DOI: 10.7860/JCDR/2023/66801.18628

Original Article

Dentistry Section

Exploring the Staining Potential of *Zingiber Officinale* Extracts (Ginger) as a Natural Alternative to Eosin in Haematoxylin & Eosin Staining: A Cross-sectional Study

MITULKUMAR RAMESHBHAI PRAJAPATI[1], PRAKHAR AGARWAL[2], BHOOMIKA JADAV[3]

ABSTRACT

Introduction: Most synthetic dyes are carcinogenic, and chronic exposure to these dyes has an impact on the health of laboratory technicians and pathologists. Eosin, a widely used synthetic dye in routine histopathological staining, poses potential risks. *Zingiber officinale* contains phenols and several colouring compounds that have the ability to stain tissues.

Aim: To explore and compare the staining efficacy of ginger extract as a natural dye with synthetic eosin dye.

Materials and Methods: This cross-sectional study was conducted at the Oral Pathology Department of Ahmedabad Dental College and Hospital from April 2023 to June 2023. Fresh rhizomes of *Zingiber officinale* were collected and air-dried. A staining solution of *Zingiber officinale* was obtained by dissolving 25g of powder in 90% alcohol. This solution was used to stain 30 sections of oral biopsy specimens. The stained slides were evaluated by two independent observers using various parameters such as nuclear and cytoplasmic detail, overall histologic appearance, intensity, and contrast. Statistical significance was determined using the Chi-square test.

Results: *Z.officinale* (Ginger) stain the cytoplasm of the cell and connective tissue elements with pale eosin colour, yellowish golden to the RBCs and deep brownish to the bony tissue. Statistical analysis comparable staining intensity, contrast, nuclear staining, cytoplasmic staining and overall histologic appearance between the two groups with p-values of 0.531, 0.917, 1.000, 0.924 and 0.7003, respectively.

Conclusion: *Z.officinale* (Ginger) can be utilised as a natural alternative to eosin in routinely used Hematoxylin and Eosin staining.

Keywords: Histopathology, Natural histopathological stain, Plant extracts, Zingiberaceae

INTRODUCTION

Nature has provided us with many resources, and one of them is colour [1]. Stains are dyes that colour tissues to facilitate the optical differentiation of tissue components. Hematoxylin and eosin are globally used stains in histopathology. Hematoxylin is a natural dye, while eosin is a synthetic dye [2]. Synthetic dyes, despite their effectiveness, have been reported to pose risks to human health. Additionally, the scientific community has experienced repeated shortages of raw materials for stains, especially during times such as pandemics. The development and use of non-toxic, environmentally friendly dyes have garnered significant attention in laboratories worldwide [3].

Impaired respiratory function, airway diseases, and conditions like emphysema are some of the toxic effects of eosin. Direct exposure to open cuts, abraded or irritated skin can lead to systemic injury with harmful effects. Furthermore, eosin can irritate the skin, eyes, and mucous membranes, leading to conditions like chelitis, stomatitis, and dermatitis [4].

The rhizome of *Zingiber officinale*, a member of the Zingiberaceae family, has a deep yellow colour. *Zingiber officinale*, also known as ginger, is commonly used as a whole medicinal plant, as a spice in cooking, in beverage manufacturing, and for making tea. Flavonoids, a polyphenolic compound, contribute to its acidic nature, and colouring compounds that impart colour are found in *Zingiber officinale* [5]. To our knowledge, there are only a few studies available that explore the staining potential of ginger. Ajileye AB et al., concluded that the natural histological dye extracted from *Z. officinale* can effectively stain both muscle fibers and the cytoplasm of a tissue section [6]. Sudhakaran A et al., conducted a study to assess the staining efficacy and shelf life of *Z. officinale* and found it to be considerably longer compared to *Curcuma longa* [5]. The current study emphasises the innovative application of ginger extract in histopathology as a natural alternative to eosin and aimed to evaluate the staining characteristics of ginger extract when used as a counterstain for hematoxylin in Hematoxylin and Eosin staining.

MATERIALS AND METHODS

During a three-month period from April 2023 to June 2023, this cross-sectional study was conducted at the Oral Pathology Department of Ahmedabad Dental College and Hospital, Ahmedabad, Gujarat, India. The study received approval from the Institutional Ethics Committee, with the number ADC/IHEC/27.

Inclusion criteria: Well-fixed pathologically diagnosed benign tissues with adequate tissue structure were included in the study.

