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HONEY AS AN ALTERNATIVE FOR ALCOHOL-BASED FIXATIVES IN TISSUE-PROCESSING

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Abstract

Alcohol-based fixatives are used to fix a wide range of tissues including its usage in oral exfoliative cytology. Ethanol is the most used fixative in labs though high in price and creating health problems like nausea, vomiting, drowsiness, unconsciousness, and affecting the eyesight of the lab technician. Because of its disadvantages, a biodegradable, and cost-efficient fixative was discovered. This review compares the efficiency and effectiveness of different concentrations of refined honey as a fixative in comparison with 95% ethanol. Fundamentally, 5 constraints are used in this review to compare the efficiency of the fixatives under consideration namely, cell structure, cytoplasmic and nuclear staining, precision in staining the tissues, and homogeneity of staining while observation. In the process of designing of the review it was studied that 20% of refined honey exhibited good results in comparison with 95% alcohol and 10% honey. In lesser concentrations honey serves to be an outstanding substitute to ethanol as a fixative. The review provides a novel approach using 20% of refined honey as a fixative, providing a positive result with respect to the above-mentioned parameters. Honey is an economical and a naturally available fixative to ethanol with less health hazards and more efficiency. Honey can also be used for a large number of persons who are required to be screened through oral cytology.

Keywords

Cytology, fixative, formalin, honey, natural fixatives, Cytological fixatives, ethanol, tissue preservation

Introduction

The branch of exfoliative cytology is a noninvasive and simple diagnostic practice that can be used for initial identification of oral malignant and premalignant abrasions. Though clinical biopsy accompanied by histopathology is recognized as the golden protocol for the analysis of oral lesions, biopsies cannot be followed in all cases. Since, some patients may be pathologically compromised and a few of them with asymptomatic lesions might not agree with the procedure of biopsy.

The oral epithelium is continually in-contact with various carcinogenic agents, and it frequently experiences the procedure of development where the old cells are incessantly substituted by the new proliferating cells. The microscopic review of these exfoliative cells by excoriating or scrapping is termed as exfoliative cytology. Exfoliative cytological has an important and central role in the analysis of lesions that are medically not perceptible or unsure for malignancy. It is examined to be a rapid, pain-free, non-invasive and a bloodless process, and is appropriate for patients wherein biopsies show contraindications or involves diagnosis that can be performed immediately. Exfoliative cytology is an addition to biopsy but, the conclusive diagnosis is only taken over biopsy however, both has its own benefits and disbenefits.

95% of ethanol is consistently used as a fixative in exfoliative cytology. Ethanol is costly and not easily obtainable so, the pathologist is constantly discovering new cytological fixatives. Methanol and lately natural sweeteners like jaggery and honey have been analytically used as a cytological fixative.

Honey chiefly comprises sugar and water which amounts for 95%–99% of honey dry substance. Major part of the sugar content includes simple sugars like glucose (31.3%)and fructose (38.2%). Honey has revealed to possess an antimicrobial property against an extensive range of fungi and bacteria and is also used as an agent for inhibiting the process of putrefaction and autolysis.

Scientific literatures examine for an economically and an environmentally-friendly cytological fixative as a substitute to ethanol which is very scarce or in experimental phases.

Therefore, an effort has been taken to regulate the efficiency of 10% honey, 20% honey, and 30% honey as an oral fixative, while equalling it with 95% ethanol.

Table: 1 Assessment criteria

Features	Scores and criteria	Scores and criteria
Nuclear staining	Acceptable=1	Unacceptable=0
	Round, smooth, and clear nuclear membrane	Granular, disintegrated, and out of focus
Cytoplasmic staining	Acceptable=1	Unacceptable=0
	Intracytoplasmic membrane and transparent cytoplasm	Disintegrated cytoplasmic membrane, granular cytoplasm, and out of focus
Cell morphology	Preserved=1	Unpreserved=0
	Absence of folds, no overlap, and maintained nuclear to cytoplasmic ratio	Overlapping cells, folded and disintegrated cells
Clarity of staining	Present=1	Absent=0
	Crispness in staining and transparency	Obliterate the nucleus and cytoplasm
Uniformity of staining	Present=1	Absent=0
	Uniformly stained throughout the individual cell	Stained in different shades of color in an individual cell

Source: Efficacy and reliability of various grades of processed honey as a fixative: A comparative study

Table: 2 Staining characteristics of each study table according to assessment criteria

