

Natural sweeteners as fixatives in histopathology: A longitudinal study

Shankargouda Patil,
Roopa S. Rao,
Ganavi B. S.,
Barnali Majumdar

Department of Oral Pathology and Microbiology, Faculty of Dental Sciences, M. S. Ramaiah University of Applied Sciences, MSR Nagar, Bangalore, Karnataka, India

Address for correspondence:

Dr. Shankargouda Patil, Department of Oral Pathology & Microbiology, Faculty of Dental Sciences, M. S. Ramaiah University of Applied Sciences, MSR Nagar, Bangalore, Karnataka, India - 560054, India. E-mail: dr.ravipatil@gmail.com

Abstract

Background: Fixation is the essential step in histopathological processing of tissues. Since formalin is a corroborated biohazard, its routine use as a fixative is a major health and safety concern and hence the quest for safer alternatives is envisaged. Natural sweeteners like jaggery and honey have proved to be effective tissue preservatives over 24 h. This pioneer eco-idea needs further research to expand its application. **Aim:** (1) To evaluate the fixative property of jaggery and honey over 6 months and ascertaining the results using hematoxylin and eosin stain (H and E). (2) To evaluate the compatibility of jaggery and honey fixed tissues for special stains - Periodic acid Schiff (PAS) and Masson–Trichrome (MT). **Materials and Methods:** Equal bits of commercially available animal mucosae were preserved in 30% jaggery, 20% honey, and 10% buffered formalin (control) over 6 months at intervals. Following which, tissues were subjected to routine H and E, special stains - PAS and MT using standard operating procedures established in our group. **Results:** Formalin, jaggery, and honey yielded satisfactory results post 6 months for H and E and special stains, jaggery was comparable to formalin in tissue preservation. **Conclusion:** We propose the use of eco-friendly jaggery and honey as alternatives to formalin for long term tissue preservation.

Key words: Formalin, honey, jaggery, natural fixatives, tissue preservation

INTRODUCTION

Formalin is traditionally a popular and widely used fixative for histopathology processing of tissues due to its ease, economic viability, fairly fast fixation, effortless processing and an array of histologic techniques that can be performed postfixation. Routine histologic staining methods employed in laboratories are based on the use of formalin fixed paraffin-embedded tissues.^[1] Despite these advantages, the health and safety risks associated with formalin use is a concern. Screening out regrettable substitutes, a handful of synthetic options like alcoholic fixatives, fixatives for nucleic acids, nonalcoholic substitutes, and fixatives with

<10% of formalin are commercially available.^[2] Natural fixatives such as honey, sugar, and jaggery are evaluated by our group as a potential alternative to formalin use.^[3] Interestingly, among these natural fixatives, jaggery syrup has shown better utility as a fixative over a 24 h period, when compared with the reliable honey.^[3] The goal of the present study was to evaluate the fixative property of jaggery and honey over a 6 months period and compare it with formalin as control using quality of H and E, Periodic acid Schiff (PAS), and Masson–Trichrome (MT) staining as benchmark.

MATERIALS AND METHODS

The materials and method adopted is summarized in Flowchart 1. The histomorphological criteria employed are indicated in Table 1. 30% jaggery, 20% honey, and 10% buffered formalin were used. Results were analyzed using Kruskal-Wallis ANOVA and Mann-Whitney U-test. Inter-observer variability was determined by Kappa statistics.

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RESULTS

Formalin fixation for 48 h resulted in significantly better results when compared to honey and jaggery. However, the quality of fixation could not be assured as the tissues fixed by all three fixatives gradually deteriorated over a period of time. Kruskal–Wallis ANOVA and Mann–Whitney *U*-test revealed no significant difference between the three fixatives by the end of 5th month. Further at the end of 6th month, all the three fixatives demonstrated similar results, with jaggery being comparable to formalin in H and E [Graph 1], PAS and MT [Graph 2] stained sections. Kappa value of 0.563 suggested moderate agreement between observers.

DISCUSSION

Although formalin is the gold standard fixative in routine histopathology, a search for its alternative actively explored, primarily due to its adverse effects on health. Recently, the potential carcinogenicity of formaldehyde has been emphasized.^[4,5] The International Agency for Research on Cancer has sufficient evidence for the carcinogenicity of formaldehyde in humans, and hence categorized formaldehyde as carcinogenic.^[6] Repeated exposure or prolonged inhalation of formaldehyde in occupational settings is a causative irritant of the mucous membrane of eyes, nose, mouth and upper respiratory tract,^[7] which has potential health hazards.^[3]

