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A comparative study of honey, jaggery an ethanol as cytological fixatives

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Abstract---The processing of cytological samples. It is the gold standard cytological fixative used in many laboratories. However, it is an expensive, volatile and flammable liquid with an irritant smell. It has also been shown to be carcinogenic in some animal models. Hence, a need to identify a safer substitute of alcohol cytological fixative is necessary. The aim of study was to analyse the efficacy of cytological smears fixed in ethanol & 20% unprocessed honey and 30% jaggery solution and to compare the efficacy between the three fixatives on staining. Three buccal smears from 50 healthy volunteers each, were collected from either cheek or tongue using wooden spatula. One of the smear was fixed in ethanol (95%) and Rapid Papanicolaou staining was done. And the other two smear was fixed in honey (20%) and Jaggery (30%) and Rapid Papanicolaou staining was done. The cytoplasmic and nuclear details were evaluated using

following parameters:- nuclear staining, cytoplasmic staining, cell morphology, clarity of staining and uniformity of staining. The results were recorded by 2 independent oral pathologists. Pearson Chi square test and Bonferroni post hoc test were used for comparison of qualitative data. 100% of Honey fixed slides, 84% of Jaggery fixed slides and 68% of alcohol fixed slides showed good nuclear staining ($p < 0.05$). 60% of Honey fixed slides, 64% of Jaggery fixed slides and 72% of alcohol fixed slides showed good cytoplasmic results ($p > 0.05$). 44% of Honey fixed slides and 40 % of both Jaggery and alcohol fixed slides showed good cell morphology ($p < 0.05$). 64% of the Honey Fixed, 52% of the jaggery and 56% of the alcohol fixed slides showed good clarity of stain ($p > 0.05$). 76% of honey-fixed, 56% of the jaggery-fixed and 58% of the ethanol-fixed smears showed uniformity of staining ($p > 0.05$). Honey can be used efficiently in cytological fixation and in preservation of cells jaggery was much better as compared to honey. Hence, honey is an excellent and efficient replacement for ethanol in cytological fixation.

Keywords---cytological fixation, unprocessed honey, jaggery, ethanol.

Introduction

Smear is a useful method for early detection of cancerous lesions and inflammatory conditions. Many important systemic disorders including hematological, dermatological, endocrinal or even rheumatological diseases manifest in the oral cavity, and thus, the oral cavity can be considered as a window to the body. Rapid turnover rate of oral mucosal cells, the exfoliated cells have a valuable role in diagnosis of aforementioned disorders as reflected by cytomorphological and nucleomorphological variations in the exfoliated cells. Exfoliative cytology is based on the monitoring the mucosal exfoliated cells through natural or artificial means. It is a minimally technique for obtaining cells to rule out the diagnosis.^{1,2}

In the present era, cytopathology is a well-accepted and valid diagnostic tool.³ Undoubtedly, diagnostic accuracy and reliability here depend greatly on the quality of collection, fixation, staining and interpretation. Inadequacy in any of these steps will adversely affect the standards of efficient diagnostic cytology.⁴ Fixative also plays a pivotal role in cytopathological diagnosis. Fixation is an initial and important step in tissue processing for microscopical examination. The primary aim of fixation is to preserve the tissues in a life-like state, prevent bacterial putrefaction, prevent autolysis, and increase there fractive index of the tissue.^{5,6}

Ethanol is an excellent fixative and dehydrant which plays an important role in the processing of cytological samples. The routine fixative used is 95% ethanol, and is proven for its efficiency. Methanol is used alternatively, the efficacy of which is yet to be documented. It is an excellent, proven fixative due to many properties such as rapid action, efficiency of fixation and wide applicability. However, it is an expensive, volatile and flammable liquid with an irritant smell. It

has also been shown to be carcinogenic in some animal models.⁷⁻⁸ Hence, a need to identify a safer substitute of alcohol cytological fixative is necessary.

