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Original Research Article

Comparing the efficacy of xylene and kerosene oil as a clearing agent during processing and staining in histopathology

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ABSTRACT

Aim of this study is to determine the efficacy of kerosene oil as a clearing agent for tissue processing, haematoxylin & Eosin stains in contrast with traditionally used xylene. The study was conducted using three types of clearing agents xylene, kerosene and mixture of xylene kerosene oil in the ratio 50:50. Hundred cases of biopsies for each clearing agent were compared on the basis of ribboning, thin section, section cutting, nuclear staining, cytoplasmic staining, differential staining, clarity and uniformity of the tissue sections. Biopsy specimen was received in the Department of Pathology G.G.S Medical College and Hospital. The investigation of all cases was compared by using the routine method of histopathology lab. Results related to the tissue processing, section cutting and staining were obtained after the microscopy investigation by the residents. The xylene processed tissue produced better thin sections and ribboning during section cutting than those processed in mixture of kerosene and xylene and kerosene alone being the least suitable agent in case of thin section cutting. The nuclear staining, cytoplasmic staining and Differential were better processed in xylene than those processed in mixture of kerosene and xylene and kerosene alone. The clarity and uniformity was not satisfactory by using the mixture of kerosene and xylene while kerosene alone than xylene processed tissues.

1. Introduction

Working in Medical laboratories, particularly in histopathology section constitutes a menace to the technicians, owing to exposure with wide range of chemical, mechanical, biological and environmental hazards. Routinely contact with chemicals at workplace can cause severe immediate or long-lasting ill effects on the health of technicians and pathologists. The precaution and prevention have always been the basic aim in health sciences. Therefore, to bring in practice the nontoxic, eco-friendly, economically viable, less bio hazardous chemicals are advised. Any attempt to replace hazardous chemicals with alternative potential agent in histology laboratories deserves to be tried.

In histopathology, a technique known as Tissue processing involves chemical solutions reacting with biological specimens. Tissues are made suitable for embedding within a supportive medium such as paraffin, and are visualized by cutting thin sections and staining with histochemicals for microscopic evaluation of tissue. This tissue processing comprises of four different procedures:

1.1. Fixation

The fixation is done to stabilize and harden tissues with minimal distortion of cells. The fixation of Biopsy and Autopsy specimen is carried out as soon as possible to

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prevent autolysis. Different types of fixatives are used for fixation depending on the type of specimen (Tissues). Formalin is the most commonly used fixative in pathology labs worldwide.[1]

1.2. Dehydration

Tissue specimen is immersed in a series of ethanol (alcohol) solutions of increasing concentration to remove water. Ethanol is miscible with water in all proportions so that the water in the specimen is progressively replaced by the alcohol. The ultimate purpose of tissue specimen treatment is to infiltrate the tissue sample in paraffin.

1.3. Clearing

The process makes tissue components receptive to the infiltrating medium (paraffin) by removal of dehydrating solution. The tissue becomes translucent when impregnated with clearing agent.[1] Xylene is the most preferred clearing agent by histologists. But, xylene is toxic and its long-term exposure is injurious to health.[1]

1.4. Infiltration

It constitutes the final step in the treatment of tissue sample. Clearing agent i.e. Xylene is replaced by embedding medium. The embedding medium thoroughly permeates the tissue in fluid and solidifies it without any damage to the tissue. Paraffin wax is used as an infiltrating medium.[2] The wax makes tissue firm for thin sections to be cut on a microtome.

1.5. Xylene

Xylene is an organic chemical compound with a formula of C8H10, also known as xylol or dimethyl benzene. It has molecular weight of 106.16 g/mol, boiling point (137-143°c), flash point (25°c), ignition point (25°), and insoluble in water.[3] It was first isolated in 1850 by the French chemist 'Auguste Cahours' from a constituent of wood tar.[4] The name Xylene comes from Greek xy'lon means wood. Xylol is colourless, flammable liquid with a sweet odour. Xylene is primarily a synthetic chemical produced from petroleum by chemical industries and occurs naturally in petroleum and coal tar.[1]

Xylene is used as a solvent in leather industries, agricultural sprays, adhesives and coatings, as an ingredient in airplane fuel, gasoline and cigarette smoke. In histological laboratories, Xylene is used for tissue processing, staining, as deparaffinising solvent, for cover slipping, as a solvent to clean objectives of microscope and tissue processors.[5]

