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A Study of Cytoplasmic Staining in Buccal Smears Using Curcuma Longa (Turmeric) Extract as an Alternate to Eosin as Compared to H & E Staining Procedure

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***Abstract*---**Eosin is an efficient synthetic dye belonging to xanthenes group. Eosin dyes are efficient but are hazardous to human and animal health. Owing to this, an alternative dye that has a good staining ability, eco-friendly, biodegradable & commercially available

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turmeric known as Curcuma longa was used. The intense yellow color of turmeric inspired us to explore its efficacy as an alternative for eosin in routine cytological procedure and compare it with routine H&E stain. The present study aims to explore the efficacy of turmeric extract as a cytoplasmic counter stain instead of eosin and a comparative assessment with routine H&E in cytological smear. A total of 30 individuals were initially included in the study, which comprised of 2 cytological smears were taken from each individual and each smear was stained with H&E and Curcuma longa respectively. All the slides were interpreted by 3 observers and findings were recorded separately. The intensity of staining was recorded as follow: 0 – Poor / no stain, 1 – Mild, 2 – Moderate, 3 – Intense. The results were analyzed using Spearsons correlation test.The results revealed that the staining intensity of Curcuma longa is similar to that of eosin. So, Curcuma longa can be suggested as an alternate for eosin in routine cytopathological procedure. Although in H&E staining procedure, Eosin is the most efficient cytoplasmic stain and a counter stain for hematoxylin. Curcuma longa also can be used as an alternative cytoplasmic stain due to its efficacy of staining cytoplasm and its eco-friendly nature. Until recently studies have been published using Curcuma longa in staining tissue sections. The present study is a preliminary attempt using Curcuma longa as a cytologic stain in cytosmears.

***Keywords*---**Fixative, Eosin, Curcuma longa.

Introduction

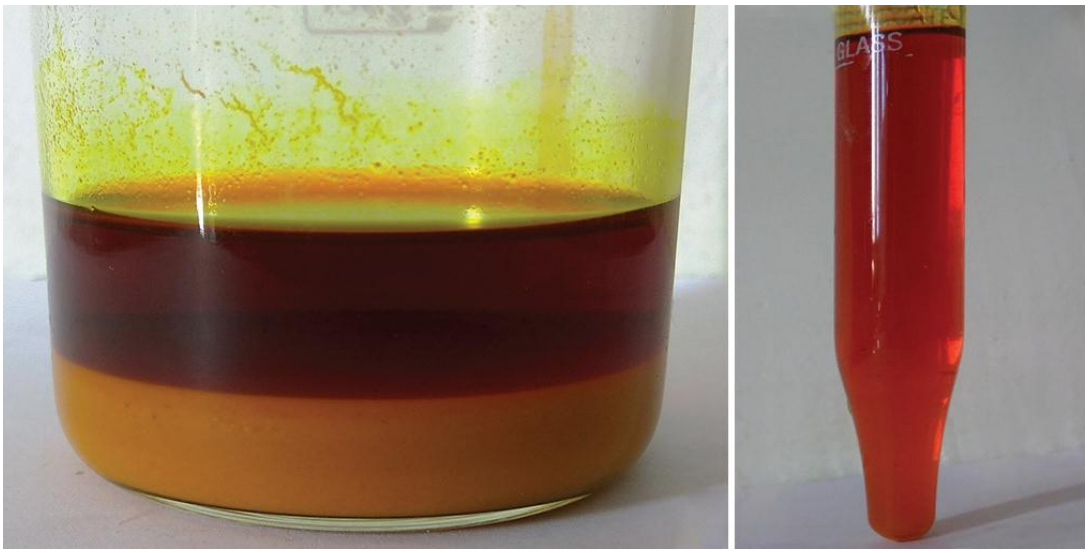
The routine Hematoxylin and eosin staining protocol has been used for several decades for staining procedures due to their characteristic feature of identifying different tissue and their good result with various fixatives. Hematoxylin is a natural dye obtained from the Mexican tree Haematoxylan campechianum, where is Eosin is a synthetic dye[1,4]. Synthetic dyes are often efficient, but due to their hazards to animals and human, many dyes were withdrawn. Due the increasing price and hazards of the synthetic dyes, many naturally occurring dyes have been demonstrated for staining procedures[1,4]. Curcuma longa, a perennial herb of Zingiberaceae (Ginger) family commonly called as turmeric has proved to be a better alternative for Eosin stains[2,4,7]. Curcuma longa has acidic reaction and thereby has a strong affinity to the cytoplasm of cells and stains it in yellow[4]. Other than its staining properties it serves as an antioxidant, hepatoprotective, anti-inflammatory, anti-carcinogenic and anti-microbial agent[1,5,6]. It is easily available, cost effective with various biological properties [1,4]. The characterisation of the constituents of a polysaccharide fraction of Curcuma longa demonstrated new acid glycans designated as Ukanons (A, B, C, D) in addition to small amounts of peptide moieties[4]. This study specifies about the efficiency of Curcuma longa as a cytoplasmic counter stain instead of Eosin and a comparative assessment with routine H& E in cytological smear.