Exclusion criteria: Biopsies that were not properly fixed, processed, or had inadequate tissue structure, as well as, normal and malignant tissues, were excluded.

From the archives of the Department of Oral and Maxillofacial Pathology, 30 paraffin-embedded tissue blocks of oral biopsy specimens were retrieved. Two sections with a thickness of 5 µm were prepared from each tissue block.

Procedure

Steps in the dye preparation of *Zingiber officinale* (Ginger) dye: Fresh rhizomes of *Zingiber officinale* were collected from the local market of Ahmedabad, cut into pieces, air-dried, and then ground into a powder using a mechanical grinder. A total of 25 g of *Zingiber officinale* powder was diluted in 100 mL of 90% alcohol. The prepared solution was left for 24 hours and then filtered using Whatman's filter paper No.1 to collect the supernatant fluid [5]. The

supernatant fluid was obtained using a micropipette and kept in a coupling jar for staining [Table/Fig-1].

Group-I (Control Group): 30 slides were stained with conventional Haematoxylin and eosin staining method (H&E) [Table/Fig-2] [7].

Group-II (Experimental Group): 30 slides were stained using Hematoxylin and Ginger (H&G) [5].

[Table/Fig-1]: Ginger solution left for 24 hours: (a) and prepared Stain of Ginger solution in coupling jar (b).

H&E staining	
Steps	**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
Harris haematoxylin	2.5 min
Tap water	5 min
1% acid alcohol	1 dip
Tap water	5 min
Ammonia water	Few dip
Eosin	1 min
Tap water	5 min
95% alcohol	1 dip
70% alcohol	1 dip
Absolute alcohol	1 dip
Xylene I	5 min
Xylene II	5 min

[Table/Fig-2]: Staining method of Haematoxylin and Eosin (H&E) [7].

Procedure for staining with *Zingiber officinale* [5]: After dewaxing the tissue sections on a hot plate, they were cleared in xylene for 15 minutes. Hydration was done using 80% alcohol, followed by rinsing with water. The sections were stained with Harris hematoxylin for five minutes and then rinsed in running water. Differentiation was performed using 1% Acid alcohol for 2-3 seconds, followed by bluing for 15 minutes in running water. The sections were counterstained with the prepared *Zingiber officinale* stain for eight minutes, rinsed in water, cleared in xylene, and mounted.

Histopathological evaluation: Two blinded oral pathologists independently evaluated the slides and scored them according to the criteria used by Raju L et al., [Table/Fig-3] [8].

STATISTICAL ANALYSIS

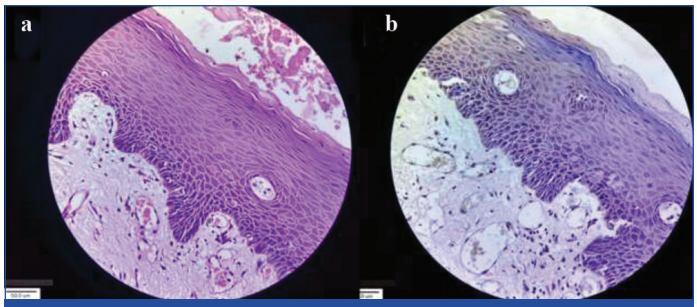
The overall scores of the two observers were analysed using a Chi-square test, with statistical significance considered when the p-value was <0.05. International Business Management (IBM) Statistical Package for Social Sciences (SPSS) software was used to conduct all statistical analyses.

Parameters	Score
Overall histologic appearance	0 (Inadequate) or 1 (Adequate)
Nuclear staining	0 (Inadequate) or 1 (Adequate)
Cytoplasmic details	0 (Inadequate) or 1 (Adequate)
Staining intensity	0 (Inadequate) or 1 (Adequate)
Contrast	0 (Inadequate) or 1 (Adequate)
Final score	
Poor	0-2
Good	3
Satisfactory	4
Excellent	5

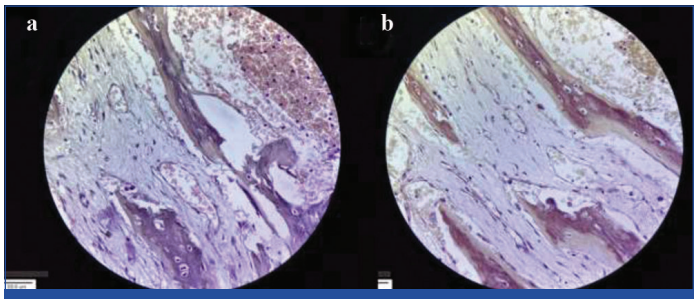
[Table/Fig-3]: Scoring criteria for assessment of stained sections [8].

