Curcuma longa extract – Haldi: A safe, eco-friendly natural cytoplasmic stain

Hema Suryawanshi, Rupali Naik, Pramod Kumar, Rolly Gupta
Department of Oral Pathology and Microbiology, Chhattisgarh Dental College and Research Institute, Rajnandgaon, Chhattisgarh, India

INTRODUCTION

A dye can be described as a colored substance that has affinity to the substrate on which it is being applied. Dyes are used to color a variety of substrates (e.g., textiles, leather, paper and hair) from liquid in which they are completely or at least partially soluble and may require a mordant to enhance its binding on the fiber. Biological stains are generally used to add color to animal and plant tissues, microbes and spores to make them optically distinct. This technique of visual labeling is known as staining. Before synthetic dyes came into use, natural stains were already available and were used for various purposes. However, their use was limited, and to compensate for their shortcomings, superior

Abstract

Background: Eosin is most widely used synthetic dye belonging to the xanthene group. These dyes are efficient but are hazardous to human and animal health. With the increasing awareness of a green earth, it is advisable to use more of eco-friendly and biodegradable material which can be effectively achieved by the use of natural dyes obtained from plants and other natural sources. Turmeric, available as Curcuma longa (domestic), has long been in use in the subcontinent as a spice and flavoring agent in most food preparations. Its health benefit as a natural antibiotic and anti-inflammatory has been successfully established by several researchers. The intense yellow color imparted by turmeric inspired us to explore its efficacy as a potential alternative for eosin in routine histopathological procedures.

Aim: The aim of this was to explore the efficacy of turmeric extract as a stand-alone counterstain for hematoxylin and its comparative assessment with routine H and E staining.

Materials and Methods: The rhizomes of C. longa were cut into small pieces, dried and milled. This powder was dissolved into alcohol and centrifuged using a centrifugal machine. The supernatant was then collected with the help of micropipette. This supernatant was used as a counterstain for hematoxylin.

Results: The data were analyzed using Mann–Whitney U test with Statistical Package for the Social Sciences version 15.0 (SPSS Inc.). The P value obtained was statistically insignificant (P > 0.05).

Conclusion: Although eosin is the most efficient counterstain for hematoxylin, turmeric can also be used as an alternative for eosin.

Keywords: Curcuma longa, hematoxylin, rhizomes, turmeric
and more effective synthetic dyes are still in use. They are superior in terms of rapidity and versatility and are widely available at an economical price.\(^2\) However, most synthetic stains are carcinogenic while others may cause hypersensitivity reactions on long-term exposures. Their toxic properties also pose a problem in their safe disposal. This consequently led to the development of stains which are not only environmentally safe but are also harmless.\(^3\)

The most sought nuclear stain, hematoxylin, is obtained from the Mexican tree Haematoxylon campechianum; it is an example of a natural dye which is widely used in histochemistry. On the contrary, its counterstain eosin is a synthetic dye which belongs to the xanthene group.\(^2\) These dyes are not only efficient but also has deleterious effects which are hazardous to human and animal health. With the increasing awareness of a green earth, it is advisable to use more of environment-friendly and biodegradable material. This can be effectively achieved by the use of natural dyes obtained from plants and other natural sources. Moreover, as many developing countries can no longer afford the ever-increasing cost of synthetic dyes, the use of cost-effective and naturally occurring dyes from plants is always a better option.\(^4\)

Turmeric, available as *Curcuma longa* (domestic) and *Curcuma aromatica* (wild), has long been in use in the subcontinent as a spice and flavoring agent in most of the food preparations.\(^5\) Turmeric is used not only as a principal spice but also has its medicinal uses in Ayurveda as natural antibiotic and anti-inflammatory. It has been successfully established by several researchers in the past.

**Contents of turmeric powder**

Turmeric powder (Haridra in Sanskrit) is basically a dried rhizome powder of *C. longa*, a perennial herb of the Zingiberaceae (ginger) family which appears morphologically similar to *Zingiber officinale* except for the intense yellow color. 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%). The chemical structure of recently identified cyclocurcumin was determined by Roughley & Whiting in 1973.\(^5,6\)

Curcumin is an orange-yellow crystalline powder which is practically insoluble in water. It forms a reddish-brown salt with alkali and is soluble in alcohol, alkali, ketone, acetic acid and chloroform. It is widely cultivated in Asia and other tropical countries. Turmeric contains protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrates (69.4%) and moisture (13.1%).\(^5\)

The ethanolic extract of rhizomes contains sodium curcuminate, \([\text{feruloyl-}(4\text{-hydroxycinnamoyl})\text{-methane}]\) and \([\text{bis-}(4\text{-hydroxycinnamoyl})\text{-methane}]\) [Figure 1].\(^6\)

This staining property of turmeric can be employed in various histochemical techniques and it can be a potential replacement of synthetic dyes. Only a couple of studies have been done in the past to evaluate the staining potential of turmeric. Thus, keeping the above facts in mind, we tried to explore the efficacy of turmeric extract as a potential alternative for eosin in routine histopathological procedures.

**MATERIALS AND METHODS**

The present experimental study was conducted in the Department of Oral Pathology and Microbiology, Chhattisgarh Dental College and Research Institute, Rajnandgaon, Chhattisgarh.