Materials and Methods

The present experimental study was conducted in the Department of Oral & Maxillofacial Pathology, Adhiparasakthi Dental College and Hospital, Melmaruvathur, Kanchipuram. A total of 30 individuas for whom 2 buccal smears were taken and each smear was fixed in 95% ethanol. All smear were stained using eosin and curcuma longa extract with conventional H&E staining procedures. Curcuma longa extract was prepared from rhizomes of turmeric. The turmeric rhizome were cut into small pieces, dried and milled to form a fine powder using a normal household mixer-grinder (Figure 1a,b). A 15 g of this powder was weighed using an electronic weighing machine and the fine powder was mixed in 70% alcohol in 100ml and was incubated for 24hrs. After 24hrs, the mixture was centrifuged (REMI LAB CENTRIFUGE) for 3000rpm for 2min. The supernatant was then collected with the help of a micropipette taken as c.longa extract (Figure 2 a,b).All the stained slides were scored for staining intensity by 3 observers.The scoring was given as 0- Poor /no stain, 1- Mild, 2- Moderate, 3- Intense.

(a) Rhizomes of *Curcuma longa*, (b) fine-dried powder of *Curcuma longa*

Figure 1



(a) Prepared solution of *Curcuma longa* in 50% alcohol,(b)centrifuged extract Figure 2(a,b)

Procedure

In the first set of slides eosin stained cytoplasmic stain was used. In the second set of slides, alcoholic extract of *C. longa* was used as cytoplasmic stain and with conventional H&E staining procedure. Sections were dipped in the cytoplasmic stain for 5–7 min followed by approximately 4–5 dips in distilled water and in xylene was finally mounted with the resinous mounting media-DPX. Sections were observed using binocular microscope (Lawrence and Mayo) at ×10 and ×40 magnification of objective lens. Figure 3(a,b,c,d)

Statistical analysis

The results were analyzed using Spearsman correlation test.

Observations and Results

We asked 3 qualified observers to evaluate and compare the staining efficacy of hematoxylin and curcumin. The results revealed that the staining intensity of *Curcuma longa* is statistically significant to that of eosin (p value – 0.003). The overall quality index was calculated by Actual score obtained /maximum score of *Curcuma longa* (0.85) is higher than eosin (0.75).

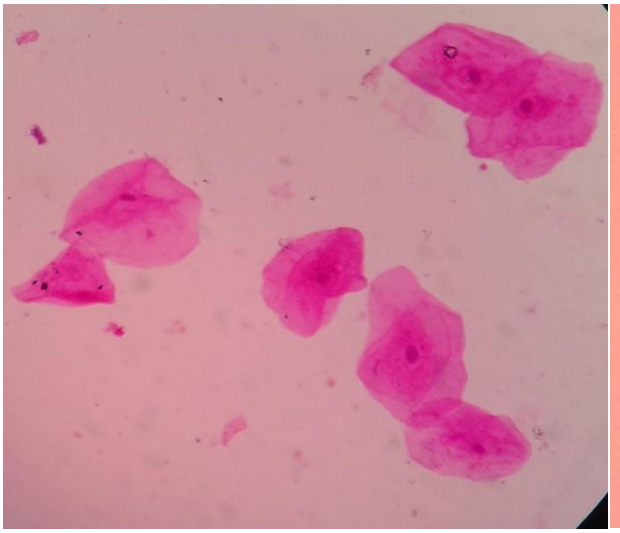


Figure 3(a)

