Original Article

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Comparison of Fixative Properties of Honey with Ethanol in Oral Cytological Smears

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ABSTRACT

Background: Fixation is an important step in cytopathological diagnosis. Ethanol is traditionally a popular and widely used fixative for cytopathological diagnosis. But ethanol is expensive and subjected to pilferage thus decreasing its ability. Ethanol denatures proteins and glycogen by precipitation. Hence in a search of better, ecofriendly and cost effective fixative, honey can be as efficient as ethanol in cytological fixation.Properties of honey such as high osmolarity, low pH and the presence of components such as ascorbic acid, hydrogen peroxide and phenol inhibine, all contribute to its anti-oxidative and antibacterial effects.

Methods: A cross sectional comparative study was carried out after ethical approval on healthy patients fulfilling the inclusion criteria referred to the Department of Pathology in BLDEU'S Shri B.M. Patil Medical College, Hospital and Research centre, Vijayapur. After informed consent two buccal smears were obtained from each individual, one was fixed in Ethanol and other was fixed in Honey (20%) for a minimum of 15 min. After staining, smears were scored for cytomorphological characters.

Results: Out of the 200 cases studied, honey fixed smears showed cytomorphological features similar to ethanol among which nuclear staining, clarity and uniformity of staining showed significant p value ($p < 0.05$) where as cytoplasmic staining, cell morphology showed no statistical difference. ($p > 0.05$)

Conclusion: The present study offers an innovative proposal of using natural eco-friendly sweeteners, as fixative in cytopathology. The results are promising and invoke extensive large multicentric collaborative work to reach a global consensus on this fixative.

Keywords: *Honey, Fixative, Oral Cytology.*

Introduction

Cytopathology in the present era is a valid and well-accepted diagnostic tool. Diagnostic accuracy always depends upon the procuring samples, fixation, staining, screening and interpretation of the specimen and quality control. Each of these steps play a vital role in diagnosis. [1] Adequate fixation is required for proper examination of tissue or cells understudy, to reach a proper diagnosis. An ideal fixative which can fix various tissues including lymphoid, neural, muscle and fatty tissue has not been identified till date. Ideal fixative must be nontoxic, cheap and easily available, should preserve tissue for long time and should be compatible with immunohistochemical and molecular techniques. Ethanol is a well known and widely accepted fixative in Cytopathology providing excellent preservation of morphology and cellular details which are the basic requirement to make cytological diagnosis. Ethanol being an alcohol fixative preserves the tissue antigens and decreases the turnaround time and cost which are required during antigen retrieval. [2]

Ethanol though an efficient cytological fixative has few disadvantages such as it is subjected to pilferage, expensive, flammable, evaporates easily and not freely available. It usually causes skin and eye irritation. [3] In search of eco-friendly and ideal fixative many natural sweeteners are being experimented, among which honey has given promising results. Many studies have proved its efficacy in histopathology. It is produced from many floral sources and contains carbohydrates, vitamins, minerals, and several trace elements. Honey has inherent antibacterial, anti-oxidative properties due to high osmolarity, low pH and the presence of components such as ascorbic acid, hydrogen peroxide and phenol inhibine. [4] Probable mechanism of fixation is due to presence of carbohydrates such as fructose which causes breakdown of aldehyde in presence of low pH. These aldehydes then cross-link with tissue amino acids which leads to tissue fixation. [5] Hence, considering this honey has also been experimented as fixative in cytology which has provided excellent cellular preservation and dehydration which are required for fixing the smears in Cytopathology.

Material and methods

A cross sectional comparative study was carried out on healthy patients fulfilling the inclusion criteria referred to the Department of Pathology in BLDEU'S Shri B.M.Patil

Medical College, Hospital and Research centre, Vijayapur. Ethical clearance was obtained from institutional ethical committee.

Two smears were collected from each subject, one smear was fixed in ethanol and other will be fixed in 20% commercially available honey (Two parts of honey+eight parts of distilled water). Smears were fixed in each fixative i.e ethanol and 20% honey for a minimum of 15 minutes. After which they were washed in tap water for 30 sec and subjected to conventional Papanicolaou staining procedure. Smears were evaluated by following criteria (Table 1)

Data analysis:- Data was analyzed using 1. Mean ± S.D, and 2 Chi square test

Inclusion criteria: All healthy individuals who visit for regular health check-up were included in the study.

