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Research Article

Evaluation of Turmeric (Curcuma longa L.) extract as a natural alternative to Eosin in histopathological staining

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Abstract

Background: Synthetic dyes like eosin are widely used in histopathology but are associated with health hazards and environmental concerns. Objective: The study aims to evaluate the staining potential of Curcuma longa L. (turmeric) extract as a substitute of eosin in standard hematoxylin and eosin (H&E) staining. Methods: Two techniques were used to slice and stain archival paraffin-embedded tissue samples: the conventional H&E process and a modified variant that substituted an alcoholic extract of turmeric for eosin. The staining quality of various tissue components such as keratin, collagen, muscle, salivary glands and epithelium was evaluated. Stained slides were assessed for cellular outline, cytoplasmic details, nuclear clarity and morphology using a three-tier grading scale (poor, good, excellent). The staining quality scores were compared using the Mann-Whitney U test. Results: The turmeric produced a distinct yellow to brownish-yellow cytoplasmic staining with excellent differentiation. Significant differences were observed for collagen ($U = 67.5$, $p = 0.0049$) and salivary gland staining ($U = 67.5$, $p = 0.0049$) while keratin ($U = 112.5$, $p = 0.0658$), epithelium ($U = 104.0$, $p = 0.7238$) and muscle ($U = 106.5$, $p = 0.778$) did not show statistically significant differences. Although eosin provided superior sharpness in some tissue structures, turmeric demonstrated comparable staining quality across most components. Conclusion: This fining support the use of Turmeric as a natural and non-toxic alternative, making it a suitable option for laboratories seeking sustainable staining methods.

Keywords: Curcuma longa L., H&E staining, Tissue staining.

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Introduction

Staining is a fundamental technique in biological microscopy, enabling enhanced visualization of cellular structures, tissues, and microorganisms. The development of synthetic dyes have greatly improved the effectiveness and dependability of staining. Among commonly used stains, hematoxylin and eosin (H&E) remain the gold standard (1). Hematoxylin, a popular nuclear stain made from Haematoxylum campechianum heartwood, is a great illustration of a natural dye that has been shown to be effective in histology. Its widely used cytoplasmic counterstain, eosin, on the other hand, is a synthetic dye based on xanthene that may pose health hazards. Both dyes have good staining qualities, but their ongoing usage raises questions regarding environmental effect and long-term safety.(2, 3). In light of these concerns, there is growing global interest in developing safer, more affordable, eco-friendly, and readily available alternatives for use in histopathology.

Natural plant extracts are biodegradable, non-toxic, and offer a renewable and potentially safer option for histological staining (4). Turmeric (Curcuma longa L.), a widely used medicinal plant and culinary spice, is known for its rich yellow pigment, curcumin. The curcumin exhibits good binding properties and has been traditionally used for its antimicrobial, antioxidant, and staining properties (5).

Although turmeric has been explored in various biomedical applications, its role as a histological stain remains relatively under-studied. The present study was undertaken to evaluate the effectiveness of turmeric extract as a natural substitute for eosin in histopathological staining.

Materials and Methods

The study was conducted after seeking approval from Institutional Ethics Committee (KIMSDU/IEC/06/2018).

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Preparation of Turmeric Extract

Fresh rhizomes of Curcuma longa L. (turmeric) were procured from a local source. The rhizomes were washed, cut into small pieces, and dried in a hot air oven at 40°C. The dried pieces were then ground into a fine powder using a standard household mixer grinder. A total of 10 g of this turmeric powder was dissolved in 80 ml of 50% ethanol. The solution was left overnight in a tightly sealed container at room temperature. After incubation, the mixture was centrifuged at 3000 rpm for 2 minutes. The supernatant was carefully collected using a micropipette and stored in an airtight container. This alcoholic extract of *C. longa* was used as a cytoplasmic counterstain in the study.

Tissue sample preparation

Paraffin embedded tissue blocks from previously diagnosed cases were retrieved from the department archives. Only those blocks that contained adequate tissue specimens for sectioning were included in the study. Blocks with insufficient tissue or those from undiagnosed cases were excluded.

Slide preparation and staining procedure

Sections of 4–6 µm thickness were obtained from each selected paraffin block using a rotary microtome. A total of 60 sections were prepared and divided into two sets of 30 slides each. Set one Stained with routine Hematoxylin and Eosin (H&E) protocol. And set two stained with the Hematoxylin and alcoholic extract of *C. longa* as a cytoplasmic counterstain.

Stained slides were evaluated under a light microscope for the following parameters cellular outline, cytoplasmic details, nuclear details and overall morphology. Each parameter was assessed and scored using the following scale (Poor) difficulty in appreciating the specific tissue structure, G (Good) adequate appreciation of the specific tissue structure and E (Excellent) clear and fine appreciation of the specific tissue structure.

