



# Grape Juice as Alternative Agent for Xylene in Hematoxylin and Eosin Staining Procedure

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<b>ABSTRACT:</b> <i>Aim:</i> To compare the grape juice as substitute for xylene agent in hematoxylin and eosin staining procedure. <i>Objective:</i> The objective is to find the use of grape juice as deparaffinizing agent as a substitute to xylene and comparing the staining characteristics in grape juice and Xylene as deparaffinizing agent in hematoxylin and eosin stain. <i>Materials and Methods:</i> The study made in 45 paraffin embedded blocks of breast fibroadenoma tissues. From each block, two sections of 4-5 microns thickness were prepared. Two different groups like A and B were separated. Tissue sections in Group A were stained with H & E method where xylene was used as deparaffinizing agent and Group B were stained with H & E with grape juice, were it used as deparaffinizing agent alternative to Xylene. Staining characteristics were compared with xylene and scoring was given. The scoring was given to the tissue depending upon the staining of the cells, 3-5 score was regarded as satisfactory for diagnosis and less than that is insufficient for diagnosis. <i>Results:</i> Impression obtained with staining characteristics in the cell eg : nuclear, cytoplasm, uniformity, clarity of the cell and crispness of staining for diagnosis was greater with grape juice than xylene. <i>Conclusion:</i> The natural method can be used as deparaffinizing agent such as grape juice an alternative to xylene.	<b>RESEARCH PAPER</b>
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## INTRODUCTION

Xylene is used in histopathology laboratories for tissue processing, staining and mounting. It is a solvent factor allows maximum displacement of alcohol and makes the tissue transparent, helps in paraffin infiltration. In staining procedures xylene is an excellent dewaxing agent, clearing agent and as an important role in contributing to brilliant stained slides. Xylene is extremely useful but it leads to various health hazards and toxicity to skin, eyes, nose, nervous system, and musculoskeletal system [1-3]. Hazards of xylene can occur through inhalation, ingestion, and eye or skin contact. The National Institute for Occupational Safety and Health recommended exposure limits 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. Elimination of xylene from tissue processing cut costs, saves time, and improves the laboratory environment [1,2]. The objective of the study is to find natural way of deparaffinizing agents such as grape juice as an alternative to xylene by comparing the staining characteristics such as nuclear

staining, cytoplasmic staining, clarity, uniformity and crispness of staining of grape juice with xylene.

## MATERIALS AND METHODS

The study comprised of 45 paraffin embedded blocks of fibroadenoma breast tissues. Two sections of 4-5 microns thickness were prepared from each block and stained with hematoxylin and eosin in to 2 groups. one group with xylene (Group A) and the other group with grape juice solution (Group B), used as deparaffinizing agents. The stained sections are graded based on the parameters of Nuclear staining, Cytoplasmic staining, Clarity of staining, Uniformity of staining and Crispness of staining.

## RESULTS

The staining characteristics like nuclear staining, cytoplasm staining, uniformity of staining, clarity of the cell and crispness of staining in diagnosis was greater with grape juice solution when compared with xylene.

## DISCUSSION

H & E stained tissue section deparaffinized with xylene is used in histological laboratories as clearing agents. Xylene is a stable fluid which rapidly removes the dehydrating agent and removes the molten wax, causes minimum tissue damage and is cost effective. Histopathological technicians commonly exposed with xylene-contaminated solvents in the workplace are the population most likely to be exposed to high levels of xylene. Xylene causes health hazards from acute exposure to chronic exposure. The type and severity of health hazards depends on several factors, including the amount of chemical and the length of time of exposure [4]. Many substitute chemicals like limonene reagents, aliphatic and aromatic hydrocarbons, and vegetable oil mixtures are being used to substitute xylene as a clearing agent during tissue processing. The main theme of using grape juice as deparaffinising agent for its solvent property used to

dissolve wax [5, 6]. The underlying principle is that the grape juice by its solvent property prevents the wax from resticking onto the slides, thus helping in deparaffinizing the sections [6]. The results shows out of 90 tissue sections, 91.1% of both Group A(Fig 1) and 75% Group B tissue sections( Fig: 2) showed adequate nuclear staining. A statistically significant up gradation of nuclear staining was noted in both Group A and B. Adequate cytoplasmic staining was seen in all the tissue sections of both Group A and Group B . Considering the adequacy of diagnosis of stained sections obtained by two staining methods on the basis of scores obtained, it was evident that 87.52% of the tissue sections of Group B stained slides were found to be adequate for diagnosis as compared to 79.84% of Group A. The results showed that gape juice had showed superior staining properties in all the parameters when compared to the xylene.

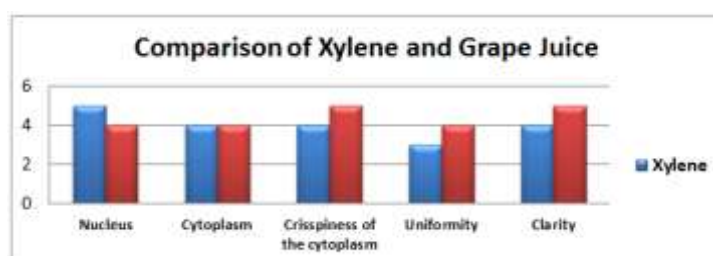


Fig-1: Section from the breast shows benign neoplasm composed of proliferating mammary ducts in a background of myxoid stroma. (In tissue section where Xylene as deparaffinizing agent)

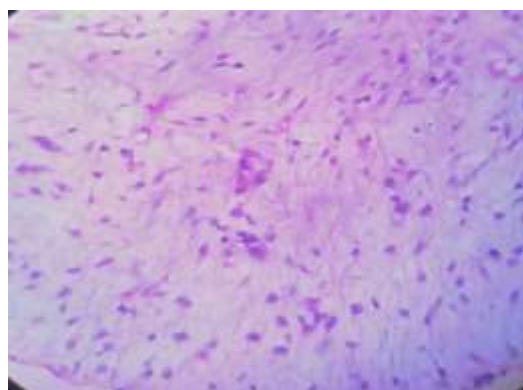


Fig-2: Section from the breast shows benign neoplasm composed of proliferating mammary ducts in a background of myxoid stroma.( in tissue section where grape juice used as deparaffinising agent)

## CONCLUSION

It is essential for the Pathologist to decrease the cost value and to minimize the usage of unsafe chemical agents used in histopathological laboratories. The quality of natural product such as Grape juice as deparaffinising agents is more efficient than xylene in H & E staining procedure. Grape juice is harmless and cost effective. The knowledge of using grape juice as natural replacements to xylene is a small step to the future xylene free histopathological laboratories.

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