Prolonged and routinely exposure to the Xylene at workplace causes ill effect on the health of biomedical laboratory workers. Xylene evaporates easily and vapors absorb readily through inhalation, oral and dermal route. Inhaling xylene vapour causes irritation in eye, skin, respiratory tract, and mucous membrane.[6] Xylene also affects the central nervous system. Mild effects includes, headache, weakness, irritability, dizziness, giddiness, loss of appetite, nausea, vomiting, shivering, and sever conditions includes, memory loss, loss of coordination and judgment, respiratory depression or difficulty in breathing, unconsciousness, coma, and possible death due to respiratory failure.[5]

Preventive measures as Personal hygiene practices and protective equipments, installing local exhaust ventilation with a proper hood and finding a substance that can perform the same function and which may lessen the hazard are practiced.[7] Much has been written about the use of safer, less expensive xylene substitutes.In general, the chemical components of potential substitutes includes: Limonene reagents (major component of citrus peel oils), aliphatic hydrocarbon mixtures (Clearite), aromatic hydrocarbon mixtures, and mineral oil mixtures.[8]

1.6. Kerosene

Kerosene, also known as lamp oil, is a thin and transparent liquid fuel with 6 to 16 carbon atoms length, obtained from the fractional distillation of petroleum between 150 °C and 275 °C. It consists of both aliphatic and aromatic hydrocarbons. It has density of 0.78-0.81g/cm3, boiling point between 150°-275°. And auto ignition temperature is 220 °C. The flash point of kerosene is between 38°C and 52 °C, which makes kerosene a relatively safe fuel to store and handle, therefore used widely in developing countries.[9]

The combustible hydrocarbon liquid is suitable for household, commercial and industrial applications. In the non-availability of electricity, kerosene is often considered as a cleaner alternative to solid fuels, biomass and coal for cooking and kerosene lamps.[9] Globally, on an average 500 million households are dependent on fuels, particularly kerosene, for lighting.[10] It is used as a fuel component for jet engines, also used as a degreaser. Kerosene is sprayed on stagnant water to prevent mosquitoes from breeding[11] Kerosene emulsions are used to kill lice on domesticated animals, which rank first in effectiveness and cheapness. Kerosene can be tested for its property as a clearing agent in histopathology lab.[12]

ACGIH lists kerosene as an A3 substance, "Confirmed Animal Carcinogen with Unknown Relevance to Humans". Ingestion of kerosene is harmful or fatal. But People can be exposed to kerosene in the work place by breathing it in, skin contact and eye contact. The prolonged exposure to kerosene causes headache, drowsiness, and irritation of the eyes, nose and lungs, also Contact dermatitis (skin irritation) may occur.[9]

According to National Institute for Occupational Safety and Health (NIOSH) the recommended airborne exposure

limit of kerosene is 100 mg/m3 averaged over a 10 hour work shift.[13] Comparatively, the recommended airborne exposure limit of Xylene is 100 ppm averaged over a 10 hours' work shift and 150 ppm, not to be exceeded during any 15 min work period.[11] The acute health risks involved in handling and using kerosene are minimal, provided that the product(s) are used in accordance with current safety practices.

Without compromising the morphology and staining characteristics of tissue sections, a less toxic, economically viable and easily available potential substitute of Xylene is the need of an hour.[12] So, this study is designed to contrast the potency of kerosene oil as a clearing agent opposite to Xylene,

2. Aim and Objective

1. To study the efficacy of kerosene oil as a clearing agent at the time of tissue processing.
2. To study the efficacy of kerosene oil as a clearing agent in Haematoxylin & Eosin staining.
3. To compare the efficacy of xylene, kerosene and mixture of xylene & kerosene as a clearing agent during tissue are processing and Haematoxylin & Eosin staining.[14]

3. Materials and Methods

1. 10% Formalin
2. Fully Automatic Tissue Processor
3. Xylene
4. Kerosene oil
5. Paraffin wax
6. Manual Rotary Microtome
7. Slides
8. Wax bath
9. Slide warming table
10. Hematoxylin and eosin stains
11. Compound microscope

3.1. Sources of data

The study was conducted on 100 histopathology specimens received in the Department of Pathology G.G.S Medical College and Hospital, Faridkot.

3.2. Inclusion criteria

Viable tissue specimens pertaining to all forms of diseases and ≥ 5cm in size were included.

