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Original Research Article

ASSESSMENT OF STAINING POTENTIALITY OF CURCUMA LONGA L. (TURMERIC) AS A NATURAL ALTERNATIVE TO EOSIN IN HAEMATOXYLIN AND EOSIN STAINING IN HISTOPATHOLOGY

Mitul Prajapati[1*], Gargi Rathod[2], Heena Parmar[3], Purvi Prajapati[4]

[1*]Assistant Professor, Department of Oral Pathology and Microbiology, Ahmedabad Dental College & Hospital, Ahmedabad, Gujarat, India

[2, 3, 4] B.D.S, Goenka Research Institute of Dental Science, Gandhinagar, Gujarat, India

Corresponding Author: Dr.Mitul Prajapati, Department of Oral Pathology & Microbiology,Ahmedabad Dental College & Hospital, Ahmedabad, Gujarat, India Email ID: opadc08@gmail.com

Abstract:

Introduction: Hematoxylin and Eosin staining is the globally practiced staining technique for histology and histopathology 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 Curcuma Longa L. (Turmeric) as annatural and eco friendly alternative staining solution to eosin in the histopathological staining procedure.

Aim and Objectives: To analyse staining ability of turmeric extract as a potent histological stain and to compare staining ability of turmeric extract with that of eosin.

Materials and Method: 30 Formalin-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 turmeric stain. Stained slides were evaluated with scoring criteria by independent obsever and statistical analysis was done.

Results: C.longastains the tissues in different shades of yellow color and there were no statistical significant differenceseen in between Haematoxylin& Eosin stain and Haematoxylin& Turmeric stain. Less time was required for haematoxylin and turmeric staining procedure (40- 45 minutes) compared to conventional staining (60-65 minutes).

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

Keywords: Turmeric, Eco-friendly stain, Counter stain

1. Introduction

Histopathology is the study of biologic tissues using a microscope to appreciate the diseased cells. Fixation, dehydration, 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 expensiveness of cost, non-biodegradability also made natural stains gaining importance to substitute the synthetic stains.(2)

Turmeric is a rhizomatous herb that belongs to the family *Zingiberaceae* and the major species is genus *C. longa*.It is a medicinal plant extensively used in Ayurveda, Unani and Siddha medicine as a home remedy for various diseases including biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism, and sinusitis. Curcumin (Diferuloylmethane), the main yellow bioactive component of turmeric has been shown to have a wide spectrum of biological actions - anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antidiabetic, antibacterial, antifungal, antiprotozoal, antiviral, antifibrotic, antivenom, antiulcer, hypotensive and hypocholesteremic activities.It has an aromatic odor and tastes somewhat bitter. The characteristic yellow color of turmeric is due to curcumoid, first isolated by Vogel in 1842, comprising of curcumin I (94%), curcumin II (6%) and curcumin III (0.3%).[3]So this study was done to see ability of turmeric extract as a natural alternative to the eosin.

2. Material & Methods

Preparation of Turmeric staining solution :

The rhizomes of *C. longa* were collected from local market of Ahmedabad, Gujarat, India. Rhizomes were then cut into small pieces, air dried. They were then milled to form fine powder using mixer, 15 g of this powdered dissolved in 100 ml of 70% alcohol in a borosil beaker and left for 24 Hrs. After 24 hrs the supernatant was collected with the help of micropipettes in a coupling jar [Figure 1] and stored in the refrigerator until the time of staining procedure.

Figure 1. Turmeric Powder left for 24 hrs and filtered

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 turmeric stain. [Table 1]

Haematoxylin & Eosin (H&E)		**Haematoxylin & Turmeric (H&T)**	
Reagent	Time	Reagent	Time
Xylene I	5 min	Xylene I	5 min
Xylene II	5 min	Xylene II	5 min
Absolute Alcohol	5 min	Absolute Alcohol	5 min
95% Alcohol	5 min	95% Alcohol	5 min
60% Alcohol	5 min	60% Alcohol	5 min
Running Water	5 min	Running Water	5 min
Hematoxylin	2.5 min	Hematoxylin	2.5 min
Running Water	5 min	Running Water	5 min
Acid Alcohol	1 dip	Ammonia Water	3 dip

Running Water	5 min	Running Water	5 min
Ammonia Water	Few dips	Turmeric Stain	1 min
Running Water	5 min	Air dry	
Eosin	1 min	Xylene I	2-3 dip
Running Water	5 min	Xylene II	2-3 dip
95% Alcohol	1 dip		
Absolute Alcohol	1 dip		
Alcohol + Xylene	3 dip		
Xylene I	5 min		
Xylene II	5 min		
Total Time Required	60-65 min	Total Time Required	40-45 min

Table 1: Conventional H&E and H&T Staining Method

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 turmeric stain.
The stained sections were evaluated independently and graded based on the criteria given by Lizbeth Raju et al. [4]
Clarity of staining (Adequate = score 1, inadequate = score 0)
Uniformity of staining (Adequate = score 1, inadequate = 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)

