

The Efficacy of the Bees Honey as Alternative for Routine Ethyl Alcohol in Cytological Fixation: Cross_ Sectional Study

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Abstract:- Fixation is an initial and essential step in diagnostic cascade for cytological specimen, in usual it carried out by using 95% Ethyl Alcohol, but this alcohol based fixation method exhibited some limitations and problems either for specimen, workers, and even the environment. New way to find ecofriendly and specimen safe alternative to alcohol is critical, the suggestion says Bees Honey is most suitable alternative. The study objective was to investigate the efficiency of using bees honey instead of ethanol fixation in buccal smear samples. Samples were collected by gently scraping donor's buccal mucosa by wooden sticks (n=60) and divided into alcohol group coded (E) and honey group coded (H). Honey group divided according to concentration into: A (10%), B (20%) and C (30%), then both groups fixed for 15 min after that stained with papanicolaou staining procedure, then examined under light microscope. The results show the honey fixed smears were same as the result of ethanol fixed ones. And the analysis of staining result which done by using Chi square analysis program the results showed good observation of cytoplasmic staining result obtained by using 10% honey fixative agent. So honey could be used as excellent alternative to ethanol cytofixative.

Keywords: *Alternative Fixative, Ethyl Alcohol (Ethanol) , Cytology, Honey.*

I. INTRODUCTION

The fixation process is an essential part in cytology diagnostic steps to preserve these cells in their natural state as much as possible [1]. The good fixative or an ideal one is that which preserve the cellular structure and prevent the autolysis and putrefaction in minimum time and at lower concentration that could be used to achieve a good result [2]. Conventional alcohol based fixative that use (95%) ethyl alcohol is the routine fixative for cytology lab due to it is good preservation of cellular morphology and gives clear staining result, but on other hand it has many disadvantage and side effects: it is carcinogenic, flammable, not freely available and it is expensive and remember that for cytological purpose need high concentration [2]. Honey is the natural sweet product, that produced by honeybees from the nectar of plants, which is formed of sugars and other compounds. It has a role in medical projects, it has an antioxidant, antimicrobial and anti-autolytic effects. Also it is able to penetrate the deepest tissue and can prevent autolysis and putrefaction, antibacterial properties of honey is due to the low water activity causing osmosis, hydrogen peroxide effect, and high acidity, this combination of high acidity, hygroscopic and antibacterial effect makes honey a plausible way to turn human cadaver into a mummy[3,4,5]. The mechanism of fixation by honey has been postulated. First, fixation occurs due to the formation of hydroxymethylfurfural (HMF) which is an intermediate product which forms cross link with the cellular component. Second, fixation occurs when the sugars, largely fructose, are broken down at acidic PH to form aldehydes which in turn forms cross links with the cellular proteins(6).

➤ **Aims and Objectives:-**

- To assess the efficiency of honey as herbal natural fixative substance to preservation of cytological specimen as alternative for ethyl alcohol.
- To detect the proper concentration of honey as cytological fixative.
- To examine the nuclear staining characteristic after fixation with different concentration of honey.

II. MATERIAL AND METHOD

120 Samples were collected after getting the approval in conventional cytological specimen collection method by using wooden sticks, from adult donor’s buccal mucosa, then samples were divided into two group based on type of fixative that use to ethyl alcohol group (E) and honey group (H), then the H group divided into three group according to honey concentration as first group fixed in 10% honey, the second one fixed in 20% honey, the last one is fixed in 30 % honey. By tacking two smears from each donor of these three groups, one for ethanol fixation which used as control and other one for honey with different concentration, all of them fixed for 15 min. After that stained with papanicolaou

stain and tested by pathologist under the light microscope (Olympus), then the results been analyzed by using Chi square analysis program.

III. RESULT

After a complete fixation process and papanicolaou staining procedures, the results that obtained from honey fixed groups and ethyl alcohol fixed ones and after visualized under light microscopy, the pathologist examined the overall maintenance of nuclear and cellular structures, and clear staining feature.

The comparison of staining result for honey fixed smears and ethanol fixed ones, according to nuclear details and cytoplasmic appearance, background stain and the overall staining features were showed as excellent, satisfy and poor results respectively as they showed below in Tables (i, ii) and Fig(1-6) .

