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Original research

COMPARISON OF ROUTINE XYLENE AND DISH WASHING SOLUTION WITH LEMON WATER USED AS DEPARAFFINIZING AGENTS

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Abstract

Background:

Xylene is an aromatic hydrocarbon known for its wide usage in tissue processing which has many hazardous effects.

Objectives:

To compare the efficacy of xylene free sections deparaffinized with dish washing soap and diluted lemon water and compare them.

Materials and Methods:

Using twenty paraffin embedded tissue blocks, three sections each were prepared. One section was stained with conventional H &E method (Group A) and the other two sections with xylene-free (XF) H & E (Groups B and C). Staining characteristics were compared with xylene and scoring was given.

Result:
There is appreciable nuclear stain, cytoplasmic staining, staining clarity with dishwash and diluted lemon water solution with p value <0.001 in comparison with xylene.

Conclusion:
This study shows good result in terms of nuclear staining, cytoplasmic staining, and clarity of staining, liquid soap with lemon can be used as alternate to xylene.

Keywords: xylene, deparaffinization, dish wash, lemon.

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INTRODUCTION

Xylene, also known as xylol or dimethyl benzene, is an aromatic synthetic hydrocarbon that forms an integral part of pathological laboratory since many years. It is available naturally in coal tar and petroleum and has derived its name from crude wood spirit (Greek xylon- wood). It is colorless, flammable liquid or gas with a sweet smell. Three forms of xylene exist called isomers in which the methyl groups vary on the benzene ring: *meta*-xylene, *ortho*-xylene, and *para*xylene (m-, o-, and p-xylene). Laboratory-grade xylene is composed of m-xylene (40–65%), p-xylene (20%), o-xylene (20%), and ethyl benzene (6–20%) and traces of toluene, thiophene, trimethyl benzene, phenol, hydrogen sulfide, and pyridine. It is used as a clearing agent in tissue processing where it causes maximum displacement of alcohol and makes the tissue transparent thus enhancing paraffin infiltration and as a deparaffinizing agent in staining and cover slipping. Although it is extremely useful, it leads to health hazards when exposed involving almost all parts of the body ranging from nausea, vomiting to death. Current Occupational Safety and Health Administration permissible exposure limit for xylene is 100 ppm as an 8-hour time-weighted average (TWA) concentration. Limonene reagents, aliphatic hydrocarbon mixtures, aromatic hydrocarbon mixtures, and mineral oil mixtures were used as alternatives for xylene in tissue processing as clearing agent. But peak exposure is during dewaxing of sections. Unrelenting usage of xylene is a paradigm of its futile replacement. [1,2] (Table 1)

Xylene free environment in laboratories can be made by substituting xylene with various substitutes such as limonene reagents, aliphatic hydrocarbon mixtures, aromatic hydrocarbon mixtures, and mineral oil mixtures are used for processing tissues. [1]

Table 1: Health hazards of xylene (Kandyala et al.)

SYSTEM	EFFECTS
CNS	100–200 ppm → nausea and headache. 200–500 ppm → dizziness, weakness, and vomiting. 800–10,000 ppm → giddiness, confusion, slurred speech, loss of balance, and ringing sound. >10,000 ppm → sleepiness, loss of consciousness, and death.
GIT	Nausea, vomiting, and gastric discomfort.
ENT	Irritation and damage to eye (accidental splash).
MUSCLES	Reduced grasping power and reduced muscle power in extremities.
SKIN	Irritation, dermatitis, dryness, and flaking and cracking of skin
CANCER	Carcinogenic in animals.
LUNGS	Irritation, chest pain, and shortness of breath (200 ppm). Pulmonary edema (extreme conditions).
REPRODUCTIVE SYSTEM	Delayed ossification and contaminates breast milk.
LIVER AND KIDNEY	Injury.

MATERIALS AND METHODS:

A total of 6 paraffin-embedded tissue block specimens were retrieved from the archives of the Department of Oral Pathology. They were previously diagnosed as oral squamous cell carcinoma. They are two groups; A & B, group A, where the xylene used as deparaffinizing and in group B liquid soap with lemon used as deparaffinizing agent, both the groups of slides were stained with haematoxylin and eosin stain. Each section was scored and analysed by two oral pathologists, they were blinded in this study. Slides were scored on the following parameters. (Table 2-6)

PREPARATION OF LIQUID DISH WASH SOAP AND LEMON WATER

- 1.5 ml - liquid dish wash soap
- 5ml – lemon juice
- 93.5 ml – distilled water

Table 2: Scoring criteria

0	Poor (no nucleus and cytoplasm staining; no adequacy in clarity of staining; more retention of wax)
1	Fair (nucleus staining present, no cytoplasm staining and mild adequacy in clarity of staining, moderate retention of wax)
2	Good (nucleus and cytoplasm staining present, moderate adequacy in clarity of staining, mild retention of wax)
3	Very good (nucleus and cytoplasm staining present with good adequacy in clarity of staining and no retention of wax)