Honey and jaggery have been used since centuries as sweetening and medicinal agents. Codex alimentarius defines honey as "A natural sweet substance, produced by honeybees from the nectar of plants, which the bees collect and transform by combining with specific substances of their own. It is then deposited, dehydrated, stored and left in honeycombs to ripen and mature."^{5,6,9,10} Honey primarily contains sugar and water. Sugar accounts for 95%-99% of honey dry matter. Majority of these are simple sugars, fructose (38.2%) and glucose (31.3%). It has been shown to have an antimicrobial action against a broad spectrum of bacteria and fungi.^{8,11} Honey has also been used as an agent for preventing autolysis and putrefaction.^{4,6,12} Both honey and jaggery are being used in pathology laboratories for tissue fixation, but their role in cytopathology is still at experimental level. Compared to alcohol, it is non hazardous, natural organic product, is odourless and does not require additional equipment. Hence, in the present study, we used unprocessed honey and jaggery solution as a substitute of alcohol cytological fixative.

Materials and Methods

The study population encompassed 50 healthy volunteers who attended the Department of Oral Medicine and Radiology, Surendera Dental College and Research Institute, Sriganganagar, Rajasthan. Three normal and healthy buccal smears were collected either from cheek or tongue using wooden spatula. One of the smear is fixed in ethanol (95%) and Rapid Papanicolaou staining is done. And the other two smear is fixed in honey (20%) and Jaggery (30%) and Rapid Papanicolaou staining is done Smear processing and staining were done. One smear was fixed in ethanol (95%) and the other two smears in 20% aqueous honey solution and 30% aqueous jaggery solution (Fixative Solutions preparation Table 1) for 15-30 min. The slides were then washed in tap water for about 30sec and the Rapid Papanicolaou staining procedure done. The cytoplasmic and nuclear details were evaluated using following parameters:- Nuclear staining, Cytoplasmic staining, Cell morphology, Clarity of staining and Uniformity of staining. The slides were categorized into poor (score 0), intermediate (score 1) and good (score 2). The results were recorded by 2 independent oral pathologists. Pearson Chi square test and Bonferroni *post hoc* test were applied using SPSS software version 22.0. The data was analyzed and the test results tabulated and evaluated.

Results

The results of Nuclear staining, Cytoplasmic staining, Cell morphology, Clarity of staining and Uniformity of staining are shown in Table 2. The results showed significant difference between three fixatives during nuclear staining ($p < 0.05$) and cell morphology ($p < 0.05$) of cytological smear. However, results showed no significant difference between three fixatives during cytoplasmic staining ($p > 0.05$), Clarity of staining ($p > 0.05$) and Uniformity of staining ($p > 0.05$) of cytological smear.

Discussion

Fixation of smears is a step of utmost significance in cytopathology laboratories as unfixed smears always yield results which are impossible to discern. Alcohol plays a significant role in cytological fixation. Good fixative is necessary for preservation of cellular details, enabling accurate cytological assessment and diagnosis. The routine fixative used is 95% ethanol, which is an efficient fixative, however, it has its own limitations which is subject to pilferage, is expensive, evaporates easily and is not freely available.¹³⁻¹⁶ Honey and jaggery have been used since centuries as sweetening and medicinal agents. Both honey and jaggery are being used in pathology laboratories for tissue fixation and as compared to alcohol, it is non hazardous, natural organic product, is odourless and does not require additional equipment.¹⁷⁻²³

In our study, 100% of Honey fixed slides, 84% of Jaggery fixed slides and 68% of alcohol fixed slides showed good nuclear staining. Chi square test gave a p value .021 which is less than significant value of 0.05, that shows a significant difference between three fixatives during nuclear staining of cytological smear. Pearson chi square value is 11.524 which is more than critical value so all three fixatives do have difference in nuclear staining adequacy. Similar results were reported by Bhattacharyya A et al in 2018⁶ with promising results using sugar and jaggery with other natural fixative for nuclear staining. Deepak Pandiar et. al.², in 2017 also reported similar results with honey fixed samples showed good staining.