Study parameters	Score	Group				Total	Fisher's exact test *P*
		Group A (%)	Group B (%)	Group C (%)	Group D (%)		
Nuclear staining	Score 0	0 (0)	2 (10.0)	1 (5.0)	2 (10.0)	5 (6.3)	0.75 (NS)
	Score 1	20 (100.0)	18 (90.0)	19 (95.0)	18 (90.0)	75 (93.8)	
Cytoplasmic staining	Score 0	3 (15.0)	3 (15.0)	3 (15.0)	6 (30.0)	15 (18.8)	0.62 (NS)
	Score 1	17 (85.0)	17 (85.0)	17 (85.0)	14 (70.0)	6 (81.3)	
Cell morphology	Score 0	0 (0)	1 (5.0)	1 (5.0)	2 (10.0)	4 (5.0)	0.90 (NS)
	Score 1	20 (100.0)	19 (95.0)	19 (95.0)	18 (90.0)	76 (95.0)	
Clarity of staining	Score 0	2 (10.0)	1 (5.0)	1 (5.0)	4 (20.0)	8 (10.0)	0.51 (NS)
	Score 1	18 (90.0)	19 (95.0)	19 (95.0)	16 (80.0)	72 (90.0)	
Uniformity of staining	Score 0	4 (20.0)	2 (10.0)	1 (5.0)	3 (15.0)	10 (12.5)	0.68 (NS)
	Score 1	16 (80.0)	18 (90.0)	19 (95.0)	17 (85.0)	70 (87.5)	

*$P<0.05$ Statistically significant, $P>0.05$ NS. NS: Nonsignificant

Table: 3 Evaluation of assessment criteria marksamong the study tables

Study parameters	Group	*n*	Mean (SD)	Range	Median (Q1–Q3)	Kruskal–Wallis test	
						χ^2	*P*
Nuclear staining	Group A	20	1.00 (0.00)	1–1	1 (1–1)	2.32	0.51 (NS)
	Group B	20	0.90 (0.31)	0–1	1 (1–1)		
	Group C	20	0.95 (0.22)	0–1	1 (1–1)		
	Group D	20	0.90 (0.31)	0–1	1 (1–1)		
Cytoplasmic staining	Group A	20	0.85 (0.37)	0–1	1 (1–1)	2.19	0.53 (NS)
	Group B	20	0.85 (0.37)	0–1	1 (1–1)		
	Group C	20	0.85 (0.37)	0–1	1 (1–1)		
	Group D	20	0.70 (0.47)	0–1	1 (0–1)		
Cell morphology	Group A	20	1.00 (0.00)	1–1	1 (1–1)	2.08	0.56 (NS)
	Group B	20	0.95 (0.22)	0–1	1 (1–1)		
	Group C	20	0.95 (0.22)	0–1	1 (1–1)		
	Group D	20	0.90 (0.31)	0–1	1 (1–1)		
Clarity of staining	Group A	20	0.90 (0.31)	0–1	1 (1–1)	3.29	0.35 (NS)
	Group B	20	0.95 (0.22)	0–1	1 (1–1)		
	Group C	20	0.95 (0.22)	0–1	1 (1–1)		
	Group D	20	0.80 (0.41)	0–1	1 (1–1)		
Uniformity of staining	Group A	20	0.80 (0.41)	0–1	1 (1–1)	2.26	0.52 (NS)
	Group B	20	0.90 (0.31)	0–1	1 (1–1)		
	Group C	20	0.95 (0.22)	0–1	1 (1–1)		
	Group D	20	0.85 (0.37)	0–1	1 (1–1)		
Total	Group A	20	4.55 (0.51)	4–5	5 (4–5)	3.80	0.28 (NS)
	Group B	20	4.55 (0.69)	3–5	5 (4–5)		
	Group C	20	4.65 (0.49)	4–5	5 (4–5)		
	Group D	20	4.15 (0.93)	2–5	4 (3.25–5)		

*$P<0.05$ Statistically Significant, $P>0.05$ NS. SD: Standard deviation, NS: Nonsignificant

Source: Efficacy and reliability of various grades of processed honey as a fixative: A comparative study

Discussion:

The process of fixing the tissues is examined to be an essential constituent in diagnostic pathology. It functions by alleviating the tissue components and conserving their morphological features. Most commonly used fixatives functions on the principle of cross-linkage of cellular proteins.