3.3. Exclusion criteria

All autolysis specimens and small biopsies were excluded.

3.4. Methodology

3.4.1. Experimental design

Specimens were fixed in formalin for 24 hours. Each tissue specimen was divided into 3 equal parts which were processed using three different methods. The groups were as following:

1. Group I – Conventional processing using Absolute Xylene.
2. Group II–Processing using absolute kerosene.
3. Group III–Processing using mixture of kerosene & xylene (50:50).

The processing for Group I was as follows:

1. Formalin 1 - 1hr
2. Formalin 2 - 1hr
3. Isopropyl Alcohol 50% - 1hr
4. Isopropyl Alcohol 70% - 1hr
5. Isopropyl Alcohol 90% - 1hr
6. Isopropyl Alcohol 100% - 1hr
7. Isopropyl Alcohol 100% - 1hr
8. Xylene 1 - 1hr
9. Xylene 2 - 1hr
10. Paraffin wax 1 - 2hr
11. Paraffin wax 2 - 2hr
12. Paraffin wax 3 - 2hr

The processing for Group II was done as follows:

1. Formalin 1 - 1hr
2. Formalin 2 - 1hr
3. Isopropyl Alcohol 50% - 1hr
4. Isopropyl Alcohol 70% - 1hr
5. Isopropyl Alcohol 90% - 1hr
6. Isopropyl Alcohol 100% - 1hr
7. Isopropyl Alcohol 100% - 1hr
8. Absolute Kerosene 1 - 1hr
9. Absolute Kerosene 2 - 1hr
10. Paraffin wax 1 - 2hr
11. Paraffin wax 2 - 2hr
12. Paraffin wax 3 - 2hr

The processing for Group III was as follows:

1. Formalin 1 - 1hr
2. Formalin 2 - 1hr
3. Isopropyl Alcohol 50% - 1hr
4. Isopropyl Alcohol 70% - 1hr
5. Isopropyl Alcohol 90% - 1hr
6. Isopropyl Alcohol 100% - 1hr
7. Isopropyl Alcohol 100% - 1hr
8. Xylene: Kerosene 1 - 1hr
9. Xylene: Kerosene 2 - 1hr
10. Paraffin wax 1 - 2hr
11. Paraffin wax 2 - 2hr

12. Paraffin wax 3 - 2hr
    (a) Processed tissue sections were embedded into paraffin wax.
    (b) Paraffin embedded tissue blocks were used to prepare sections using semi automated rotary microtome.
    (c) The quality of paraffin block prepared and the quality of section cutting by all these three methods were assessed and compared with the standard xylene processing method by using Kappa agreement.

The following parameters were used for comparing the quality of processing:

1. Adequate ribboning of the paraffin tissue section. A ribbon was called adequate when 3 or more sections were cut together and obtained like a ribbon. (Score 0,1)Paraffin tissue thin sections up to 2.5 μm by manual rotary microtome. (Score 0,1)
2. Ease of section cutting by manual rotary microtome. (Score 0,1)
3. Score of tissue processing $\geq$ 2 were considered satisfactory.
4. Ribbon sections will be floated on the water bath and subsequently on the slide.
5. Sections were dried and conventional staining procedure was used for staining with haematoxylin and eosin stain.
6. Then group I blocks were stained using Xylene as clearing agent.
7. The group II blocks were stained using absolute kerosene as clearing agent.
8. And group III blocks were stained using kerosene: xylene mixture (50:50) as clearing agent.
9. The staining of group II and group III blocks were compared with the group I by using Kappa agreement.

The following parameters were used for comparing the quality of staining:

1. Nuclear staining. (Score 0,1)
2. Cytoplasmic staining. (Score 0,1)
3. Differential staining. (Score 0,1)
4. Clarity or crispiness of staining. (Score 0,1)
5. Uniformity of staining. (Score 0,1)
6. Score of staining $\geq$ 3 was considered satisfactory.

4. Observations and Results

Table 1 shows grading of ribboning on the basis of type of clearing agent used. Ribboning of the section was grade 1 in 17/100 cases using kerosene, 81/100 cases by use of xylene and 32/100 cases by use of mixture of xylene and kerosene.

Ribboning of the section was grade 0 in 83/100 cases using kerosene, 19/100 cases by use of xylene and 68/100 cases by use of mixture of xylene and kerosene.