Similarly out of 50 cases evaluated for cytoplasmic staining 60% of Honey fixed slides, 64% of Jaggery fixed slides and 72% of alcohol fixed slides showed good cytoplasmic results. Among all the tested fixatives, smears fixed in alcohol showed the highest percent of good cytoplasmic staining. About 16% alcohol-fixed, 24% honey fixed and 28% jaggery-fixed samples showed intermediate cytoplasmic staining. Chi square test gave a p value 0.545 which is more than significant value of 0.05, that shows a no significant difference between three fixatives during cytoplasmic staining of cytological smear. Pearson chi square value is 3.078 which is less than critical value so all three fixatives do not have significant difference in cytoplasmic staining adequacy. These data were in accordance with Nerune S M et al.¹⁴ and Deepak Pandiar et. al. They also showed better cytoplasmic staining result with alcohol.

In the present case out of 50 cases evaluated for cell morphology, it was analysed in terms of size and shape of the cell, the Honey Fixed slides were slightly better than the alcohol and jaggery fixed slides. Grading of cell morphology was 44% of Honey fixed slides and 40 % of both Jaggery and alcohol fixed slides. However, 8% of the Honey fixed and alcohol whereas 20% of the Jaggery fixed slides showed unpreserved cellular morphology ascribed to the disintegration of the cell membrane and cell shrinkage. Chi square test gave a p value .0411 which is less than significant value of .05, that shows a significant difference between three fixatives during cell morphology of cytological smear. Pearson chi square value is 3.966 which is less than critical value so all three fixatives do not have significant difference in cytoplasmic staining adequacy. These data were similar to the results obtained by Nerune S M et al.¹⁴ and Deepak Pandiar et al.²

When clarity was analysed 64% of the Honey Fixed, 52% of the jaggery and 56% of the alcohol fixed slides showed good clarity of stain. Out of 50 evaluated cases of Honey fixed smear 32 were good, 16 were intermediate and 2 were poor. Similarly Jaggery showed 44% intermediate clarity and 4% were poor where as Alcohol fixed smear did not showed poor clarity of stain. The Clarity decreased in all the three fixatives, however the clarity of alcohol better compared to others. The possible cause for slightly inferior results with jaggery and honey would be due to altered cross binding with the cells.²⁴⁻²⁹ Chi square test gave a p-value 0.772 which is more than significant value of 0.05, that shows a no significant difference between three fixatives during clarity of staining of cytological smear. Pearson chi square value is 1.801 which is less than critical value so all three fixatives do not have significant difference in cytoplasmic staining adequacy. These results were in accordance with several other authors.^{4,7,10,12,13}

When uniformity was evaluated about 76% of honey-fixed smears showed good overall uniformity in staining followed by jaggery-fixed smears (56%) and ethanol-fixed smears (58%). Chi square test gave a p value .173 which is more than significant value of .05, that shows a non significant difference between three fixatives during uniformity of staining of cytological smear. Pearson chi square value is 6.370 which is less than critical value so all three fixatives do not have significant difference in cytoplasmic staining adequacy. In the present study all cellular parameters were in concordance with Singh et al. study.^{4,30,31} Although ethanol and its various concentrations have been widely used in histopathology laboratories, it has many well-known disadvantages. However, honey has many advantages and disadvantages over ethanol as drawn from the present study, which includes viscosity of honey, the fact that diluted honey has to be mixed with antifungals and easy maintenance of honey, which can be stored in an air tightbox. Therefore, it is revealed that any cytological smears in which preservation of cellular details is necessary can be adequately and efficiently assessed with fixation in 20% unprocessed honey, and jaggery which is at par with and as good as ethanol.

Conclusion

We can infer that the Honey is as efficient as ethanol in cytological fixation and in preservation of cells jaggery was much better as compared to honey. Consistent performance of jaggery and honey identified in our study is a safety milestone to advance the field of histopathology. Further in-depth research on honey and jaggery as a possible safe substitute fixative should be conducted.