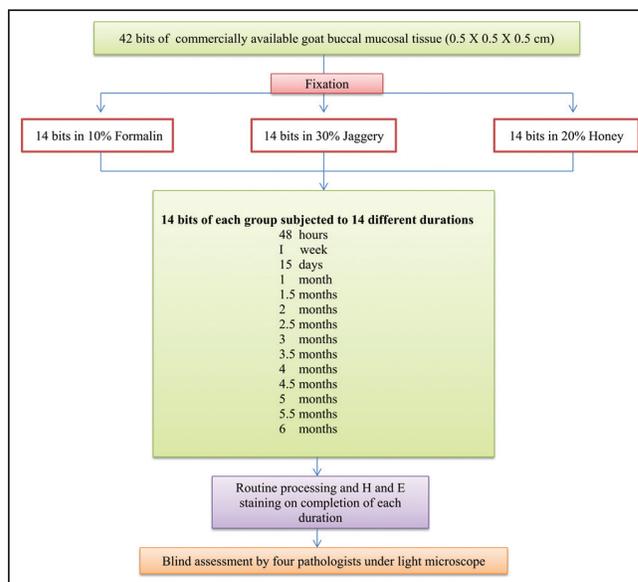
Non-formalin fixatives either contain an aldehyde component other than formaldehyde or do not contain an aldehyde component; thereby, avoiding any potential toxic effects.^[8] Currently, the natural substitutes have better scope due to their desirable results. In this regard, honey was the first proven natural fixative evaluated.^[9-12] Our group has recently reported the effectiveness of jaggery as tissue fixative and its superiority over honey.^[3]

In this study, standardized concentrations of 20% honey and 30% jaggery were taken against 10% formalin as a

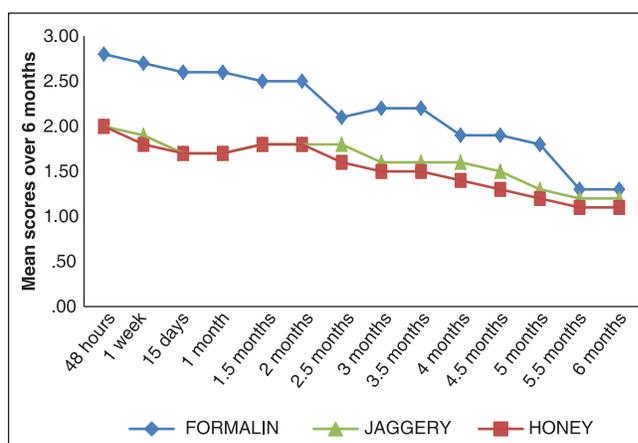
Table 1: Histomorphological criteria

Histomorphologic criteria	Rating on a scale of 1-4
For H and E staining	Poor
Cellular outline	Satisfactory
Cytoplasmic detail	Good
Nuclear detail	Excellent
Staining quality	
Overall morphology	
For PAS and MT staining	
Specificity of the stain	
Staining intensity	

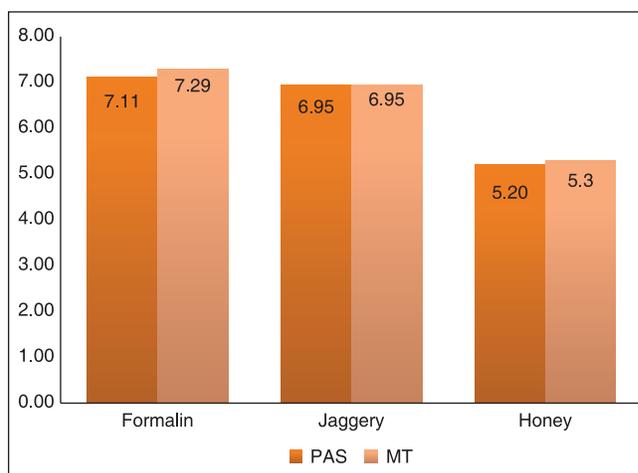
PAS: Periodic acid schiff, MT: Masson–Trichrome



Flowchart 1: Materials and method adopted



Graph 1: Mean scores of formalin, jaggery, and honey over a period of 6 months stained with H and E



Graph 2: Overall scores of Periodic acid Schiff and Masson–Trichrome stained sections fixed with formalin, jaggery, and honey

positive control. All the three reagents were subjected to testing for their efficacy using H and E, PAS, and MT over a period of 6 months. The proposed mechanism of action of honey and jaggery are displayed in Flowchart 2.^[3] Jaggery and honey maintained the high quality gross anatomy. Further, postfixation macroscopic findings are collated in Table 2. and color changes are represented in Figure 1.

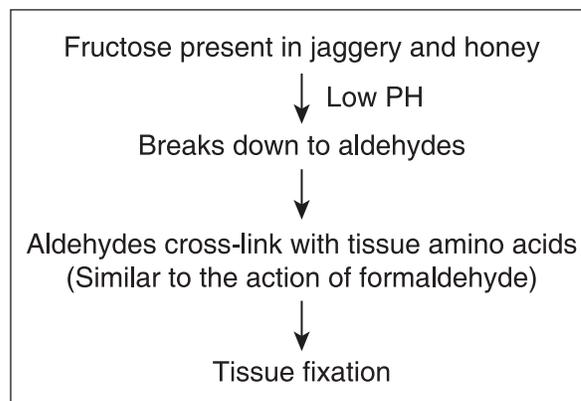
A limitation of honey and jaggery syrup was the growth of molds over a period of time. However, this can be overcome by the addition of thymol crystals. Special attention is required during sectioning of honey and jaggery fixed tissues as they tend to breach due to fragility [Table 3].