Statistical analysis

The staining scores obtained for each parameter in the two groups (H&E vs. H&T) were tabulated. The data obtained from both sets of slides (H&E vs. H&T) were compared using the Mann-Whitney U test as the data were ordinal and non-parametric. A *P* value < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA).

Results

The staining efficacy of curcumin (turmeric) in combination with hematoxylin (H and T) was evaluated and compared to the conventional hematoxylin and eosin (H and E) staining method. A total of 15 histological slides were prepared and stained with each method. The stained slides included various tissue types such as epithelium, salivary gland, collagen, muscle, blood vessels, adipose tissue, and keratin. Based on the quality of staining, the observers graded the slides as 1, 2, and 3 that is Grade 1 is poor, grade 2 is good and grade 3 is excellent quality of staining. Staining quality was evaluated for each tissue structure across both staining techniques, and the results are represented in Table 1.

Table 1: Comparison of staining ability of Hematoxylin & Eosin and Hematoxylin & Turmeric for various tissue structures (15 slides of each section stained with H & E and H & T).

Tissue	Haematoxyline and			Hamatoxyline and			P value
	Poor	Good	Excelle	Poor	Good	Excelle	
Kearatin	0	0	15	0	2	13	0.0658
Epithelium	0	2	13	0	1	14	0.723
Collagen	0	0	15	2	1	12	0.049
Muscle (Figure	0	1	14	1	1	13	0.778
Salivary gland	0	0	15	0	1	14	0.004
Total	0	3	72	3	6	66	

, **and * indicate P value <0.05, <0.01, <0.001 respectively.*

Histopathology analysis of Epithelium and keratin were consistently stained with deeper intensity in the H and T method, with a majority of the slides receiving Grade 3. Turmeric provided a deep yellow hue to epithelial cells and keratin, which allowed for clear visualization and differentiation (Figure 1).

Collagen fibers and muscle tissues stained with turmeric appeared dull yellow but were still well differentiated from surrounding structures (Figure 2 and 4). Salivary glands stained with H and T showed a brownish-yellow appearance and were generally graded as excellent (Grade 3), nearly equivalent to H and E (Figure 3). Although H and E provided more defined contrast, turmeric demonstrated comparable efficacy, with most slides graded as good.

The staining quality scores between H&E and H&T groups were compared using the Mann–Whitney U test. Significant differences were observed for collagen (U = 67.5, p = 0.0049) and salivary gland staining (U = 67.5, p = 0.0049), while keratin (U = 112.5, p = 0.0658), epithelium (U = 104.0, p = 0.7238) and muscle (U = 106.5, p = 0.778) did not show statistically significant differences.

Figure 1: Histopathological analysis of epithelium and keratin in Hematoxylin & Eosin (A) and Hematoxylin & Turmeric (B)

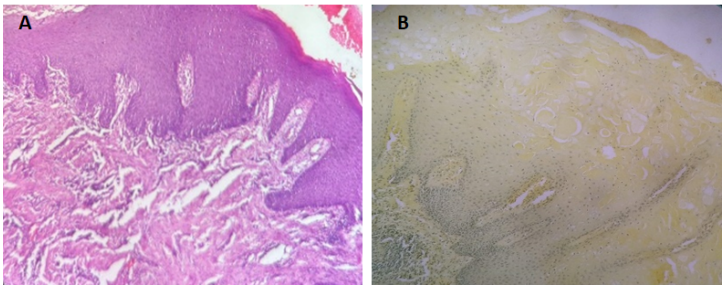


Figure 2: Histopathological analysis of collagen in Hematoxylin & Eosin (A) and Hematoxylin & Turmeric (B)

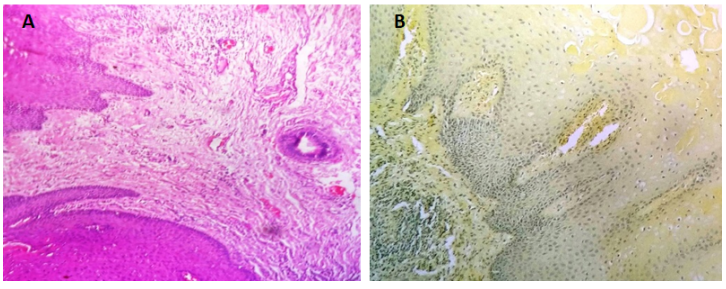


Figure 3: Histopathological analysis of salivary gland in Hematoxylin & Eosin (A) and Hematoxylin & Turmeric (B)