References

1. Kumaresan GD, Jagannathan N. Exfoliative cytology-A predictive diagnostic tool. *Int J PharmPharm Sci.* 2014;6:1-3.
2. Pandiar D, Baranwal HC, Kumar S, Ganesan V, Sonkar PK, Chattopadhyay K. Use of jaggery and honey as adjunctive cytological fixatives to ethanol for oral smears. *J Oral Maxillofac Pathol.* 2017;21(2):317. doi:10.4103/jomfp.JOMFP_224_15
3. Dhingra V, Mehrotra R. Historical development of oral cytology. In: Mehrotra R, editor. *Oral Cytology: A Concise Guide.* New York: Springer; 2013. pp. 59.

4. Singh A, Hunasgi S, Koneru A, Vanishree M, Ramalu S, Manvikar V. Comparison of honey with ethanol as an oral cytological fixative: A pilot study. *J Cytol.* 2015;32(2):113-117. doi:10.4103/0970-9371.160563
5. Culling CF. London: Butterworths & Co; 1963. Handbook of Histopathological and Histochemical Techniques: Including Museum Techniques.
6. Bhattacharyya A, Gupta B, Singh A, Sah K, Gupta V. Probing natural substitute for formalin: Comparing honey, sugar, and jaggery syrup as fixatives. *Natl J Maxillofac Surg.* 2018;9(1):14-21. doi:10.4103/njms.NJMS_57_17
7. Kumarasinghe MP, Constantine SR, Hemamali RL. Methanol as an alternative fixative for cytological smears. *Malays J Pathol.* 1997;19(2):137-140.
8. Olaitan PB, Adeleke OE, Ola IO. Honey: A reservoir for micro organisms and an inhibitory agent for microbes. *Afr Health Sci.* 2007;7:159-65
9. Rao PV, Madhusweta D, Das SK. Jaggery-A Traditional Indian sweetener. *Indian J Tradit Knowl.* 2007;6:95-102.
10. Ozkan N, Salva E, Cakalağaoğlu F, Tüzüner B. Honey as a substitute for formalin?. *Biotech Histochem.* 2012;87(2):148-153. doi:10.3109/10520295.2011.5901555
11. Olaitan PB, Adeleke OE, Ola IO. Honey: a reservoir for micro organisms and an inhibitory agent for microbes. *Afr Health Sci.* 2007;7(3):159-165. doi:10.5555/afhs.2007.7.3.159
12. Sabarinath, Balaraman & Sundharam, Sivapatha & Mayilvakanam, Sathya kumar. (2014). The effectiveness of honey as a substitute for formalin in the histological fixation of tissue (vol 29, pg 173, 2006). *Journal of histotechnology.* 37. 107-107. 10.1179/0147888514Z.00000000091.
13. Lalwani V, Surekha R, Vanishree M, Koneru A, Hunasgi S, Ravikumar S. Honey as an alternative fixative for oral tissue: An evaluation of processed and unprocessed honey. *J Oral Maxillofac Pathol.* 2015;19(3):342-347. doi:10.4103 /0973-029X.174641
14. Nerune S M, Khan M N, Potekar R M, Patil V, Natural versus synthetic fixative in oral cytological smears-A double blind study. *Indian J Pathol Oncol* 2018;5 (4):663-666
15. Piątek-Koziej K, Hołda J, Koziej M, et al. Fixative properties of honey solutions as a formaldehyde substitute in cardiac tissue preservation. *Folia Med Cracov.* 2019;59(1):101-114.
16. Srii R , Peter CD , Haragannavar VC , Shashidara R , Sridhara SU , Srivatsava S. Bee Honey as a Safer Alternative for Routine Formalin Fixation. *Kathmandu Univ Med J (KUMJ).* 2017;15(60):308-312.
17. Kumarasinghe MP, Constantine SR, Hemamali RL. Methanol as an alternative fixative for cytological smears. *Malays J Pathol.* 1997;19:137-40
18. Patil S, Rao RS, Ganavi BS, Majumdar B. Natural sweeteners as fixatives in histopathology: Alongitudinal study. *J Nat Sci Biol Med.* 2015;6:67-70.
19. Patil S, Premalatha B, Rao RS, Ganavi B. Revelation in the field of tissue preservation-A preliminary study on natural formalin substitutes. *J Int Oral Health.* 2013;5:31-8
20. Olaitan PB, Adeleke OE, Ola IO. Honey: A reservoir for microorganisms and an inhibitory agent for microbes. *Afr Health Sci.* 2007;7:159-65
21. Rao PV, Madhusweta D, Das SK. Jaggery-A Traditional Indian sweetener. *Indian J Tradit Knowl.* 2007;6:95-102