**Method of turmeric dye preparation**

The rhizomes of *C. longa* were cut into small pieces and dried in hot air oven at 40°C. They were then milled to form fine powder using a normal household mixer-grinder [Figure 2]. 10 g of this powder was weighed using an electronic weighing machine. This was dissolved in 80 ml of 50% alcohol. The prepared solution was left overnight in a tightly sealed container [Figure 3] and was centrifuged the following day, using a centrifuge machine (REMI LAB CENTRIFUGE) at 3000 rpm for 2 min. As studies revealed that curcumin is more soluble in alcohol and when equal proportions of alcohol and water are used, the solubility of curcumin increases exponentially.\(^6,7\) The supernatant was then collected with the help of a micropipette. Thereafter, the supernatant was stored and used as a regular counterstain for hematoxylin [Figure 3]. It has been observed that the turmeric and alcohol solution when immediately centrifuged and used as a stain gave inferior
results compared to the ones which were left overnight. It is because of the fact that solubility of curcumin in alcohol increases with time to a certain extent.[6]

**Procedure**

Archival wax blocks of previously diagnosed cases were taken. Two sets of each comprising of 10 slides of 4–6 μ thickness were prepared. All the slides were deparaffinized in xylene, rehydrated and first set of 10 slides was stained with the conventional H and E staining protocol. In the second set of slides, alcoholic extract of *C. longa* was used as cytoplasmic stain. Sections were dipped in the cytoplasmic stain for 5–7 min followed by approximately 4–5 dips in distilled water. All the sections were thereafter dehydrated in increasing grades of alcohol, cleared in xylene and finally mounted with the resinous mounting media-DPX (Merk specialties private LTD, Mumbai). Sections were observed using binocular microscope (Lawrence and Mayo) at ×10 and ×40 magnification of objective lens.

**Statistical analysis**

*P* value was obtained using Mann–Whitney U test with Statistical Package for the Social Sciences version 15.0 (IBM corporation, Armonk, New York, United States).

**OBSERVATIONS AND RESULTS**

We asked 7 different qualified observers to evaluate and compare the staining efficacy of hematoxylin and curcumin (turmeric; H and C) with that of hematoxylin and eosin (H and E) for 8 different tissue structures such as epithelium, keratin, collagen fibers, muscles, adipocytes, blood vessels and red blood cells (RBCs), cartilage and bone [Figures 4-10]. Set of 10 slides was evaluated. Based on the quality of staining, the observers graded the slides as 1, 2, 3 [Table 1].

Grading for each set of 10 slides was done for both H and E [Table 2] and H and C [Table 3] and comparison between the staining ability of H and E and H and C was made [Table 4].

On the relative comparison between H and E and H and C, we found that the staining ability of epithelium, keratin, muscles, adipocytes, blood vessels and RBCs with curcumin was almost as good as eosin. whereas for collagen fibers, cartilage and bone staining ability of curcumin was not as good as eosin with *P* < 0.05 , which suggests that curcumin stains these structures in a different manner imparting a yellowish hue to them.
DISCUSSION

Natural dyes offer an important alternative as they are safer to use with no health hazards, have easy disposability, are biodegradable and can be used to make compost for agricultural purposes after they have been extracted. Thus, the use of nonallergie, nontoxic and eco-friendly natural dyes has become a matter of significant importance due to the increased environmental awareness in order to avoid some hazardous synthetic dyes.

Curcumin is the principal coloring pigment which imparts characteristic yellow color to fabrics and Indian cuisines. Phytochemical screening of the dye confirmed the presence of saponins, tannins, flavonoids and alkaloids. Among these compounds, tannins and flavonoids are the substances which can give the color. Tannins are primarily recognized as the pigments responsible for the autumnal burst of hues and the many shades of yellow, orange and red in flowers and food. 90% of all yellow
dyes are flavonoids. The fastness of these yellow dyes is greatly affected by the mordant and the photosensitivity of the chromophores. Saponins are known to reduce surface tension and this property also enhances staining.\[1,9\] The turmeric solution is acidic and stains the basic parts of the cell. It contains flavonoids, which are typically polyphenolic compounds. Phenols are acidic, due to their ability to release the hydrogen from their hydroxyl group and hence the ability of \textit{C. longa} to stain the basic parts of the cell, mainly protein part of cytoplasm.\[10\] The dye tissue interactions are determined by certain factors, one of which is ionic interactions. Acidic structures will take up basic stain and vice versa.\[2,4\]

Curcumin, besides imparting its yellowish coloration when used in combination with hematoxylin, resulted in contrast that was well appreciated. Above reasons inspired us to try curcumin as an alternative to eosin.

Results obtained were immensely fascinating. \textit{C. longa} extract stains tissue structures in a remarkably different and distinct pattern giving different shades of yellow. It stains epithelium and keratin with deep yellowish orange, collagen and muscle dull yellow, RBCs with bright yellow and bone deep yellow. Results were similar to previous study by Kumar \textit{et al.}, where turmeric had shown good and comparable staining to eosin for collagen and skeletal muscle fibers.\[11\] In our study, also, turmeric has shown comparable staining to eosin in case of muscle fibers. Even striations are well appreciated.

Although statistically eosin has proved to be better over turmeric, turmeric has shown almost equivalent staining to eosin for many tissue structures such as adipocytes and muscle fibers. Our study showed that in the epithelium, melanocytes are well appreciated and properly differentiated in curcumin staining where the melanocytes appeared bright brown against the light yellow background of the epithelium. Blood vessels along with RBCs were well differentiated from rest of the tissues with a characteristic yellowish hue imparted by turmeric, although at some occasions few observers found it difficult to differentiate them from the background staining.

Undoubtedly, eosin has proved to be better over turmeric, but turmeric being a safer alternative has shown almost equivalent staining to eosin and it also has the following advantages.

- Cost-effective
- Eco-friendly
- Easy availability
- Less technique sensitive
- No harmful chemicals
- Nonallergic and nontoxic
- Non carcinogenic
- Safely disposable.

CONCLUSION

\textit{C. longa} extract is a revolutionary and effective histological dye that is not only cost-effective but readily available. It can be an effective, natural and safe alternative to eosin which is a relatively more expensive synthetic dye.

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Conflicts of interest
There are no conflicts of interest.

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