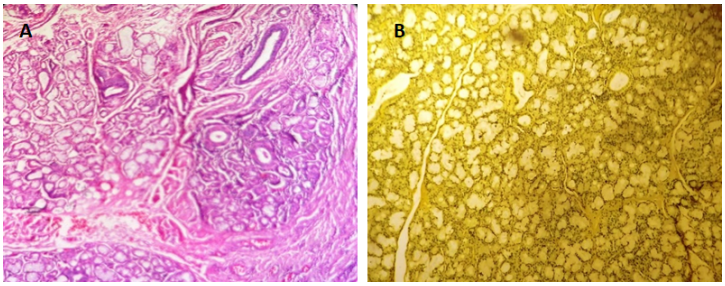
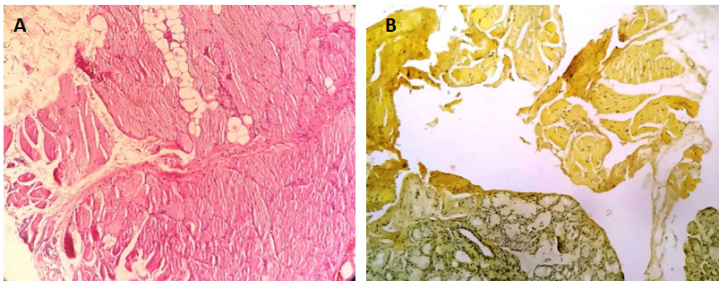


Figure 4: Histopathological analysis of muscle in Hematoxylin & Eosin (A) and Hematoxylin & Turmeric (B)



Discussion

In the present study, turmeric was evaluated as a natural alternative to eosin for histological staining. The findings suggest that turmeric staining provided clear differentiation of tissue components with staining quality nearly comparable to that of eosin.

Curcumin, when used alongside hematoxylin, provided a well-appreciated contrast, staining tissue structures with distinguishable shades of yellow. Curcumin, besides transmitting its yellowish coloration when used in combination with hematoxylin, resulted in contrast that was well appreciated. The results demonstrated that turmeric was capable of providing good staining quality across multiple tissue types. Most notably, turmeric exhibited a strong affinity for cytoplasmic components, with tissue structures such as epithelium and keratin displaying distinct deep yellow coloration. Melanocytes within the epithelium were clearly differentiated as bright brown cells against a light yellow background, indicating effective visualization and contrast. The difference in staining efficacy between H and T and H and E was statistically significant. This suggests that while H and E remains the gold standard in terms of staining sharpness and contrast, H and T was almost as effective in staining different tissue types, particularly muscle fibers and adipocytes.

Since long turmeric or *C. longa* is valued for its principle colouring constituent Curcumin, which imparts yellow colour to food and textile fibers. The ability of a dye to stain specific tissue structures is determined by certain factors, one of which is the acidity of the stain (6). Acidic structures would be stained by basic dyes while basic structures would be stained by acidic dyes. Owing to the strong affinity of *C. longa* for the cytoplasm, it can be deduced that the *C. longa* extract dye is acidic in nature (7). This contemplation is corroborated by the phytochemical analysis of the effective column fraction. It composed of flavonoids, which are typically polyphenolic compounds (8). Phenols are acidic, due to their property to release the hydrogen from their hydroxyl group, hence the property of *C. longa* to stain the basic parts of the cell (9).

Tannins are the most significant ingredients which are essential for colouring reagents. Flavonoids are primarily identified as the pigments responsible for the autumnal burst of hues and the many shades of yellow, orange and red in flowers and food. Approximately 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 (10).

Turmeric probably acts by scavenging free radicals, decrease lipid peroxidation and stimulating DNA damage repair enzymes in healing oral sub mucous fibrosis a precancerous condition and also by inhibiting proliferation of fibroblasts, which proves its stronger affinity for collagen (11). This fact is also supported by another research work. Turmeric also has an ancient medicinal role in muscle sprains and injuries which suggests its stronger affinity for muscle fibers (12) (13).

While the results of this study demonstrate the potential of turmeric (curcumin) as a natural alternative to eosin. The study has some limitations. Limited tissue variety only a selected range of tissue types was examined. The staining performance of turmeric on other specialized tissues (e.g., nervous tissue, lymphoid organs) remains to be explored. Turmeric stains may be less stable over time and may vary slightly depending on the extraction method and concentration and the Standardization of preparation and application protocols is necessary to improve reproducibility and consistency. Further studies involving larger sample sizes, diverse tissue types, and long-term stability assessments are recommended to validate and standardize its use in histopathology.

Conclusion

The present study found that turmeric offers satisfactory staining clarity in multiple tissue components and provides adequate contrast when used along with hematoxylin. Although eosin remains superior in providing sharpness and consistency, turmeric shows promising performance, particularly for collagen, keratin and salivary gland staining. Turmeric showed consistent affinity for cytoplasmic elements, likely due to its acidic polyphenolic constituents such as flavonoids and tannins. These findings support the potential use of *C. longa* extract as a natural histological stain, offering a cost-effective, eco-friendly and non-toxic alternative to conventional synthetic eosin dyes.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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