22. Sabarinath B, Sivapathasundharam B, Sathyakumar M. Fixative properties of honey in comparison with formalin. *J Histotechnol.* 2014;37:21–5
23. Patil S, Rao RS, Agarwal A, Raj AT. Instant transport media for biopsied soft tissue specimens: A comparative study. *Scientifica (Cairo)* 2015;2015:876531.
24. Al-Jabri AA, Nzeako B, Al Mahrooqi Z, Al Naqdy A, Nsanze H. *In vitro* antibacterial activity of Omani and African honey. *Br J Biomed Sci.* 2003;60:1–4
25. Wakefield JC. London: Health Protection Agency (HPA); 2008. Formal dehydrotoxicological overview.
26. Al Somai N, Coley KE, Molan PC, Hancock BM. Susceptibility of helicobacter pylori to the antibacterial activity of manuka honey. *Russ Med J.* 1994;87:9–12
27. Allen KL, Molan PC, Reid GM. A survey of the antibacterial activity of some New Zeal and honeys. *J Pharm Pharmacol.* 1991;43:817–22
28. Gunter M, Bryant P. Immunohistochemical evaluation of ductal carcinoma in breast after preservation in honey. *J Histotechnol.* 2009;32:54–9
29. Atlanta, US: US Department of Health and Human Services; 1999. Toxicological Profile for Formaldehyde. Agency for Toxic Substances and Disease Registry (ATSDR)
30. Molan PC. Why honey is effective as a medicine. Its use in modern medicine. *Bee World.* 1999;80:80–92
31. McCarthy J. The antibacterial effects of honey. *Am Bee J.* 1995:171–2

Table 1
Fixative solution preparation

95% Ethanol	95 ml of ethanol mixed with 5 ml of distilled water
20% honey	20 ml honey mixed in 80 ml of distilled water
30% jaggery solution	30 g of jaggery dissolved in 70 ml of distilled water

Table 2
Criteria used for analysis of efficacy between the three fixatives on staining

Parameters		20% Honey	30% Jaggery	95% Ethanol	Chi square test (P value)	Pearson Chi- Square test
Nuclear staining	2 - Good	100	84	68	0.021*	11.524
	1 -Intermediate	0	12	12		
	0 -Poor	0	4	20		
Cytoplasmic staining	2 - Good	60	64	72	0.545	3.078
	1 -Intermediate	24	28	16		
	0 -Poor	16	8	12		
Cell morphology	2 - Good	44	40	40	0.041*	3.966
	1 -Intermediate	44	40	52		
	0 -Poor	8	20	8		
Uniformity of staining	2 - Good	64	52	56	0.772	1.801
	1 -Intermediate	32	44	44		
	0 -Poor	4	4	0		

Clarity of staining	2 - Good	76	56	48	0.17	6.370
	1 -Intermediate	20	40	52		
	0 -Poor	4	4	0		