Table 2 shows grading of paraffin tissue thin section on the basis of type of clearing agent used. Thin section of the paraffin tissue was grade 1 in 22/100 cases using kerosene, 81/100 cases by use of xylene and 39/100 cases by use of mixture of xylene and kerosene.

Thin section of the paraffin tissue was grade 0 in 78/100 cases using kerosene, 19/100 cases by use of xylene and 61/100 cases by use of mixture of xylene and kerosene.

Table 3 shows grading of ease of section cutting on the basis of type of clearing agent used. Ease of section cutting of the paraffin tissue was grade 1 in 16/100 cases using kerosene, 79/100 cases by use of xylene and 41/100 cases by use of mixture of xylene and kerosene.

Ease of section cutting of the paraffin tissue was grade 0 in 84/100 cases using kerosene, 21/100 cases by use of xylene and 59/100 cases by use of mixture of xylene and kerosene.

Table 4 shows grading of nuclear staining on the basis of type of clearing agent used. Nuclear staining of the paraffin tissue section was grade 1 in 22/100 cases using kerosene, 79/100 cases by use of xylene and 38/100 cases by use of mixture of xylene and kerosene.

Nuclear staining of the paraffin tissue section was grade 0 in 78/100 cases using kerosene, 21/100 cases by use of xylene and 62/100 cases by use of mixture of xylene and kerosene.

Table 5 shows grading of cytoplasmic staining on the basis of type of clearing agent used. Cytoplasmic staining of the paraffin tissue section was grade 1 in 31/100 cases using kerosene, 74/100 cases by use of xylene and 63/100 cases by use of mixture of xylene and kerosene.

Cytoplasmic staining of the paraffin tissue section was grade 0 in 69/100 cases using kerosene, 26/100 cases by use of xylene and 37/100 cases by use of mixture of xylene and kerosene.

Table 6 shows grading of differential staining on the basis of type of clearing agent used. Differential staining of the paraffin tissue section was grade 1 in 25/100 cases using kerosene, 82/100 cases by use of xylene and 32/100 cases by use of mixture of xylene and kerosene.

Differential staining of the paraffin tissue section was grade 0 in 75/100 cases using kerosene, 18/100 cases by use of xylene and 68/100 cases by use of mixture of xylene and kerosene.

Table 7 shows grading of clarity on the basis of type of clearing agent used. Clarity of the staining of the paraffin tissue section was grade 1 in 24/100 cases using kerosene, 75/100 cases by use of xylene and 36/100 cases by use of mixture of xylene and kerosene.

Clarity of the staining of the paraffin tissue section was grade 0 in 76/100 cases using kerosene, 25/100 cases by use of xylene and 64/100 cases by use of mixture of xylene and kerosene.

Table 1: Grading of ribboning on the basis of type of clearing agent used by section cutting using manual rotary microtome

Parameter	Grading	Type of reagent			Total no. of specimen
		Kerosene	Xylene	Xylene: Kerosene	
Ribboning	0	83	19	68	170
	1	17	81	32	130
Total no. of specimen		100	100	100	300

Table 2: Grading of thin section on the basis of type of clearing agent used by section cutting using manual rotary microtome

Parameter	Grading	Type of reagent			Total no. of specimen
		Kerosene	Xylene	Xylene: Kerosene	
Thin section	0	78	19	61	158
	1	22	81	39	142
Total no. of specimen		100	100	100	300

Table 3: Grading of ease of section cutting on the basis of type of clearing agent used by section cutting using manual rotary microtome

Parameter	Grading	Type of reagent			Total no. of specimen
		Kerosene	Xylene	Xylene: Kerosene	
Section cutting	0	84	21	59	164
	1	16	79	41	136
Total no. of specimen		100	100	100	300

Table 4: Grading of nuclear staining on the basis of type of clearing agent used

Parameter	Grading	Type of reagent			Total no. of specimen
		Kerosene	Xylene	Xylene: Kerosene	
Nuclear staining	0	78	21	62	161
	1	22	79	38	139
Total no. of specimen		100	100	100	300

Table 5: Grading of cytoplasmic staining on the basis of type of clearing agent used

Parameter	Grading	Type of reagent			Total no. of specimen
		Kerosene	Xylene	Xylene: Kerosene	
Cytoplasmic staining	0	69	26	37	132
	1	31	74	63	168
Total no. of specimen		100	100	100	300