Exclusion criteria: Nil

Results

A total of 200 cases were collected out of which 120 cases (60%) were male and 80 cases (40%) were female. Honey fixed smears (HF) showed acceptable overall cellularity and results of all cellular parameters were very much satisfactory and as good as ethanol fixed (EF) smears.

Out of 200 cases 193 (96.5) cases EF and 186 (93%) cases of HF slides showed acceptable nuclear staining and 7 (3.5) cases of EF and 14 (7%) cases of HF slides showed unacceptable nuclear staining which was statistically significant with p value of 0.008. Similarly acceptable cytoplasmic staining was seen in 178 (89%) cases of EF and 160 (80%) cases of HF slides and 22 (11%) cases of EF and 40 (20%) cases of HF slides showed unacceptable cytoplasmic staining which showed no statistical difference between both fixatives with p value of 0.821. (Table 2)

Well preserved cell morphology was noted in 181 (90.5%) cases of EF and 188 (94%) cases of HF slides which showed no statistical difference between both fixatives with p value of 0.092. Clarity of staining and uniformity of staining was present in 190 (95%) cases EF, 176 (88%) cases of HF slides and 191 (95.5%) cases EF and 184 (92%) cases of HF slides respectively which was statistically significant with p value of <0.005. HF smears revaluated after a period of 6 months showed unchanged cellular parameters as described above in comparison to EF smears. (Table 2)

Table 1: Evaluation criteria.

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

Table2: Distribution of cases comparing various cytomorphological features of Ethanol fixed smears and Honey fixed smears.

Staining	Scale	Ethanol fixed		Honey fixed		p value
		N	%	N	%	
Nuclear staining	Unacceptable	7	3.5	14	7	0.008*
	Acceptable	193	96.5	186	93	
Cytoplasmic staining	Unacceptable	22	11	40	20	0.821
	Acceptable	178	89	160	80	

Staining	Scale	Ethanol fixed		Honey fixed		p value
		N	%	N	%	
Cell morphology	Unpreserved	19	9.5	12	6	0.092
	Preserved	181	90.5	188	94	
Clarity of staining	Absent	10	5	24	12	0.005*
	Present	190	95	176	88	
Uniformity of staining	Absent	9	4.5	16	8	<0.001*
	Present	191	95.5	184	92	
Total		**200**	**100**	**200**	**100**	

*Note:*significantly associated at 5% level of significance*

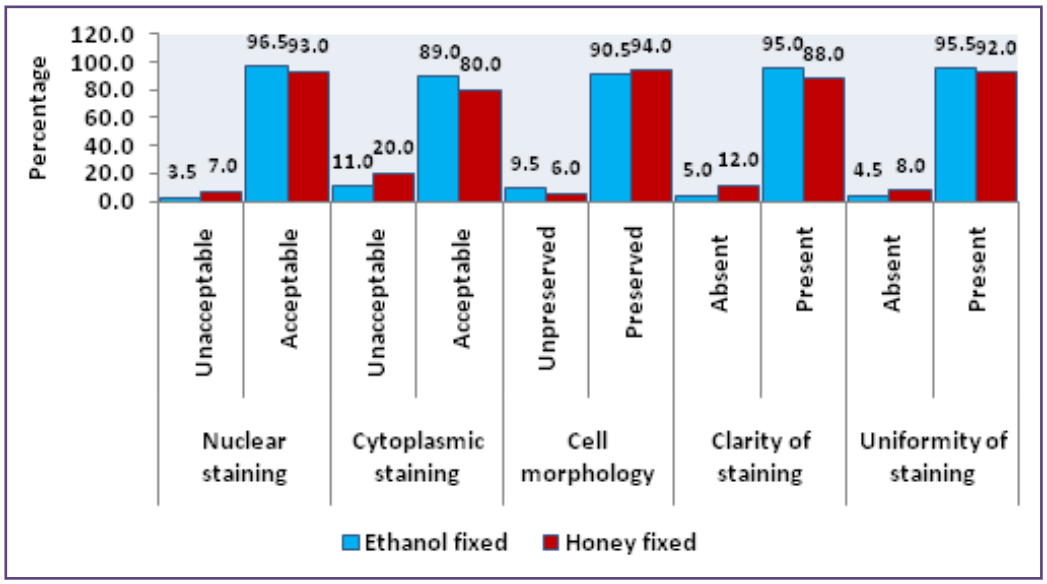

Fig. 1: Distribution of percentage of acceptable and unacceptable honey and ethanol fixed smears.

