

# HONEY AS A CYTOLOGICAL FIXATIVE: A COMPARATIVE STUDY WITH ETHANOL

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## **ABSTRACT:**

**Background:** Tissues are fixed and preserved and protected from putrefaction and autolysis before routine histopathological or cytological examination. Ethanol is commonly used as a cytological fixative due to its better preservation of nuclear details. Honey, a natural product which possess antibacterial; acidic; dehydrative properties which classify it as fixative than a preservative. **Aim:**The aim of this study is to compare ethanol with honey as a cytological fixative. **Materials and Methods:**Smear samples of buccal mucosa from patients submitted to the Outpatient Department of Oral Pathology and Microbiology, Meenakshi Ammal Dental College and Hospital, Chennai, were included in the study group. The study consisted of two groups. Group I that used alcohol as the fixative comprising of 30 smear samples and Group II that used 10% honey to fix 30 smear samples. A double-blind study was conducted. **Statistical Analysis:**Independent t-test was the statistical method applied for the study using SPSS software (Version 17). **Result:** The results are discussed. **Conclusion:** Honey can be used as an alternate fixative to alcohol in cytopathology.

**Keywords:** Honey, Ethanol, Cytology, Fixative, Natural

## **INTRODUCTION**

Tissues are fixed and preserved and protected from putrefaction and autolysis before routine histopathological or cytological examination. Fixation helps in retaining the cellular components and enables in a defined microscopic appearance. Dehydration, heat effects, cross-linked acid effects, or the latter variations can be listed as fixatives. By causing methylene bridges between amino acids, formalin satisfies this requirement of being a cross-link fixative and is also the most widely used fixative used in standard histopathological analysis.<sup>[1]</sup>

However, alcohol fixes by denaturing the protein and reducing their soluble nature and by disrupting the hydrophobic bonds. This process is different than that of aldehyde fixatives. Alcoholic fixatives function by preserving nuclei acids better as they bring about minimal

chemical change. This minimal loss of tissue concentration is due to the rapid tissue penetration and low molecular weight. Ethanol and Methanol for this purpose are more commonly used for frozen sections and smears.<sup>[2]</sup>

Nevertheless, an ideal fixative should be easy to use, preserve sufficient material and allow high quality histology. Some literature studies prove the beneficial effects of honey in histology based on its antibacterial, acidic and dehydrative properties<sup>[3]</sup> along with its anti-autolysis and tissue hardening property.<sup>[4]</sup> These properties classify honey as a fixative more than a preservative. Tests have been carried out<sup>[5]</sup> and are still being carried out on the effectiveness of honey as a histological fixative, but not many studies on the use of honey as a cytological fixative are current. This pilot study was undertaken to assess the role of honey as an adjunct to alcohol in cytology.

## **MATERIALS AND METHODS**

The research group included smear samples of buccal mucosa collected from patients who after receiving written permission, referred to the Oral Pathology and Microbiology Outpatient Department, Meenakshi Ammal Dental College and Hospital, Chennai. As the study focused on demonstrating the honey's fixative powers, lesion patients were ruled out. The study consisted of two groups. Group I that used alcohol as the fixative comprising of 30 smear samples and Group II that used 10% honey to fix 30 smear samples. Each smear category was set for 30 minutes in its accompanying solution, alcohol or honey, and then subjected to routine cytological processing. The tissues were then stained and subjected to examination under the compound microscope with hematoxylin and eosin. There was a double-blind analysis carried out. All 60 slides were analyzed by three observers for criteria such as nuclear staining and particulars, cytoplasmic staining and information, and context staining. The methodological approach used for the analysis using SPSS tools was the unbiased t-test (Version 17)

## **RESULTS**

Similar staining features were seen in the smears fixed in honey and alcohol. The overall tissue architecture for honey fixed smears was close to that of alcohol fixed smears in specifics such as cell and nuclear size. The nuclear staining was excellent for honey in 14 cases compared to 9 cases of alcohol used as fixative. However, the cytoplasmic staining and cytoplasmic detailing was at par for honey with alcoholic fixatives. Only 3 slides showed fair nuclear details for honey fixatives with good to excellent detailing compared to alcoholic fixatives. Background staining was absent in 20 slides for honey fixation which was slightly lesser than that of alcohol.

## **DISCUSSION**

Honey has been used for the treatment of ulcers and bed sores or other surface infections arising from burns and wounds because of its antibacterial and therapeutic properties.<sup>[6]</sup> Multi resistant bacteria led to the rediscovery of these properties of honey that was ignored by current day medicine.<sup>[3,7-9]</sup> The usage of honey in routine histopathological or cytological fixation is still debatable. For this cause, the research was performed to test honey's efficacy as a cytological fixative.