The overall result was good staining results were achieved after using honey with different concentration as cytological fixative.

Table 1 The Percentage of Nuclear Staining Characteristic of Ethyl Alcohol and Honey Groups 10%, 20% and 30%.

Nuclear stain	Fixatives						
	Characteristics	95% Ethanol	10% Honey	95% Ethanol	20% Honey	95% Ethanol	30% Honey
Poor		2 (20%)	1 (10%)	0	1 (10%)	0	0
Satisfy		1 (10%)	1 (10%)	1 (10%)	0	3 (30%)	4 (40%)
Excellent		7 (70%)	8 (80%)	9 (90%)	9 (90%)	7 (70%)	6 (60%)
Total		10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
P.Value		0.8		0.4		0.3	

Table 2 The Percentage of Cytoplasmic Staining Characteristic of Ethyl Alcohol and Honey Groups 10%, 20% and 30%.

Cytoplasmic Stain	Fixatives						
	Characteristics	95% Ethanol	10% Honey	95% Ethanol	20% Honey	95% Ethanol	30% Honey
Poor		2 (20%)	2 (20%)	0	0	0	0
Satisfy		3 (30%)	8 (80%)	3 (30%)	2 (20%)	3 (30%)	4 (40%)
Excellent		5 (50%)	0%	7 (70%)	8 (80%)	7 (70%)	6 (60%)
Total		10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
P.Value		0.03		0.6		0.6	



Fig 1 The Figure Shows Group A Buccal Smear which Fixed in 10% Honey.

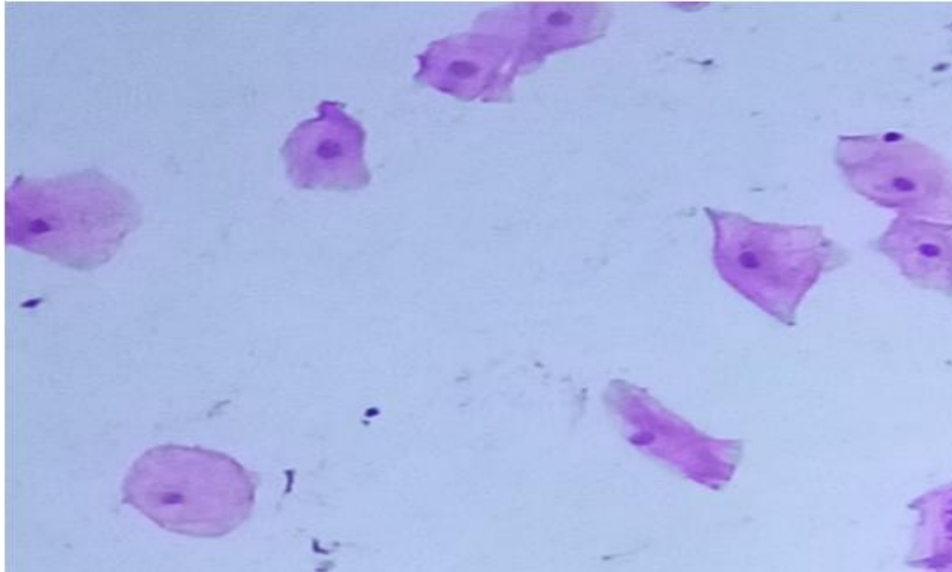


Fig 2 The Figure Shows Group A Buccal Smear which Fixed in 95% Ethanol.

