

Grape Juice As Alternative Agent For Xylene In Hematoxylin And Eosin Staining Procedure

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ABSTRACT:

Aim: To substitute grape juice for xylene as deparaffinizing agent in routine Hematoxylin and Eosin staining.

Objective: In this study we compare the use of grape juice with xylene as a deparaffinizing agent in hematoxylin and Eosin staining.

Materials and Methods: This study was conducted in the histopathology lab at Sree Balaji medical college and hospital using 45 paraffin embedded blocks of uterine fibroid tissue. From each tissue block two sections were taken simultaneously, one section was labelled as group A and the other as group B. Sections labelled as group A were stained with H&E stain using xylene as deparaffinizing agent and sections labelled group B were stained with H&E stain using grape juice as deparaffinizing agent. After staining histopathology slides scoring was done depending on staining quality which includes nucleus stain, cytoplasm stain, crispiness of staining, uniformity and clarity of the smear.

Results: Scoring given was according to H&E staining characteristics of the tissue that include, nuclear staining, cytoplasm staining, uniformity of staining, clarity of the cell and crispiness of staining. It was observed that the scoring of Group B (grape juice as deparaffinizing agent) was better than the scoring of Group A (xylene as deparaffinizing agent).

Conclusion: The natural product grape juice can be used as a potent deparaffinizing agent alternative to xylene in routine hematoxylin and eosin staining thereby reducing the toxic effects of xylene in the histopathology lab.

KEY WORDS:

Grape juice, Xylene, Deparaffinizing agent, Hematoxylin and Eosin Stain, Fibroid tissue.

INTRODUCTION:

Xylene is used in histopathology laboratories for tissue processing, as a deparaffinizing and clearing agent, to increase the quality of staining and for mounting of slides. It acts like a solvent and allows penetration of alcohol, makes the tissue transparent and helps in paraffin embedding. In H and E staining procedures, xylene is an important deparaffinizing agent for wax removal in the tissue and has an important role in contributing to bring out brilliantly stained slides. Xylene is a very useful reagent used in tissue staining but it causes health hazards like contact dermatitis to skin, chronic conjunctivitis of eyes, allergic rhinitis, weakness of nervous system, and weakness of musculoskeletal system [1-3]. Hazards of xylene occur through inhalation, ingestion, and eye or skin contact. The National Institute for Occupational Safety and Health recommended the limits of exposure for xylene at 100 ppm as a Time-Weighted Average for up to a 10-h work shift and a 40-h work week and 200 ppm for 10 min as a short-term limit. Reducing and eliminating xylene from tissue processing and staining decreases the costs and creates a hazardous free environment in the laboratory [1,2]. The aim of the study is to find an alternative way for deparaffinizing tissue sections for routine H&E staining with natural products like grape juice. In this study, grape juice is used as an alternative to xylene and the staining characteristics such as nuclear and cytoplasm staining, clarity of cell, uniformity of staining in these sections and crispiness of staining are graded according to morphometric analysis.

MATERIALS AND METHODS:

The study was conducted in Sree Balaji medical college and hospital in histopathology lab with 45 paraffin embedded blocks of fibroid tissue. Two sets of fibroid tissue sections were taken from each tissue block. One section was labelled as group A and the other as group B. Sections labelled as group A were deparaffinized with xylene and sections labelled group B were deparaffinized with grape juice. After staining tissue, histopathology slides were scored depending on staining quality which includes nucleus staining, cytoplasm staining, crispiness of staining, uniformity and clarity of the stain.

RESULTS:

Scoring of 1 to 3 was given according to H&E staining characteristics of the tissue for each parameter that include, nuclear staining, cytoplasm staining, uniformity of staining, clarity of the cell and crispiness of staining. The results from the analysis of the slides from group A and group B are listed in Table no:1 (fig 1). It was observed that group B had better uniformity and crispiness of staining compared to group A (fig 2,3). The percentage of the scoring of group B is 88.67% and that of group A is 83.33%.

Table no: 1

SL.NO	CHARACTERS	GROUP A- XYLENE AS DEPARAFFINIZING AGENT	GROUP B- GRAPE JUICE AS DEPARAFFINIZING AGENT
1	Nuclear stain	2.6	2.6
2	Cytoplasm stain	2.8	2.8
3	Uniformity of staining	2.2	2.5
4	Clarity of the cell	2.8	2.8
5	Crispiness of staining	2.1	2.6
	Total	12.5(83.33%)	13.3(88.67%)

Morphometric analysis: score ranges

Score 1-5 -Good

Score 6-10 -Very good

Score 10- 15– Excellent

Group B Grape juice as deparaffinizing agent has a better score of 13.3 when compared to group A xylene as deparaffinizing agent with score of 12.5.

