oil.

An effective clearing agent must penetrate tissues rapidly . Low viscous solutions penetrate faster than highly viscous solutions. According to Bernoulli's Principle of fluid dynamics viscosity of the fluid is indirectly proportional to temperature. Coconut oil is more viscous compared to xylene. To decrease viscosity of this oil and increase penetration into tissues deparaffinisation was done using a hot-air oven at 60°C .

Wajid Sermadi, et al concluded that coconut oil is an efficient substitute for xylene, as it is nonhazardous, cheaper and causes less shrinkage of the tissue. It can be used as a de-alcoholization agent in the histopathological laboratory, without losing the quality of the histological details [7]. Madhuri R Ankle et al reported that xylene methanol free staining procedure carried out using a simple diluted liquid dish washing soap solution is at par with conventional H and E procedure, and produced crisp nuclear and cytoplasmic staining [8]. Beusa et al concluded that tissue processing with mineral oil (pure and mixed with ethanol and isopropyl) is equivalent to processing tissue with xylene, but much safer to both personnel and the environment [9]. Our analysis showed that 100% of coconut oil processed tissues appeared translucent after clearing . This indicates that coconut oil has similar clearing properties to xylene.

The clearing agent employed has effect on the ease of section cutting . Easy microtomy with good serial sections was observed in 100 % of the coconut oil processed tissues as compared with xylene. Definitive criteria were used to assess staining quality namely: nuclear staining, cytoplasmic staining, clarity , intensity and uniformity of staining. Coconut oil processed sections produced excellent nuclear and cytoplasmic staining.. Clarity of staining and retention of histological details is attributed to the ability of coconut oil at 60C to de-paraffinize the sections allowing good penetration of stains .

In addition, the use of 1.7% dish washing soap solution at 60°C for degreasing the sections after dewaxing in coconut oil contributed to clarity of staining observed. The viscosity of coconut oil is reduced at 60C facilitating its easy emulsification and removal by the soap solution. There was no observable changes attributed to 1.7% dish washing soap solution on the quality of staining since it has been used as a dewaxing solution at 90°C to replace xylene during staining.(8) During the study, exposure to xylene was further reduced by allowing the stained slides to air dry before cover slipping, thereby eliminating the need for dehydration in alcohol and clearing in xylene before mounting.

Conclusion

Our study revealed better results using coconut oil in tissue processing as the sections were more transluscent. As a deparaffinising agent in routine H&E staining it yielded equal results, validating its use as an alternative to xylene. Additional advantages are that of being nontoxic, nonflammable, non hazardous, economical, ecofriendly, easy to handle and dispose.

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