Figure 3(b)

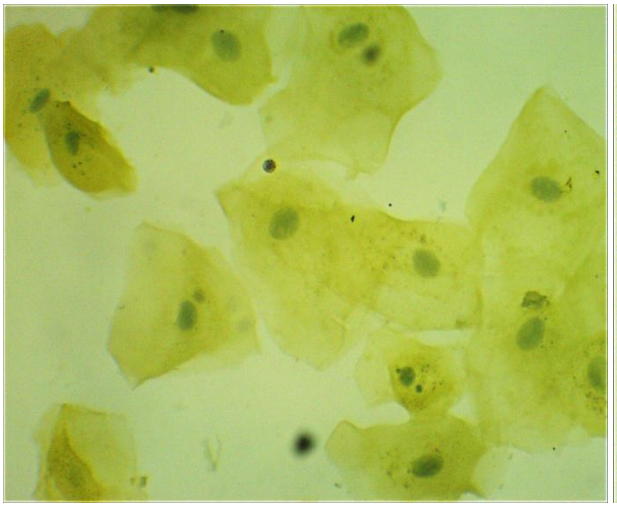


Figure 3(c)

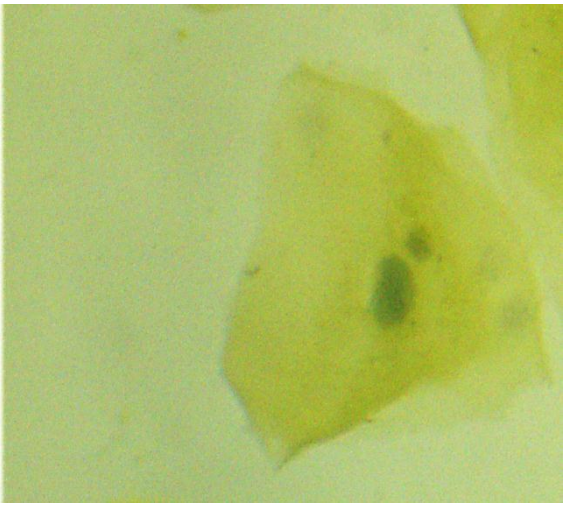


Figure 3(d)

Discussion

Natural dyes offer an important alternative as they are safer to use with no health hazards. Turmeric a rhizomatous herb that belongs to the family of Zingiberaceae and the major species is genus C. Longa is used as an alternative to the Eosin which is a synthetic dye. Curcumin is a principle coloring pigment in C. Longa which imparts yellow color to the cytoplasmic component of the cell.[1,7] The ability of the dye to stain specific tissue structures is determined by certain factors, one of which is acidity of the stain. Acidic structures would be stained by basic dye while basic structures would be stained by acidic dyes. As C. Longa stains the cytoplasmic structures it is deduced to be acidic in nature.[3] C.Longa contains sapponins, tannins, flavannoids – polyphenolic compounds and

alkaloids.[1,2] Tannins are the most important ingredient which are necessary for dyeing. Flavonoids are primarily recognised as pigments responsible for autonomal burst of hues and the many shades of yellow, orange and red. Saponins are known to reduce surface tension and this property also enhance staining. The phenol component are acidic due to their ability to release hydrogen from their hydroxyl group and hence the ability of C. Longa to stain the basic part of the cell, mainly protein part of cytoplasm.[1] In our study, we compared the staining intensity of C. Longa and Eosin, in which C. Longa showed better result than that of Eosin. Rather than the staining property C. Longa has properties such as anti-inflammatory, anti oxidant, anti carcinogenic, anticoagulant, antidiabetic, antibacterial, antifungal, antiprotozoal, antiviral and antifibrotic properties.[1,7]
C. Longa is a safer alternative which has shown almost equivalent staining to eosin and it also has the following advantages such as:

- Cost effective
- Eco- friendly
- Easy availability
- Less technique sensitive
- No harmful chemicals
- Nonallergic and non-toxic
- Safely disposable

Conclusion

Curcuma longa also can be suggested as an alternative cytoplasmic stain due to its efficacy of staining cytoplasm and its eco-friendly nature. Until recently studies have been published using **Curcuma longa** in staining tissue sections.The present study is a preliminary attempt using **Curcuma longa** as a cytological stain in cytosmears.

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