Results and Observation :
C. longa stained the tissue in various shades of yellow color. Epithelium and keratin with deep yellowish orange, dull yellow shade in collagen and muscle fibres and RBCs with golden yellow hue. [Figure 2]

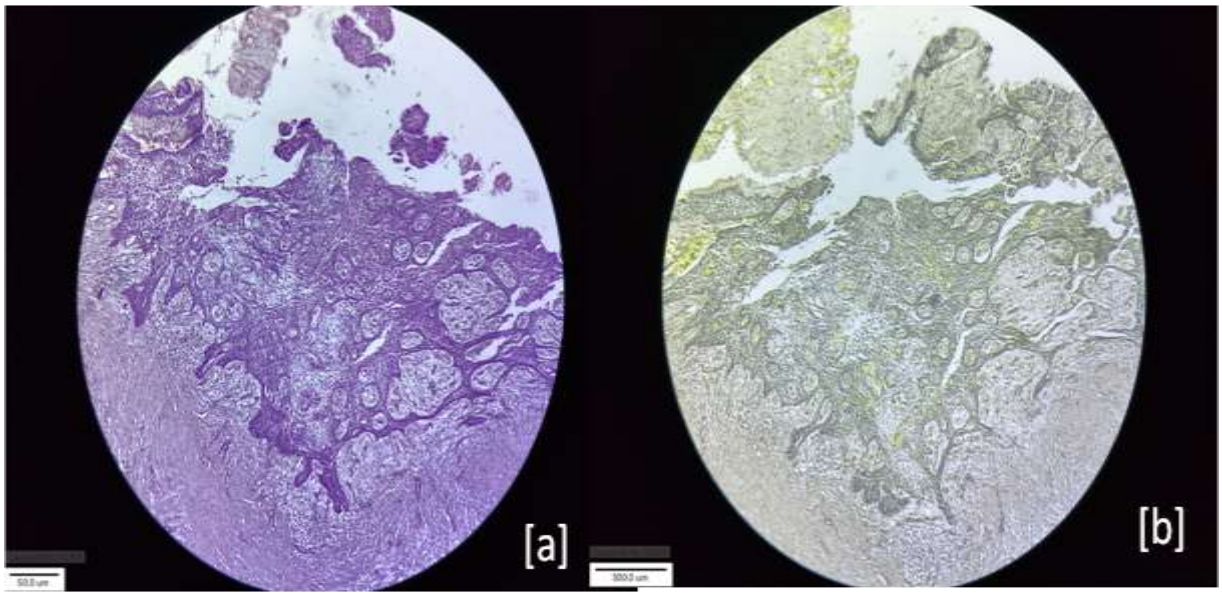

Figure 1[a]. Haematoxylin and Eosin Stain (10x) and [b] Haematoxylin and Turmeric Stain (10x)

All 60 stained slides were independently examined by experts. They assessed the slides based on criteria.Overall scores 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 . In the

present study we found that there was no statistical significant difference in Clarity of staining (p=0.542) , Uniformity of staining (p=1.000), Cytoplasmic staining (p=0.448), nuclear staining (p=0.640) and Adequacy of staining (p=0.301) between Group I (Haematoxylin and Eosin) and Group II (Haematoxylin and Turmeric). [Table 2]

Histopathological Criteria	Group	(0) Inadequate (N = 30)	(1) Adequate (N = 30)	Mean	Standard deviation (SD)	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		

Table 2: Comparison and statistical analysis of histopathological criteria in between Group I and II.

3. Discussion

Hematoxylin and Eosin (H&E) is a globally practiced staining technique for Histology and Histopathology studies. In a typical tissue, nuclei are stained blue by the basic dye hematoxylin, whereas the cytoplasm and extracellular 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. [5]The turmeric dye was standardized by using 5 grams, 10 grams, and 15 grams of turmeric powder in 100 ml of 70% alcohol. However, the best result was obtained with 15 grams of turmeric powderthis was in accordance with the study conducted by Kumar et al (2014) and Bondoc Carloc C (2018) [6,7].According to Marin Abraham et al (2017) and Kumar et al (2014), C. longa concentrations less than 15 mg did not provide adequate staining, which was comparable to our study[8,6]. In the present study we discovered that C. longa stained the tissue in various shades of yellow. Observations of deep yellowish orange were made in epithelium and keratin, dull yellow shade in collagen and muscle fibres, deep yellow shade in bone while a golden yellow shade with RBCs was observed which was in accordance with the study conducted by Kumar et al (2014) , Suryawanshi et al (2017) and Sudhakaran et al (2018) [6,9,10]

Conclusion

In the present study we concluded that turmeric stain the sections with sufficient clarity, uniformity, cytoplasmic staining, nuclear staining and adequacy of staining and can be used as annatural , eco – friendly , safer , readly available and biodegradable alternative to the Eosin.

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