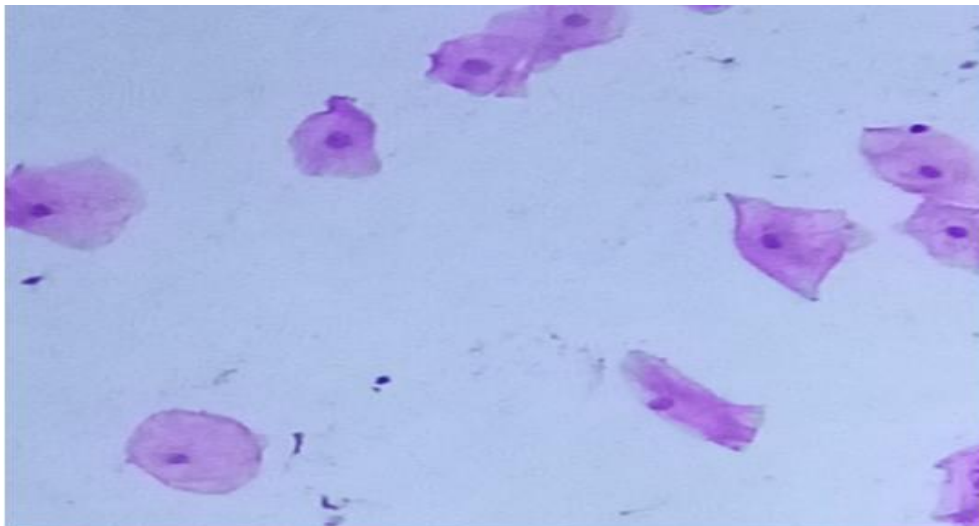


Fig 3 The Figure Shows Group B Buccal Smear which Fixed in 20% Honey.

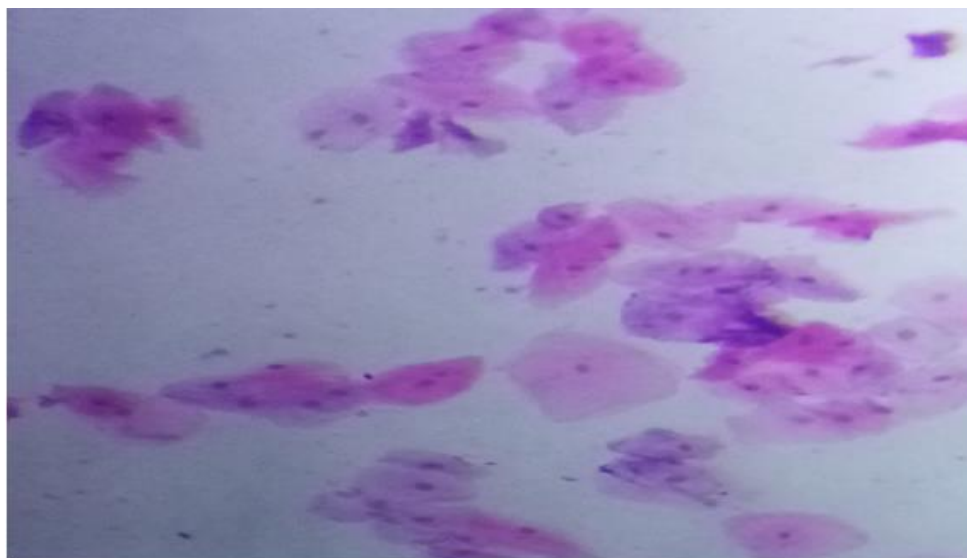


Fig 4 The Figure Shows Group B Buccal Smear which Fixed in 95% Ethanol.

