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Beta vulgaris – as an alternative to eosin in histopathological staining procedure.

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Abstract

Introduction: Hematoxylin and Eosin staining is the globally practiced staining technique for histology and his to pathology studies. Hematoxylin is a natural stain while Eosin is a synthetic dye. Despite all its uses and advantages in the field, Eosin Y is considered hazardous to health. Accordingly, the goal of this study is to find an alternative to Eosin, and develop the extract of Beetroot (Beta Vulgaris) into an alternative staining solution to eosin in the his to pathological staining procedure.

Aim and Objectives: To evaluate the staining qualities of Beta Vulgaris (Beetroot) extract as a natural substitute of eosin in Hematoxylin-Eosin staining procedure. To identify safe natural alternative of Eosin. To analyse staining ability of beetroot extract as a potent histological stain and to compare staining ability of Beetroot extract with that of eosin.

Materials and Method: 30 For mal in - fixed paraffin - embedded blocks were retrieved and 2 slides were prepared from each block. One slide was stained

with conventional Hematoxylin and Eosin staining method and the other with Hematoxylin and Beetroot stain.

Results: The staining procedure carried out using Beta vulgaris (beetroot) extract as alternative to eosin in his to pathological staining, was at par with the conventional Hematoxylin and Eosin staining procedure.

Less time was required for Beetroot staining procedure (40- 45 minutes) compared to conventional staining (60-65 minutes).

Conclusion: Beetroot extract has the advantage of being non-toxic, non - inflammable, non-hazardous, economical, and easy to handle. So beetroot stain can be used as an alternative to eosin in routine his to pathological procedures.

Keywords: Beetroot, Eco-friendly stain, Counterstain

Introduction

His to pathology is the study of bio logic tissues using a microscope to appreciate the diseased cells. Fixation, de hydration, clearing, embedding, sectioning and staining are the processes involved in converting unstained tissues to stained sections. [(1)] There are two types of stains, natural stains and synthetic stains. Hematoxylin is a natural dye, obtained from the Mexican tree Hematoxylin campechianum while the Eosin is a synthetic dye. Eosin is a synthetic Xanthene dye but it is hazardous to animal and human health. The continuous exposure of chemicals from synthetic stains can affect the health of pathologists, technicians etc. Eosin can causes irritation to skin, eye and mucosa which may cause chelitis, stomatitis and dermatitis. The other disadvantages of synthetic stains like expensive ness of cost, non – biodegradability also made natural stains gaining importance to substitute the synthetic stains. [(2)] Beetroot (Beta vulgaris L.) is crop belonging to the Chenopodiaceae family having, bright crimson colour. It is famous for its juice value and medicinal properties. The main pigment found in Beetroot is recognized as Beta lain and it is one of the richest sources of Beta lain which is used for imparting a desirable red color. It produces purple to red color. [(3)] So, this study was done to see ability of Beet root Extract as a natural alternative to the eosin.

Aims & Objectives

To evaluate the staining qualities of Beta Vulgaris (Beet root) extract as a natural substitute of eosin in Hematoxylin-Eosin staining procedure and to compare staining ability of Beetroot extract with that of eosin.

Material & Methods

Total 30 paraffin embedded tissue blocks of oral biopsy specimen were retrieved from archives of Department of Oral and Maxillofacial Pathology, Ahmedabad Dental College and Hospital, Gandhinagar. Two slides were prepared from each block. One slide was stained with conventional Hematoxylin and Eosin staining method and the other with Hematoxylin and Beetroot stain.

- Group I (Control Group): 30 slides were stained with conventional Hematoxylin and Eosin staining method.
- Group II (Experimental Group): 30 slides were stained with Hematoxylin and freshly prepared Beetroot Extract stain.

Preparation of Beetroot staining solution

Beetroots were sufficiently washed, peeled and cut into small pieces. These pieces were then blended in a food processor with blender. A relatively non-contaminated juice was obtained which was then filtered to obtain thin consistency of juice. The pure watery extract was obtained, which was dark red in colour due to rich content of betalains present in the beetroot. The fresh extract obtained was transferred to an airtight container and stored in the refrigerator until the time of staining procedure.

Figure 1: Fresh Beetroot and Prepared Staining Solution.

Table 1: Procedure for Hematoxylin and Beetroot staining.

Reagent	Time
Xylene I	5 min
Xylene II	5 min
Absolute Alcohol	5 min
95% Alcohol	5 min
60% Alcohol	5 min
Running Water	5 min
Hematoxylin	2.5 min
Running Water	5 min
Ammonia Water	3 dip
Running Water	5 min
Beetroot Stain	1 min
Xylene I	3-4 dip
Air dry	-
Xylene II	1 dip
Total Time Required	40-45 min

The stained sections were evaluated separately, and graded, based on the criteria Clarity of staining (Present = score 1, absent = score 0) Uniformity of staining (Present = score 1, absent = score 0) Nuclear staining (Adequate =score 1, inadequate = score 0) Cytoplasmic staining (Adequate =score 1, inadequate = score 0) Adequacy of staining (Adequate =score 1, inadequate = score 0) The stained sections were given a score from 0-5 where above given criteria were compiled and summation of above five criteria was done as per criteria used by Lizbeth Raju et al. [4]

Total Score: 0-2: Poor; 3: Good; 4: Satisfactory.