RESULTS

The authors observed that *Z. officinale* stained the tissue in a manner closer to eosin. *Z. officinale* stained the cytoplasm of the cells and connective tissue components like nerve bundles, muscle fibers, collagen fibers, and blood vessels with a pale eosin colour [Table/Fig-4]. It imparted a yellowish-golden colour to the red blood cells and a deep brown colour to the bony tissue [Table/Fig-5].



[Table/Fig-4]: Histopathological image shows components of epithelium and connective tissue like keratin, collagen fibers and blood vessels stained by H&E (a) and H&G (b) (x40).



[Table/Fig-5]: Histopathological image shows bony tissue and RBCs stained by H&E (a) and H&G (b) (x40).

Observer 1 scored 29 (96.67%) slides of H&E and 27 (90%) slides of H&G as having an adequate overall histologic appearance [Table/Fig-6]. Observer 2 scored 27 (90%) slides of H&E and 25 (83.33%) slides of H&G as having an adequate overall histologic appearance [Table/Fig-7]. Observer 1 scored 13 (43.33%) slides of H&E and 12 (40%) slides of H&G as excellent [Table/Fig-8]. Observer 2 scored 13 (43.33%) slides of H&E and 14 (46.67%) slides of H&G as excellent [Table/Fig-8].

Cross-tabulation of scores showed no significant difference between H&E and H&G groups for staining intensity, contrast, cytoplasmic and nuclear staining, and overall histologic appearance for both observer 1 [Table/Fig-6] and observer 2 [Table/Fig-7] (p>0.05). The total scores between the H&E and H&G groups were comparable (p>0.05) [Table/Fig-8]. The comparison of mean scores for staining intensity, contrast, cytoplasmic and nuclear staining, and overall

Criteria	Group	(0) Inadequate (N=30)	(1) Adequate (N=30)	Mean	SD	Chi-square value	p-value
Staining intensity	I	6	24	0.8	0.41	0.373	0.542
	II	8	22	0.73	0.45		
Contrast	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		
Overall histologic appearance	I	1	29	0.96	0.182	1.071	0.301
	II	3	27	0.9	0.305		

[Table/Fig-6]: Statistical analysis of histopathological criteria with number of slides in Group-I and II by Observer 1.
*SD: Standard deviation; Group 1: Control Group; Group II: Experimental Group

Criteria	Group	(0) Inadequate (N=30)	(1) Adequate (N=30)	Mean	SD	Chi-square value	p-value
Staining intensity	I	5	25	0.8	0.379	1.491	0.222
	II	9	21	0.7	0.466		
Contrast	I	7	23	0.77	0.43	0.098	0.754
	II	6	24	0.8	0.406		
Cytoplasmic staining	I	4	26	0.86	0.345	0.741	0.389
	II	2	28	0.93	0.253		
Nuclear staining	I	2	28	0.93	0.253	0.00	1
	II	2	28	0.93	0.253		
Overall histologic appearance	I	3	27	0.9	0.305	0.577	0.448
	II	5	25	0.8	0.379		

[Table/Fig-7]: Statistical analysis of histopathological criteria with number of slides in Group-I and II by observer 2.
*SD: Standard deviation; Group 1: Control Group; Group II: Experimental Group

Observer	Score	0-2	3	4	5	Total	Mean	SD	Chi-square value	p-value
Observer 1	Group I	0	3	14	13	30	4.3	0.66	2.077	0.557
	Group II	2	3	13	12	30	4.03	1.27		
Observer 2	Group I	0	4	13	13	30	4.3	0.7	3.87	0.276
	Group II	3	2	11	14	30	4	1.48		

[Table/Fig-8]: Statistical analysis of total scores of histopathological criteria with number of slides in Group-I and II by observer 1 and 2.
*SD: Standard deviation

histologic appearance showed no significant difference between the H&E and H&G groups, with p-values of 0.531, 0.917, 1.000, 0.924, and 0.703, respectively [Table/Fig-9].

Criteria	Group-I (Control group) (Mean)	Group-II (Experimental group) (Mean)	Chi-square value	p-value
Staining intensity	0.81	0.71	0.391	0.531
Contrast	0.77	0.78	0.011	0.917
Cytoplasmic staining	0.88	0.88	0.0001	1.000
Nuclear staining	0.91	0.93	0.009	0.924
Overall histologic appearance	0.93	0.83	0.148	0.7003

[Table/Fig-9]: Comparison of mean score and statistical analysis of histopathological criteria with total no. of slides in Group-I and II by observer 1 and 2.