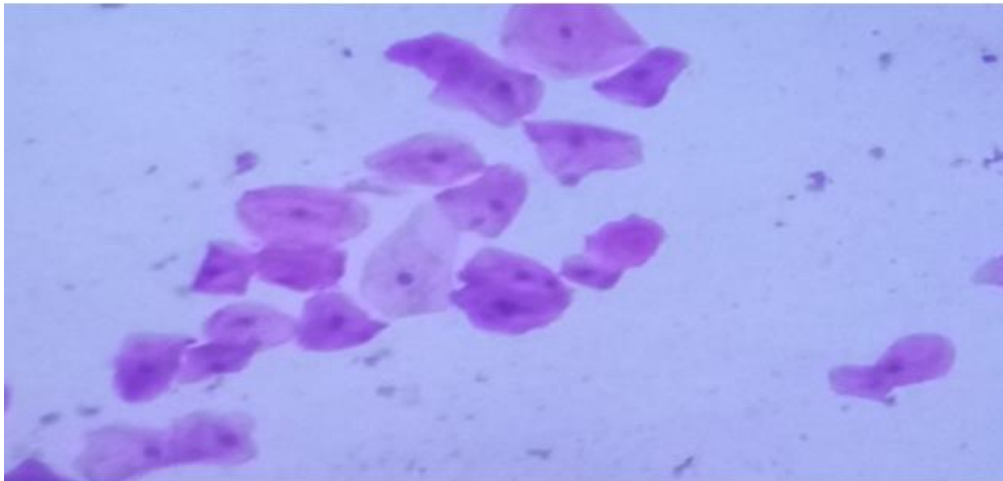


Fig 5 The Figure Shows Group C Buccal Smears which Fixed in 30% Honey.

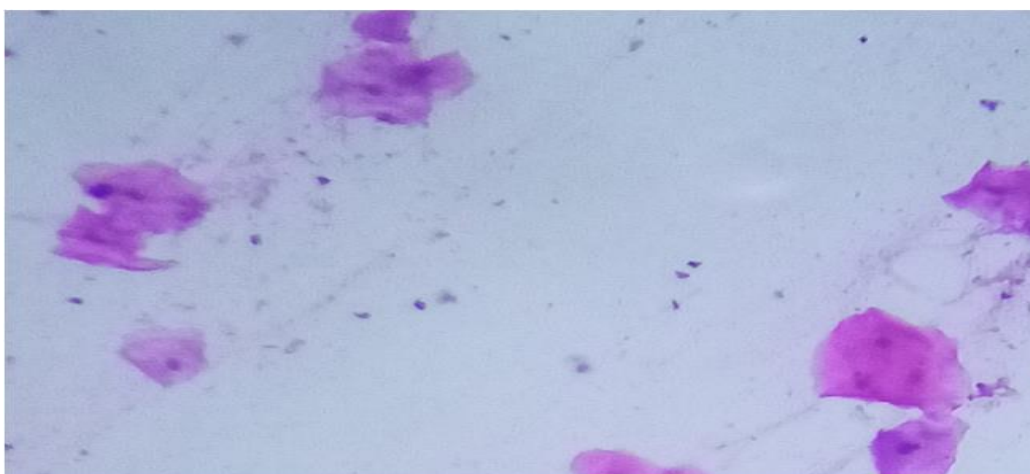


Fig 6 The Figure Shows Group C Buccal Smear which Fixed in 95% Ethanol.

Analytical results showed best result for ethanol and 20% honey followed by 10& 30% but no statistical differences; in 10% honey the results were showed very good cytoplasmic staining results. Fig below (7, 8, 9).

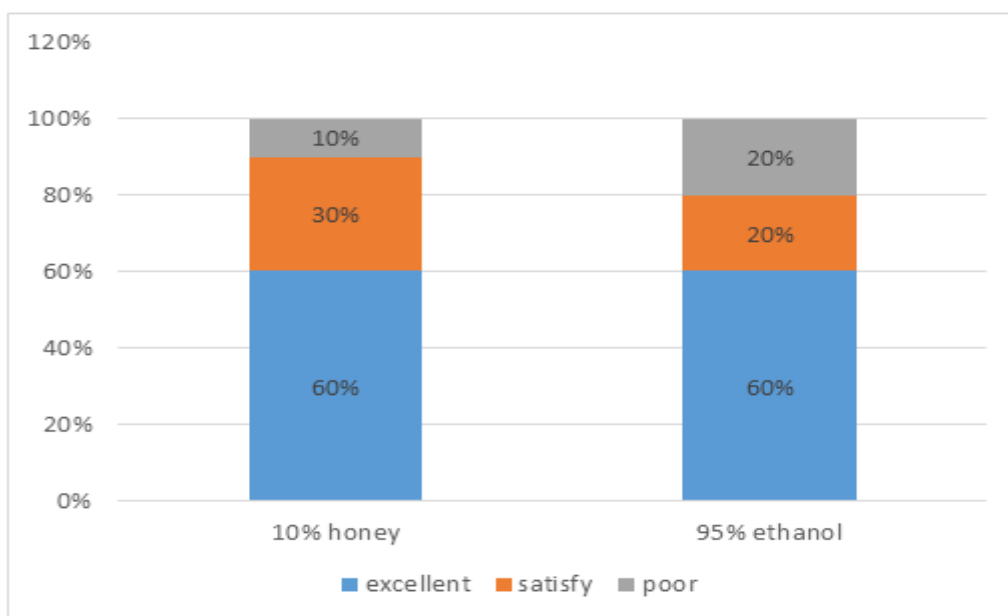


Fig 7 Which Show the Overall Cytomorphological Features of Group (A) Ethanol (Right) and 10% Honey (Left) Fixation Percentage Results.