Table 3: comparison of nucleus staining in conventional xylene and dish washing soap with lemon water

Nucleus Staining	**Group**			
	control		**DLW**	
	N	**%**	**N**	**%**
Poor	0	0	0	0
Fair	1	10	2	20
Good	6	60	6	60
Very good	4	40	2	20
Total	10	100	10	100

Chi-Square Test	Value	p-Value
Fisher's Exact Test	104.010	<0.001

Table 4: comparison of cytoplasm staining in conventional xylene and dish washing soap with lemon water

Cytoplasm Staining	Group			
	control		DLW	
	N	%	N	%
Poor	0	0	0	.0
Fair	1	10	2	20
Good	7	70	7	70
Very good	2	20	1	10
Total	10	100	10	100

Chi-Square Test	Value	p-Value
Fisher's Exact Test	104.010	<0.001

Table 5: comparison of clarity of staining in conventional xylene and dish washing soap with lemon water

Clarity of staining	Group			
	control		DLW	
	N	%	N	%
Poor	0	.0	0	0
Fair	0	0	1	20
Good	8	80	7	70
Very good	2	20	2	20
Total	10	100	10	100

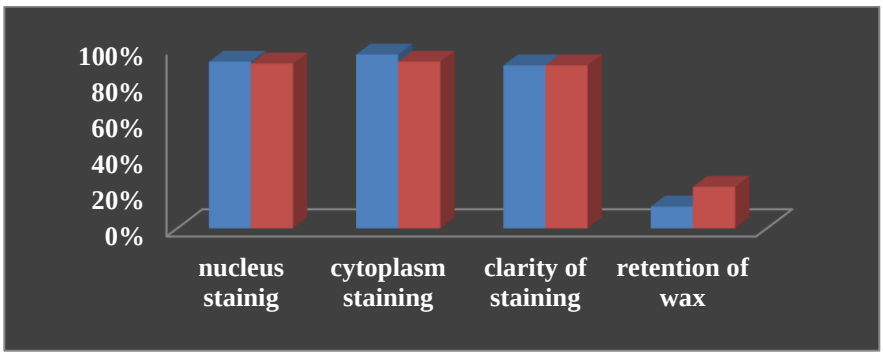
Chi-Square Test	Value	p-Value
Fisher's Exact Test	104.010	<0.001

Table 6: comparison of retention of wax in conventional xylene and dish washing soap with lemon water

Retention of wax	Group			
	control		DLW	
	N	%	N	%
Severe	0	0	0	.0
Moderate	0	0	3	30
Mild	1	10	3	30
No retention of wax	9	90	7	70
Total	10	100	10	100

Chi-Square Test	Value	p-Value
Fisher's Exact Test	104.010	<0.001

Graph 1: - Graphical representation of the group A(Xylene)(blue) and group B (liquid soap with lemon water) (red) used as deparaffinization.



Photomicrographs shows deparaffinization with liquid soap with lemon water fig.1- 4X, fig 2- 10X, fig 3. 40X