DISCUSSION

Eosin, which is a synthetic Xanthene dye, is a highly effective stain but has harmful effects on both animal and human health. Constant exposure to chemicals from synthetic stains, such as eosin, can lead to health issues for pathologists, technicians, and others. Long-term exposure to eosin can result in cheilitis, stomatitis, dermatitis, and other health problems due to its irritating effects on the skin, eyes, and mucous membranes. In order to minimise health hazards, consumers are now more inclined to use natural dyes, as plant products are readily available [9]. Natural dyes can be derived from various parts of plants such as leaves, flowers, fruits, barks, and roots. The search for biodegradable and safe dyes from natural sources has been prompted by the negative impact of chemicals and synthetic dyes on the environment [10]. Additionally, the rising cost of synthetic dyes has made the use of cheaper naturally occurring dyes more popular, especially in developing countries [11]. Natural dyes have been widely used in histology, histochemistry, and histopathology for diagnostic purposes [12]. The flavonoids present in *Z. officinale* are polyphenolic compounds with an acidic nature that have the ability to release hydrogen and can stain the basic structure of cells [6].

Several researchers have conducted studies on natural dyes for use in histopathology. [Table/Fig-10] provides a comparison of parameters using different natural dyes in various studies, including

Parameters	Present study	Rubina MP et al., [11] (India, 2020)	Sudhakaran A et al., [5] (India, 2018)	Suryawanshi H et al., [13] (India, 2017)	Abraham M et al., [12] (India, 2017)	Kumar S et al., [14] (India, 2014)
Total number of cases	30	100	25	10	40	10
Natural dyes used in place of eosin	Ginger	Turmeric	Ginger, Turmeric	Turmeric	Turmeric	Turmeric

Tissue employed	Epithelium, keratin, Collagen fibers, blood vessels, RBCs, and Bony tissue	Epithelium, Collagen, Muscle, Bone, Adipose tissue	Buccal mucosa, Lichen planus, fibroma, salivary gland and oral Squamous cell carcinoma	Epithelium, keratin, muscles collagen fibers, adipocytes,RBCs, blood vessels RBCs, bone and cartilage	Squamous cell carcinoma and Normal oral mucosa	Epithelium, keratin collagen, muscle fibers, nerves, blood vessels, bone, adipose tissue
Solvent employed	90% alcohol	70% alcohol	90% alcohol	50% alcohol	70% alcohol	70% alcohol
Inference	Ginger can be used as an Efficient counterstain in place of eosin for histopathology	Staining of epithelium, muscle and collagen fibers superior than the bony and adipose tissue.	Ginger staining was better than turmeric	H&E was better than turmeric	Maceration method was better than Soxhlet method	H&E was better than turmeric

[Table/Fig-10]: Comparison of staining parameters in various studies [5,11-14].

the present study [5,11-14]. To avoid bias, the slides were assessed by two independent observers, and they found that the quality of staining with *Z. officinale* did not interfere with the diagnostic process, and the stain did not fade even after a month. *Z. officinale* stained the cytoplasm and components of connective tissues like collagen fibers, muscle fibers, nerve bundles, and blood vessels, with a pale eosin colour and RBCs with a yellowish-orange shade, which was similar to the findings of Sudhakaran A et al., and Ajileye AB et al., [5,6].

Sudhakaran A et al., found that ginger staining was superior to turmeric staining, and the p-values for overall histologic appearance, crispness of staining, background staining, accuracy for diagnosis, and intensity of stain were 0.364, 0.154, 0.071, 0.583, and 0.583, respectively [5]. In the present study, the statistical analysis showed p-values for staining intensity (p=0.531), contrast (p=0.917), nuclear staining (p=1.000), cytoplasmic staining (p=0.924), and overall histologic appearance (p=0.7003) that were greater than 0.05. This suggests that ginger extract could be a potential alternative to eosin in histopathological staining procedures.

Limitation(s)

Due to the three-month duration of the present study, the durability of the stained slides was not evaluated. Additionally, the study did not include normal tissues or malignant neoplasms.

CONCLUSION(S)

The staining potential of H&G was comparable to H&E, indicating that *Z. officinale* (Ginger) could be an efficient counterstain in place of eosin for demonstrating various structures in histopathology. It proves to be cost-effective, readily available, and biodegradable. However, further studies are needed to evaluate the utility of ginger in staining normal and malignant tissues, as well as to assess the durability of the stained slides.