A pilot study was conducted with a total of 60 smears divided equally among the two groups of alcoholic fixative and honey fixative (Commercially available Dabur Honey, Dabur India Limited, Solan, India,) for 30 min. The tissues were then stained with hematoxylin and eosin and

three observers were subjected to an evaluation. The findings of this analysis revealed comparative results of mild differences in context staining for honey and alcohol.

Fixatives react with tissue constituents either altering their configuration or by introducing artifacts in them. <sup>[10]</sup> Low pH do not favour the staining of cytoplasm but honey contain hydrolytic enzymes like lysozymes. <sup>[11]</sup> which are active at low pH and act as a potent antibacterial agent. <sup>[12]</sup> Apart from the antibacterial effect honey seems to contain minerals, trace elements, vitamins like sodium, potassium, calcium, chlorine, Vitamin B complex and vitamin C, and so on. <sup>[13]</sup> The putrefactive nature of honey could be attributed to the presence of fatty acids, lipids, amylases and hydrogen peroxide. <sup>[14-17]</sup> Due to the above mentioned properties of honey, the nuclear and cytoplasmic specifics were well noted in the present analysis (Figure 1a and Figure 1b)

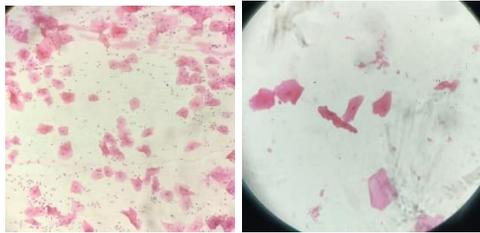


Figure 1a. 4x view of cytological smear fixed with honey showing clear details of nuclear & cytoplasmic staining

Figure 1b. 10x view showing polygonal squamous cells with prominent nuclear & cytoplasmic staining

Rahma AI-Maainil and Philip Bryant <sup>[3]</sup> in their study used 10% honey as a fixative in comparison with formalin which proved to have similar results. In a study conducted by the fixed tissue sections showed satisfactory results in terms of cytoplasmic and nuclear study similar to that observed in our current study. <sup>[18]</sup> Various studies have been conducted in the past on the effectiveness of honey as a fixative and results have revealed honey preserved tissues show adequate fixation and preservation of architecture upto 12 hours beyond which tissue quality was compromised due to cell swelling and nuclear shrinkage. <sup>[6]</sup>

In a study conducted by Godwin et al <sup>[19]</sup>, the properties of honey in wound healing and autolysis and putrefaction protection were evaluated. It was seen honey showed similar properties of formol saline whereas in a similar study by Shankargouda et al <sup>[20]</sup> The cytoplasmic and nuclear details were satisfactory, but uneven staining areas were also seen. In contrast the present study showed the staining was seen uniform similar to that of alcohol. (Figure 2a and 2b)

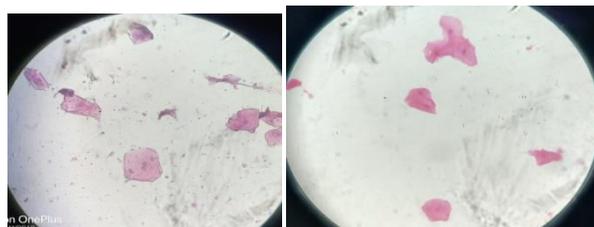


Figure 2a. 10x view of cytological smear fixed with alcohol showing polygonal squamous cells with prominent nuclear & cytoplasmic staining

Figure 2b. 10x view of cytological smear fixed with honey showing polygonal squamous cells with prominent nuclear & cytoplasmic staining

It was seen in this study the quality of fixation with honey did not differ considerably with that of alcohol (Figure 3). Though alcohol has been proved to be an effective fixative, an adjunct is always beneficial with the current advancements of histotechnology. Honey is readily available in India but more studies with larger sample size has to be conducted for better results.

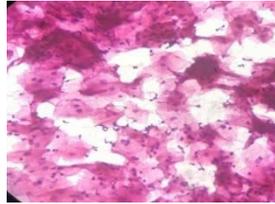


Figure 3. 10x view of cytological smear fixed with honey showing polygonal squamous epithelial cells with prominent nuclear & cytoplasmic staining

### CONCLUSION

The above study has concluded so far as an effective cytological fixative. Honey, with no known toxicity, is readily available. This can be used as an alternate fixative to alcohol in cytopathology.

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