5: Excellent.

Results and Observation

All 60 slides stained were independently examined by experts. They assessed the slides based on criteria of Clarity of staining, Uniformity of staining, Cytoplasmic staining, nuclear staining and Adequacy of staining. Overall scores of two observers were analyzed statistically by chi square test of independence to verify whether Observed score of Group II are significantly different or not different from Group I as per scoring of the observer.

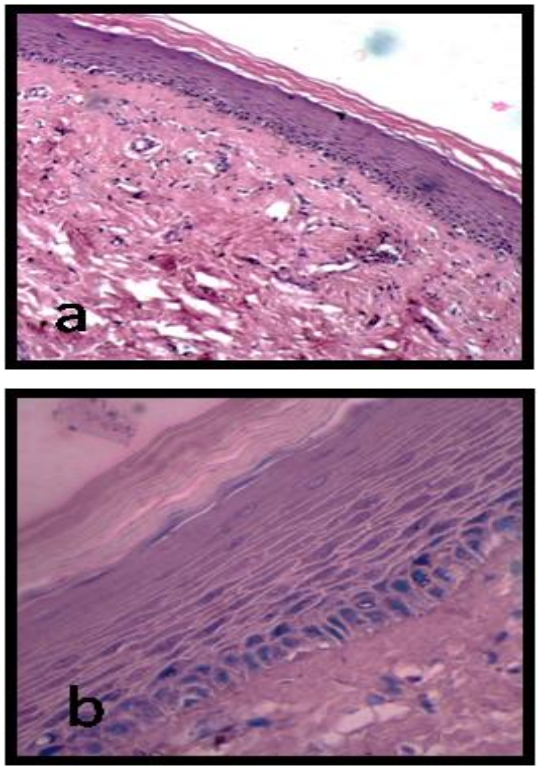

Figure 1: Haematoxylin & Beetroot Staining (a).10x and (b) 40x in Oral Submucous Fibrosis.

Table 1: Comparison of total score of histopathological evaluation with no. Of slides in group i and group ii.

Criteria of His to pathological evaluation	Group	(0) Inadequate (N = 30)	(1) Adequate (N = 30)	Mean	Standard deviation	Chi square value	p value
Clarity of staining	I	6	24	0.8	0.41	0.373	0.542
	II	8	22	0.73	0.45		
Uniformity of staining	I	7	23	0.77	0.43	0.000	1.000
	II	7	23	0.77	0.43		
Cytoplasmic staining	I	3	27	0.9	0.305	0.577	0.448
	II	5	25	0.8	0.379		
Nuclear staining	I	3	27	0.9	0.305	0.218	0.640
	II	2	28	0.93	0.253		
Adequacy of staining	I	1	29	0.96	0.182	1.071	0.301
	II	3	27	0.9	0.305		

Discussion

Hematoxylin and Eosin (H&E) is a globally practiced staining technique for Histology and His to pathology studies. In a typical tissue, nuclei are stained blue by the basic dyehematoxylin, whereas the cytoplasm and extra cellular matrix have varying degrees of pink staining with acidic Eosin. Nowadays, the availability of the plant products has greatly increased the consumer preferences for using natural dyes to minimize the health hazards caused by synthetic and inorganic dyes. Ola MA [(5)], Sridhara SU [(6)] studied the efficacy of Hibiscus as an alternative to eosin. While the contrast and intensity were comparable to eosin-stained sections, the time required for staining was comparatively longer. In contrast, in our study, the time taken for adequate staining by beetroot extracts was 1 minute, which is much less than that required by hibiscus. Sutra Dhar P [(7)] studied the staining potential of beetroot on fungal structures. They concluded that due to slight acidic nature of Beta lain pigments, they can stain the fungal cell wall. Moreover, a citric acid buffer and ph 5 intensified the red color of extract. This is similar to our study. The results concluded that beta vulgaris can be potential stain for wet mount preparation in mycology. Singnarpi S [(8)] performed a study to stain oral smear in exfoliative cytology with extract from Beetroot. Their study showed that staining intensity was similar in beetroot and HE. They concluded that beetroot can be used as a stain in exfoliative cytology and as a cytoplasmic stain in place of eosin. Obeta U [(9)] studied the efficacy of beetroot and turmeric as alternative to hematoxylin and eosin staining.

They concluded that beet root extracts has hematoxylin like staining characteristics, and can be used as a counterstain to eosin, Jasphin[(10)] did screening of natural stains from Indian plants in rat skin tissue. Similar to Sutra Dhar P [(7)], Singnarpi S [(8)], they concluded that beta vulgaris showed better staining ability as a cytoplasmic stain. Current study of staining by beetroot extract on sample of 30 tissue sections has identified beetroot extract as a potential alternative to eosin in his to pathological staining procedure.

Conclusion

From our study we concluded that Beetroot stain the sections with sufficient clarity, uniformity, cytoplasmic

staining, nuclear staining and adequacy of staining and can be used as an alternative to the Eosin.

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