REFERENCES

[1] Meenakshi D, Saxena S, Sireesha Sundaragiri K, Sankhla B, Bhargava A, Rawat M. A comparative cytological study of Eosin & eco-friendly cytoplasmic stains-A pilot study. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS). 2021;20(4):44-49.
[2] Itodo SE, Oyero S, Umeh EU, Ben A, Etubi MD. Phytochemical properties and staining ability of red onion (Allium cepa) extract on histological sections. Journal of Cytology & Histology. 2014;5(6):01-04.
[3] Sachdev SS, Chettiankandy TJ, Sonawane SG, Sardar MA, Kende PP, Pakhmode V. Toward developing natural histologic stains using anthocyanins: A novel approach. J Oral Maxillofac Pathol: JOMFP. 2021;25(1):199.
[4] Cruz PE, De Vera AP, Villa AD. The efficiancy of Santan flower (Ixora occinea Linn.) as an alternative stain to eosinY in Wright-Giemsa stain. LPU-Laguna Journal of Allied Medicine. 2018;3(1):11-21.
[5] Sudhakaran A, Hallikeri K, Babu B. Natural stains Zingiber officinale Roscoe (ginger) and Curcuma longa L. (turmeric)-A substitute to eosin. Ayu. 2018;39(4):220-25.
[6] Ajileye AB, Iteire AK, Arigi QB. Zingiber officinale (ginger) extract as a histological dye for muscle fibers and cytoplasm. International Journal of Medical Science and Public Health. 2015;4(10):1445-48.
[7] Suvarna KS, Layton C, Bancroft JD. Bancroft's theory and practice of histological techniques. Elsevier Health Sciences; 2018 Feb 27.
[8] Raju L, Nambiar S, Augustine D, Soumya SV, Haragannavar VC, Babu A, et al. Lawsonia inermis (henna) extract: A possible natural substitute to eosin stain. J Interdiscip Histopathol. 2018;6(2):54-60.
[9] Jasphin S, Raghavendra AP, Solomon MC, Amuthan A, Singh BM, Kairanna NV, et al. Screening of natural stains from Indian plants in rat skin tissue for histological applications. Journal of Pharmaceutical Negative Results. 2022;3:757-68.
[10] Mohandas R, Ramani P, Sherlin HJ, Gheena S, Ramasubramanian A, Don KR, et al. Organic stains used in histopathology-A systematic review. Drug Invent Today. 2019;11(2):426-32.
[11] Rubina MP, Khrisnan AM, Riyas BK, Mohammed ST, Soumya V. Assessment of staining quality of curcumin as a substitute for eosin in hematoxylin and eosin staining in histopathology. J Res Med Dent Sci. 2020;8(5):146-50.
[12] Abraham M, Nambiar S, Charagannavar V, Augustine D, Sowmya SV, Babu A, et al. Comparison of staining efficacy between turmeric and eosin: A histological study. Journal of Clinical & Diagnostic Research. 2017;11(11):05-08.
[13] Suryawanshi H, Naik R, Kumar P, Gupta R. Curcuma longa extract–Haldi: A safe, eco-friendly natural cytoplasmic stain. Journal of Oral and Maxillofacial Pathology: JOMFP. 2017;21(3):340-44.
[14] Kumar S, Singh NN, Singh A, Singh N, Sinha RK. Use of Curcuma longa L. extract to stain various tissue samples for histological studies. Ayu. 2014;35(4):447-51.

PARTICULARS OF CONTRIBUTORS:
1. Associate Professor, Department of Oral Pathology and Microbiology, Ahmedabad Dental College and Hospital, Ahmedabad, Gujarat, India.
2. Private Practitioner, Private Dental Clinic, Chittorgarh, Rajasthan, India.
3. Homeopathic Practitioner, Dabhva, Gujarat, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Mitulkumar Rameshbhai Prajapati,
Associate Professor, Department of Oral Pathology and Microbiology,
Ahmedabad Dental College and Hospital, Near Science City, Bhadaj,
Ahmedabad, Gujarat, India.
E-mail: opadc08@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Aug 03, 2023
- Manual Googling: Sep 09, 2023
- iThenticate Software: Sep 23, 2023 (5%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jul 31, 2023**
Date of Peer Review: **Sep 08, 2023**
Date of Acceptance: **Sep 30, 2023**
Date of Publishing: **Oct 01, 2023**