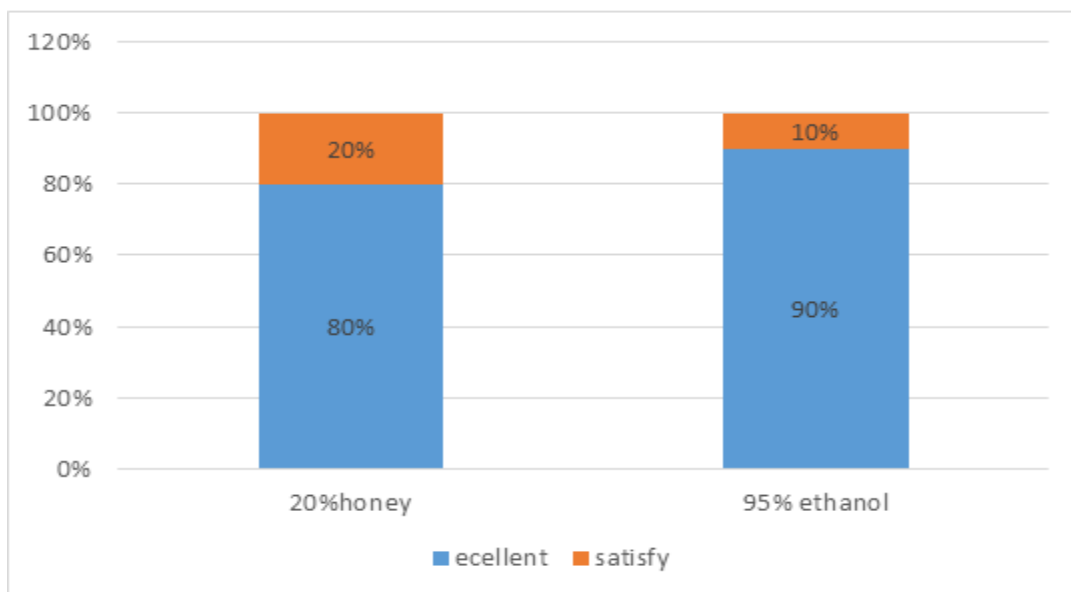


Fig 8 Which Show the Overall Cytomorphological Features of Group (B) Ethanol (Right) and 20% Honey (Left) Fixation Percentage Results.

