

Manjistha as an Alternative to Eosin in Staining

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ABSTRACT

Background: The emergent need in promoting usage of safe and environmentally friendly chemicals in clinical laboratories.

Aim: To organize a dye extract from *Rubia cordifolia* (Manjistha) to stain the histological sections as a substitute for eosin in terms of low cost and simple obtaining natural material.

Methodology: Tissue sections are stained with dye obtained from roots of *Rubia cordifolia* instead of eosin.

Results: The cells in the epithelium and connective tissue were stained red in colour. The cellular morphology was preserved, and clarity of cellular details are moderate.

Conclusion: Manjistha can be used as an alternative stain for eosin.

Keywords: *Rubia cordifolia*, Manjistha, Organic dye.

INTRODUCTION

Histopathology is the study of biologic tissues using a microscope to appreciate the diseased cells. Fixation, tissue processing, embedding followed by sectioning and staining are the processes involved in converting un-stained tissues to stained sections. Exposure to the chemicals used in these processes can cause various health hazards to the laboratory technicians, pathologists and scientists who frequently get exposed.¹

The emergent need and campaign of the international agencies in promoting greener and cleaner environment should urge different agencies including clinical laboratories in using safe and environment friendly chemicals.²

Natural Alternatives Used for Staining

The materials used in the histopathology lab have been undergoing transformation. Historical staining techniques by early pathologists were borrowed from a 17th century scientist Anton Von Leeuwenhoek, who used substances such as madder, indigo and saffron to stain tissues and used rudimentary microscopes to study them.^{3,4}

Haematoxylin (*Haematoxylon campechianum*) is a stain which is commonly used in histopathology lab is also derived from natural source¹, whereas the easily obtainable commercial forms of eosin such as Eosin Y, Ethyl eosin and Eosin B are xanthene dyes.

Rubia cordifolia, also known as Indian madder, is a species of flowering plant in the coffee family, Rubiaceae. It has been cultivated in many

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regions of Asia, Africa and Europe as an important source of a red pigment which is derived from the roots.⁵

The roots of the plant contain an organic compound known as Alizarin, which gives red colour and is used as natural food colourants and natural hair dyes. Dyes derived from natural sources have emerged as important alternatives to synthetic dyes.⁶

The aim of the present study was to organize a dye extract from *Rubia cordifolia* (Manjistha) to stain the histological sections as a substitute for eosin in terms of low cost and simple obtaining natural material.

MATERIALS AND METHODOLOGY

Stain preparation

The stain was prepared by mixing commercially available Manjistha powder with distilled water and set to boiling followed by filtration.

Different concentrations of solutions were prepared using varying amounts of powder and distilled water and it was standardized at powder water ratio of 1:2. One more solution was also prepared where ethanol was added to the combination of powder and distilled water.

Three sections were prepared from each of 10 tissue blocks obtained from the archives of Department of oral pathology, SVS Institute of Dental Sciences, Mahabubnagar, Telangana. The obtained sections were stained, one with haematoxylin and eosin, one with Manjistha powder and distilled water and the other with the stain in which ethanol was added after hematoxylin staining. Later dehydration is done in different grades of ethanol followed by clearing in xylene. Sections were mounted with DPX and coverslips were placed.

RESULTS

On observation under the microscope all the stained tissue sections were analysed under the following criteria:

1. The preservation of tissue architecture and cellular morphology.

2. The clarity of cellular details.

3. The Staining intensity.

The tissue sections processed with Manjistha powder and distilled water were stained red in colour as evident in the figure 1. The preservation of tissue architecture and cellular morphology is same as that of eosin. The clarity of cellular details and staining intensity was better in sections stained with eosin.

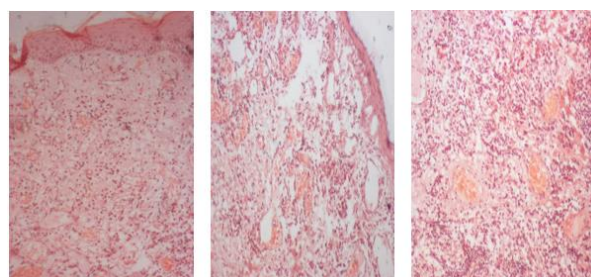


Fig 1: Sections under low power magnification stained with manjistha powder and distilled water.

The tissue sections processed with the Manjistha stain to which ethanol was added showed same colour as that of eosin stained sections as shown in Figure 2. The preservation of tissue architecture and cellular morphology is same as that of eosin. The clarity of cellular details and staining intensity was slightly better in sections stained with eosin.

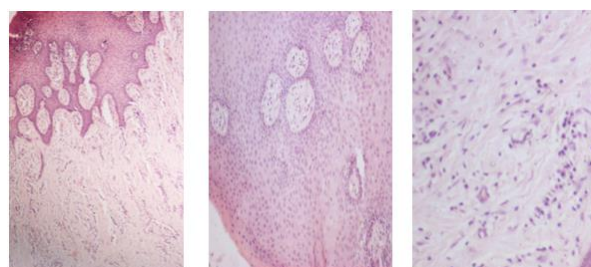
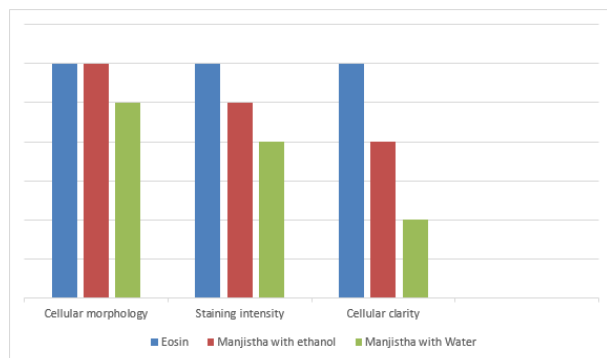


Fig 2: Section under low power magnification stained with manjistha powder, distilled water and ethanol.

The overall results showed that the staining efficacy of Manjistha is on par with eosin which is depicted in Graph 1.



Graph 1: Graph showing comparison of three stains.

DISCUSSION

Rubia cordifolia, commonly known as “Manjistha” is a spready shrub found in the hilly region of the country. The dye obtained from the roots has been used as natural food colorant and hair dye apart from its medicinal value.

The tissue sections processed with Manjistha powder and distilled water were stained red in colour because of the natural red colour pigment producing nature of Manjistha. Ethanol was added to the solution in order to make the composition same as eosin. The tissue sections treated with the solution containing ethanol were stained pink which was similar to eosin.

The preservation of tissue architecture and cellular morphology is similar to that of eosin. The clarity of cellular details and staining intensity was slightly better in eosin than Manjistha.

CONCLUSION

Based on the study conducted the dye that is produced from Manjistha can stain the tissue sections. The stain obtained from the root extract can be used as a counter stain in terms of low cost

and simple obtaining natural material. So, it can be an alternative to the routinely used xanthene dye eosin.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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