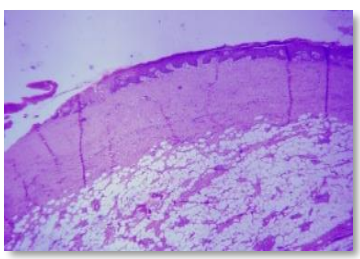


Fig-1

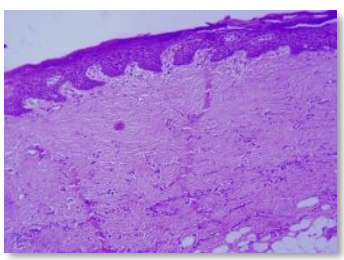


Fig-2

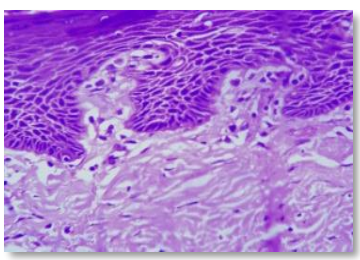


Fig-3

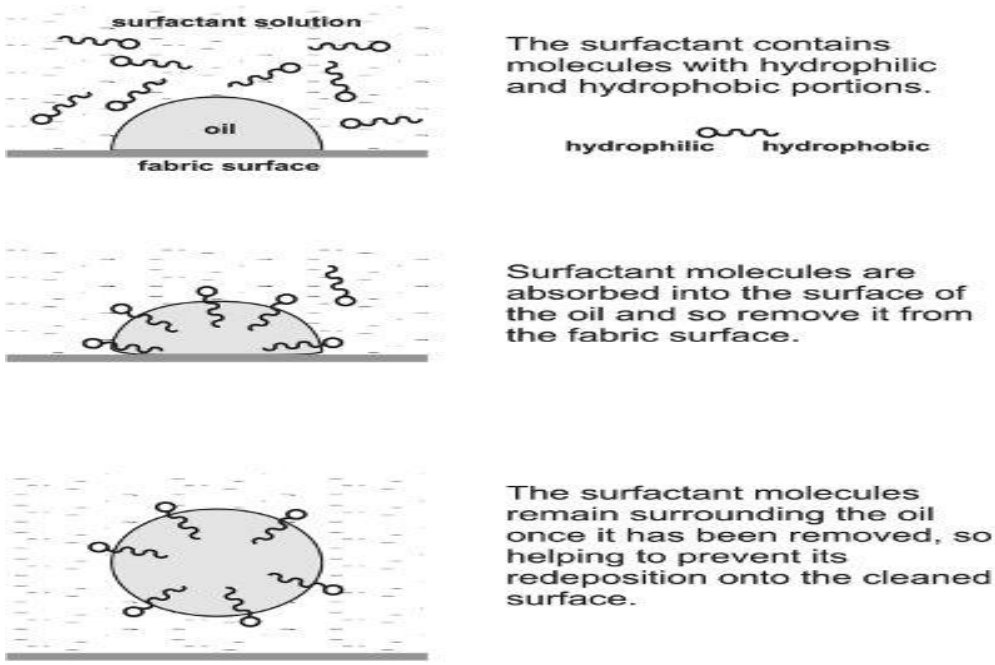
DISCUSSION

The word “Surfactant” is a contraction of the three words “Surface Active Agents.” Surfactants are materials that lower the surface tension (or interfacial tension) between two liquids or between a liquid and a solid. In the general sense, any material that affects the interfacial surface tension, can be considered a surfactant, but in the practical sense, surfactants may act as wetting agents, emulsifiers, foaming agents, and dispersants.[3]

Surfactants function by breaking down the interface between water and oils and/or dirt. They also hold these oils and dirt in suspension, and so allow their removal.(Fig.4) They are able to act in this way because they contain both a hydrophilic (water loving) group, such as an acid anion, ($-CO_2^-$ or SO_3^-) and a hydrophobic (water hating) group, such as an alkyl chain. Molecules of water tend to congregate near the former and molecules of the water-insoluble material congregate near the latter. [4]

Soaps were the earliest surfactants and are obtained from fats which are known as glycerides because they are esters formed by the trihydric alcohol, propane-1,2,3-triol (glycerol), with long chain carboxylic acids (fatty acids). the glycerides are hydrolyzed by heating with sodium hydroxide solution to form soaps, the sodium salts of the acids, and propane-1,2,3-triol. the process is known as saponification. [4]

Fig.4- Action of the surfactant



Liquid dish wash soap is a highly foaming mixture of surfactants principally made up of alkylbenzene sulfonates with low skin irritation and is principally used for hand washing of cutlery, glasses, cooking utensils, and plates. In earlier studies, it was successfully demonstrated as an alternate for xylene in deparaffinizing tissue sections. Lemon juice is customarily used to brighten up copper cookware, as a sanitary kitchen deodorizer, and to remove grease, polish, and wood cleaner, and so forth. Review of literature showed no study till date where DLW was used as deparaffinising agent. The novel concept of using DLW as deparaffinising agent was from its solvent property used to dissolve old wax [5,6].

The underlying principle is that the high temperature of 90 to 94º centigrade will help in removing the wax and dish wash by its surfactant property reduces the surface tension along with lemon water by its solvent property prevents the wax from re -sticking onto the slides, thus helping in deparaffinizing the sections. [1]

The study showed that out of 10 sections, adequate nuclear staining was noted in all the sections of both Group A (92%) and B (91%) and cytoplasm staining n group A (96%) and group B (92%). Adequate cytoplasmic staining and clarity were noted in all the sections in both the groups ($P > 0.001$); retention of wax was in group A (10%) and group B (30%).(Graph- 1) (Fig1-3)
Several studies by Ananthaneni et al, Pandey P et al, Aparna B et al and Thajudeen A et al have propounded the good qualities of dishwashing solution and diluted lemon water as deparaffinizing agents. Thajudeen A concluded that coconut oil is not a good deparaffinizing agent.[7,8,9,10]

CONCLUSION

Xylene free environment in laboratories can be made by substituting xylene with various substitutes such as limonene reagents, aliphatic hydrocarbon mixtures, aromatic hydrocarbon mixtures, and mineral oil mixtures in the processing of tissues. Dishwashing liquid along with lemon water proved to be a good deparaffinizing agent. Therefore, this composition can be further tested in laboratory environment on a wide scale to aid in refinement, patenting and marketing of this deparaffinizing agent.

Financial support and sponsorship:

Nil

Conflicts of interest

There are no conflicts of interest

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