Table 6: Grading of differential staining on the basis of type of clearing agent used

Parameter	Grading	Type of reagent			Total no. of specimen
		Kerosene	Xylene	Xylene: Kerosene	
Differential Staining	0	75	18	68	161
	1	25	82	32	139
Total no. of specimen		100	100	100	300

Table 7: Grading of clarity on the basis of type of clearing agent used

Parameter	Grading	Type of reagent			Total no. of specimen
		Kerosene	Xylene	Xylene: Kerosene	
Clarity	0	76	25	64	165
	1	24	75	36	135
Total no. of specimen		100	100	100	300

Table 8: Grading of uniformity on the basis of type of clearing agent used

Parameter	Grading	Type of reagent			Total no. of specimen
		Kerosene	Xylene	Xylene: Kerosene	
Uniformity	0	78	23	58	159
	1	22	77	42	141
Total no. of specimen		100	100	100	300

Table 8 shows grading of uniformity on the basis of type of clearing agent used. Uniformity of the staining of the paraffin tissue section was grade 1 in 22/100 cases using kerosene, 77/100 cases by use of xylene and 42/100 cases by use of mixture of xylene and kerosene.

Uniformity of the staining of the paraffin tissue section was grade 0 in 78/100 cases using kerosene, 23/100 cases by use of xylene and 58/100 cases by use of mixture of xylene and kerosene.

5. Discussion

The current study aims at comparative evaluation of efficacy of Xylene, Kerosene and combination of Xylene & Kerosene in ratio 50:50, as clearing agent in histopathological tissue processing as well as Haematoxylin & Eosin staining of paraffin tissue section.

100 histopathology specimens received in the Department of Pathology G.G.S Medical College and Hospital, Faridkot. Specimens were fixed in formalin for 24 hours. Each tissue specimen was divided into 3 equal parts which was processed using three different methods. The groups were as follows:

1. Group I – Conventional processing using absolute xylene.
2. Group II–Processing using absolute kerosene.
3. Group III–Processing using mixture of kerosene & Xylene (50:50).

The various parameters compared were ribboning of sections, thin section, and ease of section cutting, nuclear staining, cytoplasmic staining, differential staining, clarity and uniformity. First of all ribboning of sections was compared and graded. The present study showed that ribboning of sections was better obtained when xylene was used as clearing agent. This finding was in concordance with the study done by David Ofusori et al.[2] in Nigeria in 2009, which also showed better ribboning of section with xylene as clearing agent. But the grading of ribboning of the sections in context to the comparison of xylene with xylene: kerosene mixture was in disconcordance. While in present study the sections cleared in xylene gave better ribboning than those processed in xylene: kerosene mixture, their study showed that xylene: kerosene mixture was better than xylene alone.

Grading of thin section in the present study was better with tissues when xylene was used as clearing agent than with the tissue where kerosene was used as clearing agent. This finding was in concordance with the study done by David Ofusori et al.[2] in Nigeria in 2009. While in context to the comparison of xylene with the mixture of xylene & kerosene. The two studies were in disconcordance. The present study showed the sections cleared in xylene gave better thin section than those processed in xylene & kerosene mixture, but their study showed that xylene kerosene mixture was better than xylene alone.

In case of ease of section cutting, the present study shows that it was better with tissues when xylene is used as clearing agent than with the tissue where kerosene was used as clearing agent. The study done by David Ofusori et al.[2] in Nigeria in 2009 also showed xylene is a better clearing agent than kerosene. But the grading of ease of section cutting of the sections in context to the comparison of Absolute xylene with mixture of xylene & kerosene was in disconcordance. While in present study the sections cleared in xylene gave better cutting than those processed in xylene & kerosene mixture, their study showed that xylene kerosene mixture was better than xylene alone.

The grading of nuclear staining was better with tissues when xylene was used as clearing agent than with the tissue where kerosene was used as clearing agent. This finding of present study and the study done by David Ofusori et al.[2] in Nigeria in 2009 is in concordance. But again the grading of nuclear staining of the sections in context to the comparison of xylene with xylene & kerosene mixture was in disconcordance. While in the present study the sections cleared in xylene gave better nuclear staining than those processed in xylene & kerosene mixture, their study showed that xylene kerosene mixture was better than xylene alone.