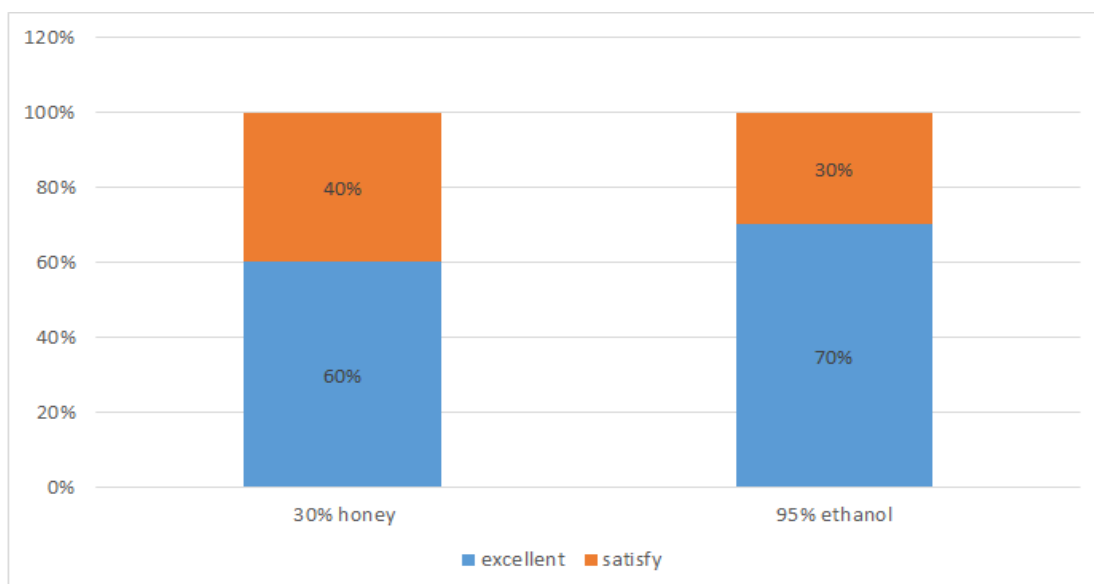


Fig 9 Which Show the Overall Cytomorphological Features of Group (C) Ethanol (Left) and 30% Honey (Right) Fixation Percentage Results.

IV. DISCUSSION

Since the fixation and fixatives have been discovered till update they have passed through many developments. Alcoholic fixatives have been used routinely for cytological examination mainly ethanol, but it has many disadvantages it is flammable, irritant induce it is highly expensive and volatile, not freely available and carcinogen [2]. And it is the most important to use safe substance which could preserve the structure also give satisfy results. Honey is natural sweet, yellowish-brown fluid made by honeybees from nectar that had been collected from plant and mainly flowers. After collection it has modified by honey bees and stored in honeycombs [3].

Honey is assumed to prevent autolysis and putrefaction, many studies also have approved that is due to it is antibacterial property, and it contains hydrogen peroxide, several tetracycline derivatives, lipids, ascorbic acid, fatty acids and amylases[7,8].

Honey plays as fixative agent at lower concentration not like ethanol, for 10min minimally. The result showed most of the cytological structures have been fixed well in 20% honey with overall cytological staining criteria about 80% compared to 90% of 95% ethanol, and in 10% & 30% honey fixed ones they get 60%. These percentages occurred when we considered that only excellent result shows the optimum fixation and preservation of cellular structures and give clear staining results.

The comparison of present study with previous studies[9,10] suggest that bee honey gives similar reaction to ethanol by using 20% honey, and our study also approved that bees honey may be the best substitute to ethanol and could give excellent result for cellular preservation and staining, and gives similar results to previous study.

Other previous studies also mentioned in literature review[11,12] suggest that 10% honey could be used as alternative fixative agent for ethanol, our study also approve that and it gives satisfy result about 60% for 10% honey, additionally in[12] previous study they found that 30% of honey concentration gives poor fixation results, but our study dis approve this assumption because it gets a satisfied result of 30% honey fixed smears, and analytically the results percentage was 60% same as in 10 % honey fixed ones.

All these variations between previous studies and our study may be due to the sample size that used, or the duration in fixative for example: in 10% may 15 min not adequate for complete fixation with this low concentration. Even the type of honey that used may affect in the fixation quality.

Eventually from all these studies, the bee honey has all the fixative properties that an ideal fixative should have and can be used as an alternative fixative to ethyl alcohol.

V. CONCLUSION

Since the diagnostic cytology has been discovered, alcoholic and mainly Ethanol has used as routine fixative. In this present time, it is most important to move towards natural and ecofriendly substitute for ethyl alcohol fixation, that could give better or similar result to alcohol fixed cytological specimen and be able to solves alcohol fixation problems, and bees honey is the most suitable one for this issue.

Honey is naturally available substance, ecofriendly and act more efficiently at low concentration (10% 20% 30%) if it compared with ethanol 95%, and it acts same as ethanol time for 15 min to fix the cytological smears.

More studies using large sample size are required to obtain more conclusive and generalizable results, with use of different duration to find out the suitable duration for each honey concentration. The application of such new technique helps to generate more healthier and safe work environment, with good and satisfy work results.

Further studies are useful to investigate if the honey has role in diagnostic cytopathology other than fixation.

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