DISCUSSION:

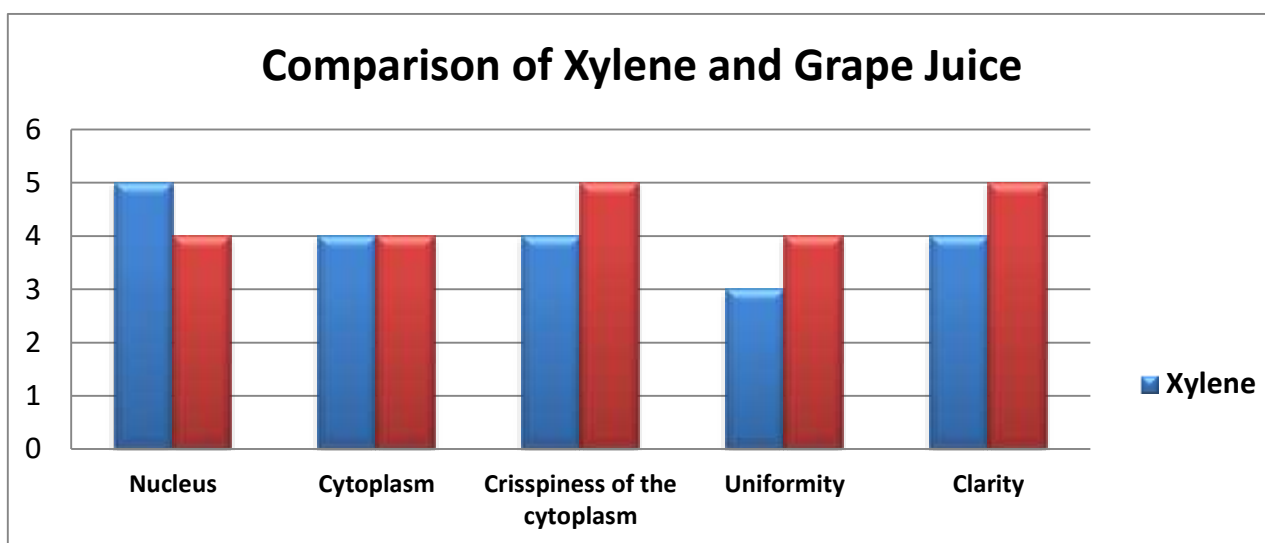
Xylene is an important chemical used in the histopathology laboratory for dehydrating tissue and to removes the wax in Hematoxyline and Eosin staining.Xylene helps in decreasing the tissue damage during processing. It is easily available and cost-effective material for use in laboratory but has some side effects. Histopathological technicians are frequently exposed to xylene and other reagents in the laboratory during the histopathology processing and staining procedure. There are high chances for the technicians to get exposed to varying levels of xylene while doing the tissue processing and staining procedure leading to airway obstructive disease. Exposure to small amounts of xylene also causes health hazards from acute to chronic disease. The severity of health hazards depends on several factors like duration of exposure, intensity of the chemical, and toxicity of the xylene [4].

Many alternative chemicals like limonene reagents, aliphatic and aromatic hydrocarbons, and vegetable oil mixtures are being used to substitute xylene as a deparaffinizing agent during tissue processing. Grape juice contains citric acid in small proportion which is being utilized in household cleaning of copper cookware, removes grease and polish, as a wood cleaner and to remove cloth stains. Grape juice as deparaffinising agent has solvent property which is used to dissolve wax [5-6]. The principle is that, grape juice with its physical solvent property prevents the wax from sticking back onto the slides and thereby helps in deparaffinizing the sections [6-8].

In our study we used grape juice as deparaffinizing agent in H&E staining. Two sections were taken from 45 paraffin wax embedded block of uterine fibroid tissue and grouped as Group A and Group B. On morphometric analysis the percentage score of H&E staining using grape juice as deparaffinizing agent was higher (88.67%) than tissues deparaffinised with xylene (83.33%). On comparing the tissue staining, group B shows precedence in the quality of staining than in group A, which is an important parameter in histopathology which will aid the pathologist in analysing the tissue to arrive at the diagnosis.

Thus, according to this study grape juice can be used as a non-toxic alternative to xylene. Ghosh S et al also suggested use of biofriendly xylene substitutes in histopathology, which has proved that natural products can be used as a substitute to xylene (3). In a study conducted by Ananthaneni A et al, they evaluated the 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 (5). Pandey P et al did a comparative study to evaluate liquid dish washing soap as an alternative to xylene and alcohol in deparaffinization in hematoxylin and eosin staining showed around 90% efficacy for nuclear and cytoplasmic staining compared to our study which showed 88.67% staining efficacy (6).

Fig no 1: Comparison of various parameters assessed in group A and Group B



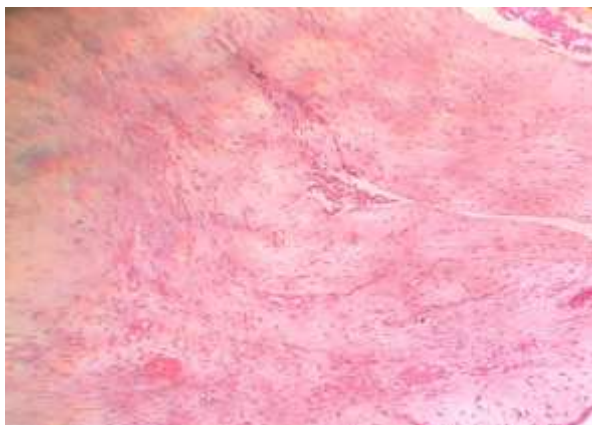


Fig no.2:Section from the fibroid shows benign neoplasm composed of spindle shaped cell with elongated nuclei in a background of myxoid stroma.(in tissue section where grape juice as deparaffinizing agent)



Fig no.3 :Section from the leiomyoma shows benign neoplasm composed of spindle shaped cell with elongated nuclei in a background of myxoid stroma.(in tissue section where from grape juice used as deparaffinising agent)

CONCLUSION:

Grape juice can be used as a potent deparaffinizing agent alternative to xylene in routine hematoxylin and eosin staining thereby reducing the toxic effects of xylene in the histopathology lab.

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