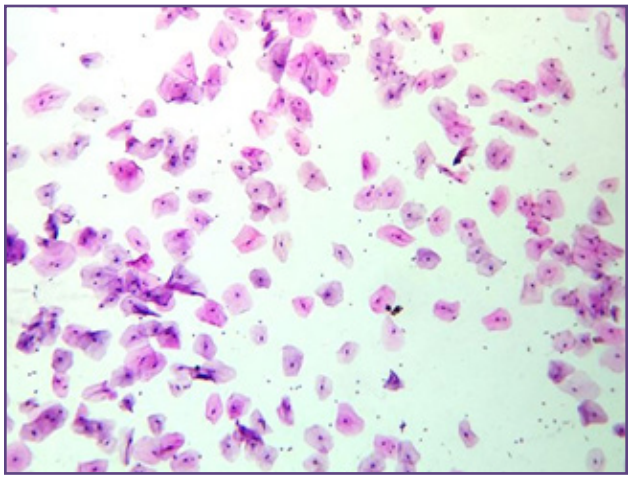

Fig. 2a: Ethanol fixed smear showing adequate cellularity, acceptable nuclear and cytoplasmic staining. - PAP 100X.

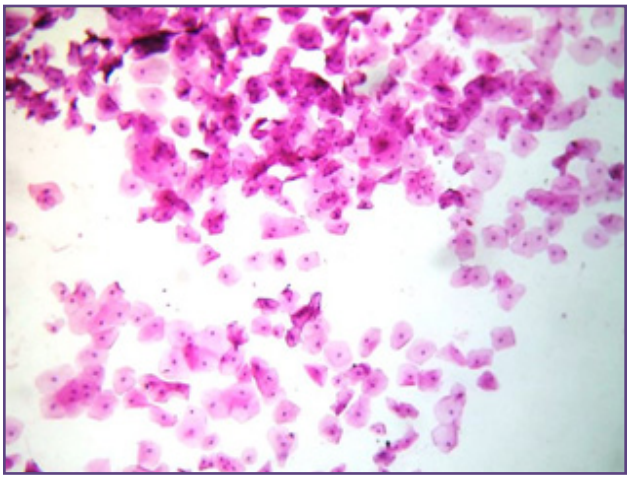

Fig. 2B: Honey fixed smear showing adequate cellularity, acceptable nuclear and cytoplasmic staining. - PAP 100X.

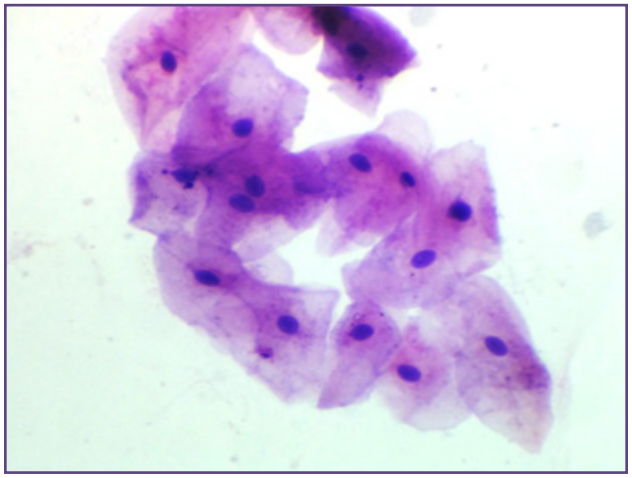

Figure 3A: Ethanol fixed smear showing preserved cell morphology, acceptable uniformity and clarity of staining nuclear staining - PAP 400X.