Ethanol is the most frequently used fixative in oral exfoliative cytology and possess its own limitations like subjection to filching, costly, combustible, and vaporises easily, it also entails a permit for its procurement. When in-contact with skin and eye, ethanol can also cause irritations. Because of these drawbacks, an examination for an economically and an environmentally-friendly cytological fixative was trialed by few researchers identifying the fixative capability of honey.

Honey has been used as a fixative ever since the Egyptian times. The Egyptians made use of honey throughout the process of preserving of dead bodies. According to the reports, the body of Alexander the Great was conserved in honey for almost2 years before being concealed. Honey is also identified to possess the properties of antiautolytic, antioxidant, antimicrobial, and is also used in tissue hardening process. The likely procedure of fixation through honey is by the incidence of fructose in honey which causes breakdown of aldehydes, and these aldehydes cross-links with the tissue amino-acids which results in tissue fixation.

In the current review, it was experimental that 20% aqueous honey solution (v/v) was significant in tissue fixation. 20% aqueous honey solution (v/v) depicted total capable outcomes in comparison with other concentrations of honey. Tissue fixation using 95% ethanol (v/v) depicted clear nuclear staining trailed by20% aqueous honey solution (95%) and 10% aqueous honey solution (90%). But, the variance amongst these fixatives was not methodically substantial with Fishers' exact trial: $P = 0.75$, and Kruskal–Wallis trial: Chi-square values 2.32, $P = 0.51$. Samples fixed by using 95% ethanol, 10% aqueous honey solution and 20% honey solution depicted clear cytoplasmic staining (85%) in comparison with 30% aqueous honey solution (70%). But, the variance amongst these fixatives was not methodically substantial with Fishers' exact trial: $P = 0.62$, and Kruskal–Wallis trial: Chi-square values 2.19, $P = 0.53$.Samples fixed by using 95% ethanol depicted tremendous cellular physiology, trailed by 10% aqueous honey

solution, 20% aqueous honey solution (95%), and Group 30% aqueous honey solution (90%). But, the variance amongst these fixatives was not methodically substantial with Fishers' exact trial: P = 0.90, and Kruskal–Wallis trial: Chi-square values 2.08, P = 0.56. Samples fixed with 10% aqueous honey solution and 20% aqueous honey solution depicted better transparency of staining (95%), trailed by the usage of 95% ethanol (90%) and 30% aqueous honey solution (80%). But, the variance among these fixatives was not methodically substantial with Fishers' exact trial: P = 0.51, and Kruskal–Wallis trial: Chi-square values 3.29, P = 0.35. Samples fixed with 30% aqueous honey solution depicted better consistency of staining (95%), followed by 10% aqueous honey solution(90%), 30% aqueous honey solution (85%), and 95% ethanol (80%). But, the varianceamong these fixatives was not methodically substantial with Fishers' exact trial: P = 0.68, and Kruskal–Wallis trial: Chi-square values 3.80, P = 0.28.

Sabarinath et al. (2014) equalled10% formalin with that of 10% honey and 10% aqueous honey solution. Patil et al. compared 30% jaggery and 20% honey as a tissue fixative and remarked the observations at an interlude of 6 months. These researchers projected that the usage of environmentally-friendly honey and jaggery as a substitute to formalin for longstanding tissue conservation. Singh et al. compared ethanol 20% with that of unrefined honey and reported the inconsequential variance among alcohol fixed smears and honey and proposed that honey is also an effective fixative. Pandiar et al. compared 20% honey with that of ethanol and 30% aqueous jaggery as a cytofixative and instituted no methodical important variance among these fixatives. These researchers reported that these fixatives can be utilized as an alternate fixative for oral smears.In fine-needle aspiration cytology, Ishaq et al. compared 20% honey and 95% alcohol fixative solutions. It was observed that no methodical substantial variance was reported among the fixative characteristics of alcohol and honey. These researchers reported that the honey can securely be used as an alternate to the alcohol as a fixative. The review is in configuration with the preceding review studies.

In the current study, 30%,10%, and 20% concentrations of refined honey were utilized as a fixative and equalled with that of 95% ethanol. Preceding studies have utilized only 20% of honey or 10% of honey or in combinations. Honey has numerous benefits in comparison to that of 95% ethanol. Few of the advantages of honey includes its nonvolatile property,

environmentally-friendly, not very expensive in comparison to alcohol, easily obtainable, not exposed to filching, and can be easily repudiated without any biohazard.

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