The overall morphology of the tissues fixed in jaggery and honey was relatively intact even at the end of 6 months. However, the cellular and nuclear clarity gradually decreased in all the three fixatives. Evident cellular and nuclear shrinkage was observed with jaggery and honey as compared to formalin during the final stages of the study. Staining properties, although deteriorated, was sufficiently discernible in all the three reagents [Figure 2]. The possible cause for slightly inferior results with jaggery and honey would be due to altered cross binding with the tissue as compared to formalin.

Jaggery and honey are at an experimental level, and are yet to be on par with formalin, to be used as a fixative in the long run. Nevertheless, at the end of 6 months all the three fixatives were equally good on H and E stained sections. This proves the long-term efficacy of jaggery and honey as tissue preservatives. Except for sectioning difficulties, they performed well in consequent steps of tissue processing [Table 4]. Interestingly, the specificity and staining intensity

using PAS and MT on jaggery and honey fixed tissues although adequate was not optimal. Nevertheless, jaggery surmounted honey in all aspects [Figure 3].

Use of natural alternatives can be attempted in screening camps, as an instant choice for biopsied tissues in private clinics and as a transporting media. This idea can equally be used for preservation of museum specimens, in the forensic field wherein stored tissue has to be occasionally retrieved for histological examination.



Flowchart 2: The possible mechanism of fixation by honey and jaggery



Figure 1: Macroscopic appearance of tissues after 6 months of fixation with: (a) Formalin, (b) jaggery, and (c) honey

Table 2: Postfixation macroscopic findings

Reagent	Formalin	Honey	Jaggery
Gross morphology	Well-preserved	Well-preserved	Well-preserved
Color	No change	Light brown	Dark brown
Consistency	Firm	Less firm	Less firm
Odor	Pungent	No significant odor	No significant odor

Table 3: Problems encountered with different fixatives with and their remedial measures

Problem	Fixatives	Remedy
Breach in continuity of the section along with folds	Honey	Re-impregnate the tissue for another hour
	Jaggery syrup	Use new blades/apply ice for 15 min on the cut surface of the embedded tissue
Intense staining with eosin	Honey	Handle the sections with care
		Minimize the staining time with eosin

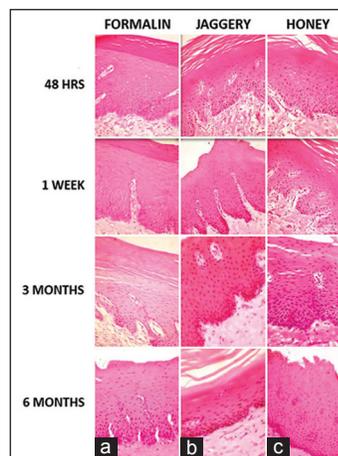


Figure 2: Photomicrographs of tissues fixed with (a) formalin, (b) jaggery, and (c) honey stained with H and E (x40)

Table 4: Pros and cons of jaggery and honey in comparison with formalin

Fixative	Formalin	Jaggery	Honey
Health aspects	Hazardous	Nonhazardous	Nonhazardous
Environmental aspects	Readily biodegradable	Readily bio-degradable (nature's boon)	Readily bio-degradable (nature's boon)
Performance aspects	Best fixative	Strong contender to formalin	Strong contender to formalin
Cost aspects	Relatively high cost	1/20 th of formalin	1/4 th of formalin
Technical feasibility	Easy handling	Easy handling	Easy handling

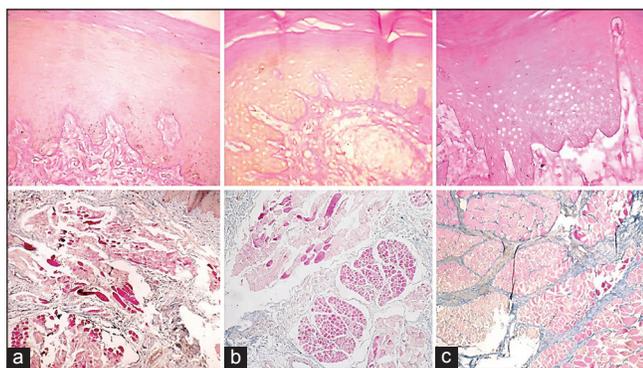


Figure 3: Photomicrographs of tissues fixed with (a) formalin, (b) jaggery, and (c) honey stained with Periodic acid Schiff (x40) and Masson-Trichrome (x10)

CONCLUSION

Implementing eco-friendly fixatives in routine histopathology is necessary. Although, extensive research in this field is required, current evidence encourages the use of jaggery and honey as alternatives to formalin. Further, the consistent performance of jaggery and honey identified in our study is a safety milestone to advance the field of histopathology.

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