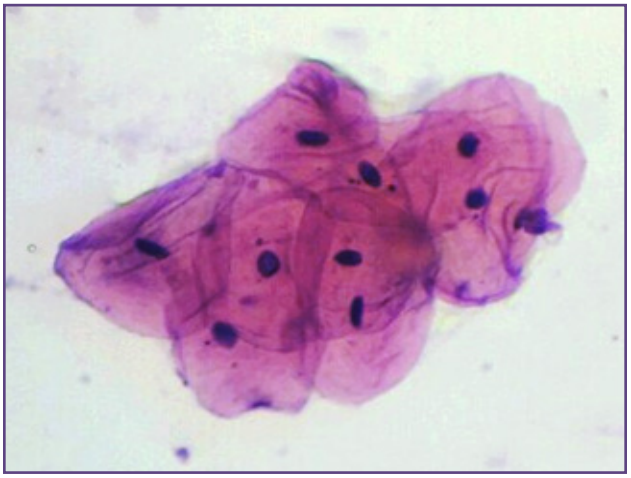

Figure 3B: Honey fixed smear showing acceptable preserved cell morphology, acceptable uniformity and clarity of staining nuclear staining PAP 400X.

Discussion

Group of cells which are building blocks of living organism unite to form a tissue which perform specific function. Microscopic study of individual cell in a smear is called cytology and study of tissue is called histology. Fixation preserves the cells similar to living state, when these are subjected to staining aids in cytological examination and diagnosis. Though many fixatives are used in both cytology and histology, each of them has certain advantages and disadvantages. Ethanol is a gold standard fixative widely used as cytological fixative in many laboratories. Advantages are rapid fixation, antibacterial properties and acceptable preservation of cytological details, but major disadvantage being not freely available, costly and inflammable which prevents it from being an ideal fixative. [6] So in search of an ideal fixative honey could be a natural, cheap and safe alternative to ethanol as it has all inherent properties which are required for fixation due to its low pH, high osmolarity and antibacterial properties. [7,8]

Many different studies have already been done to compare honey as fixative in histopatholgy in comparison to formalin, which has provided convincing and appreciable results. [9-11] In honey fixed smears one could very clearly appreciate all cellular details such as nuclear, cytoplasmic staining, cellular morphology, clarity and uniformity of the staining which are almost equivalent to ethanol fixed smears. Present study in concordance with Singh A, *et al* [4] showed that cellularity and cell morphology were well preserved in honey which provides adequate cytological material for diagnosis.

In present study comparison of ethanol and honey fixed smears for nuclear staining (p value = 0.008), clarity of staining (p value = 0.005), uniformity of staining (p value < 0.001) were statistically significant. This is in discordance with Singh A, *et al*[4] and Ishaq R *et al* [12] study in which nuclear staining, clarity of staining, uniformity of staining showed no statistical difference between both fixatives.

In present study comparison of ethanol and honey fixed smears for cytoplasmic staining (p value = 0.821), preservation of cell morphology (p value = 0.092) showed no statistical difference between both fixatives. This is in accordance with Singh A, *et al* [4] and Ishaq R *et al* [12] study in which nuclear staining, clarity of staining, uniformity of staining also showed no statistical difference between both fixatives. In present study different routine cytological smears from malignant lesion, lymphnode, necrotizing lesions etc were not studied, hence outcome of cellular fixation and cellular details in such honey fixed smears needs to be studied in detail.

Similar studies have also been done to compare fixative ability of honey in comparison to formalin in histopathology. Ozakan N et al [2] study which compared honey with neutral buffered formalin and alcohol formalin various lesion in histopathology. Nuclear morphology showed no statistically significant difference between alcoholic formalin (3.25±0.13) and honey (2.83 ±0.2) fixation (p >0.05). Similarly there was no significant difference among these fixatives with regard to cytoplasmic detail (p>0.05) Even immunohistochemical comparison done in Ozakan N et al [2] study for honey fixed and formalin fixed paraffin embedded tissue with Vimentin and Ki67 showed convincing results. There were no statistically significant differences among the various fixatives compared.(p > 0.05) The present study showed that honey fixed smears showed almost similar results when compared to ethanol fixed smears. Background

of honey fixed slides was clear as comparable to ethanol fixed slides and most of the cells showed well defined nuclear chromatin, nuclear membrane and intact cytoplasm. Even Immunohistochemistry could be done on honey fixed slides as it fixes tissue without damaging or altering the antigens present in the tissues. [13,14]

Conclusion

Honey being a natural, economical and pleasant smelling, easily available, eco-friendly innovative fixative with antibacterial properties. Honey as fixative has shown cytomorphological features comparable to ethanol. Using honey also improves the safety and work environment in the laboratory. In rural areas, health camps, public health service centres and in absence of alcohol fixatives, honey can be used as a successful alternative.

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