Current study shows that grading of cytoplasmic staining was better with tissues when xylene is used as clearing agent than with the tissue where kerosene was used as clearing agent. In context to grading of cytoplasmic staining with xylene as compared to xylene & kerosene mixture, the results of two studies were dissimilar. Although the level of difference between the cytoplasmic grading of xylene & kerosene mixture was very minimal but still. Present study indicated xylene as better clearing agent than the mixture of xylene & kerosene while the study done by David Ofusori et al[2] in Nigeria in 2009 showed xylene kerosene mixture to be better than xylene.

In context to grading of differential staining our study shows that the grade was better with tissues when xylene was used as clearing agent than with the tissue where kerosene was used as clearing agent. This finding is in concordance with the study done by David Ofusori et al[2] in Nigeria in 2009. But the grading of differential staining in context to the comparison of xylene with xylene & kerosene mixture was in disconcordance.[2] While in our study the sections cleared in xylene gave better differential staining than those processed in xylene & kerosene mixture, their study showed that xylene kerosene mixture was better than xylene alone.

Grading of uniformity of staining is also better with tissues when xylene is used as clearing agent than with the tissue where kerosene was used as clearing agent. The study done by David Ofusori et al.[2] in Nigeria in 2009 also showed similar result. But the grading of uniformity of the staining in context to the comparison of xylene with xylene: kerosene mixture was in dissimilar. While in our study the sections cleared in xylene gave better uniformity of staining than those processed in xylene: kerosene mixture, their study showed that xylene kerosene mixture was better than xylene alone.

The grading of clarity of staining in the present study was better with tissues when xylene was used as clearing agent than with the tissue where kerosene was used as clearing agent. This finding is also in concordance with the study done by David Ofusori et al.[2] in Nigeria in 2009. While in the present study the sections cleared in xylene gave better clarity of staining than those processed in xylene & kerosene mixture, their study showed that xylene kerosene mixture was better than xylene alone. So, this parameter was in disconcordance.

6. Summary

100 tissue specimens were received in histopathology laboratory G.G.S Medical College and Hospital, Faridkot, fixed in 10% formalin and separated in to three groups (I, II, III) and processed for light microscopic study using Haematoxylin & Eosin staining. The groups are as follows:

Group I – Conventional processing using absolute xylene

Group II – Processing using absolute kerosene

Group III – Processing using mixture of kerosene & Xylene (50:50)

The aim of this study was to compare the efficacy of kerosene and xylene as a clearing agent during tissue processing and Haematoxylin & Eosin staining.[15] The findings of the study are as follows.

6.1. Tissue processing

1. The tissue processed in xylene produced more satisfactory ribboning than those processed in kerosene & xylene mixture while those processed in kerosene alone were least satisfactory.
2. The xylene processed tissue produced better thin sections on tissue cutting than those processed in mixture of kerosene & xylene and kerosene alone being the least suitable agent in case of thin section cutting.
3. The section cutting was also better when the tissues were processed in xylene than the mixture of kerosene & xylene and kerosene alone.

6.2. Staining

1. The nuclear staining was best in tissues processed with xylene as clearing agent than the mixture of kerosene & xylene while kerosene alone being the least satisfactory.
2. The xylene processed tissue gave better cytoplasmic staining than those processed in mixture of kerosene & xylene and kerosene alone being the least suitable agent in view of cytoplasmic stain, although the difference in cytoplasmic staining levels was minimal amongst the three when compared to other criteria.
3. The differential staining was also better when the tissues were processed in xylene than the mixture of kerosene & xylene and kerosene alone.
4. The xylene processed tissue gave better clarity than those processed in mixture of kerosene & xylene and kerosene alone being the least suitable agent in case of clarity.
5. The uniformity was best in tissues processed with xylene as clearing agent than the mixture of kerosene & xylene while kerosene alone being the least satisfactory.

7. Conclusion

In the present study xylene is found to be better clearing agent than the mixture of xylene & kerosene as well as kerosene alone used as clearing agent, whereas the mixture of xylene & kerosene is found to be better than kerosene alone. This findings is based on the quality of tissue processing involving different variables like ribboning, thin section, ease of section cutting as well as variables involved in Haematoxylin & Eosin staining like nuclear staining, cytoplasmic staining, differential staining, clarity and uniformity.

8. Source of Funding

None.

9. Conflict of Interest

None.

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