*: statistically significant

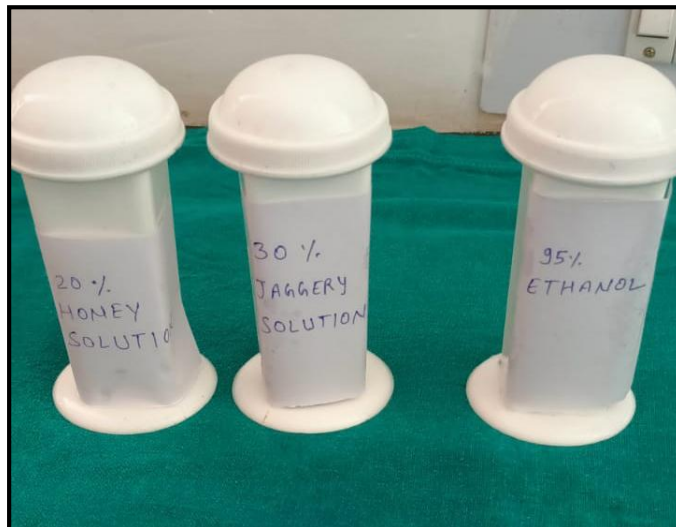


Fig 1. Fixative Agent used in the Study



Fig 2. Different content of the Stain

Nuclear staining

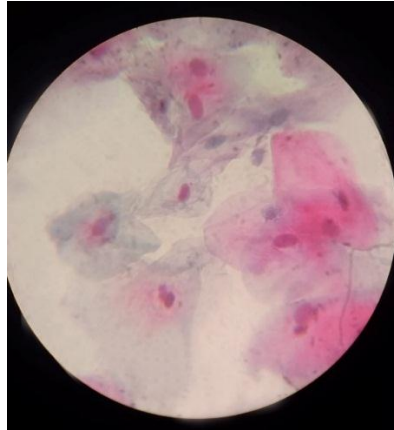


Fig 3. Ethanol used as Fixative Agent

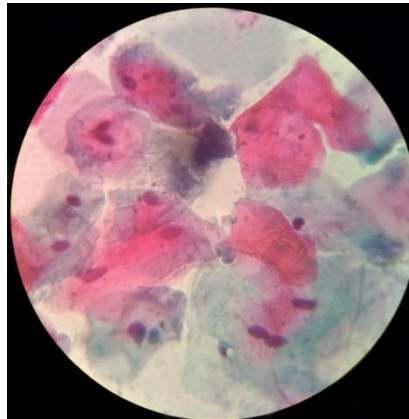


Fig 4. Honey used as Fixative Agent

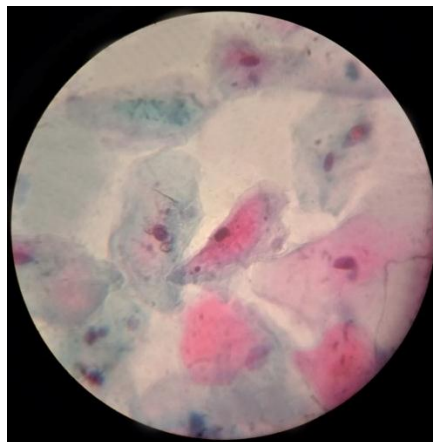


Fig 5. Jaggery used as Fixative Agent

Cytoplasmic staining

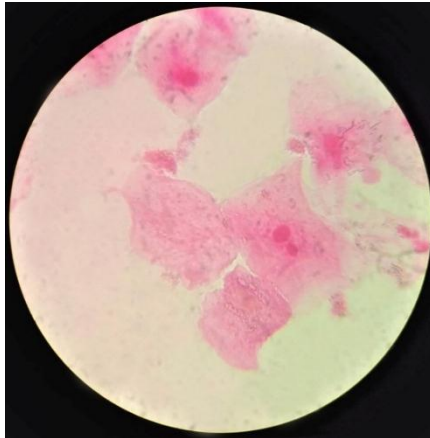


Fig 6. Ethanol used as Fixative Agent

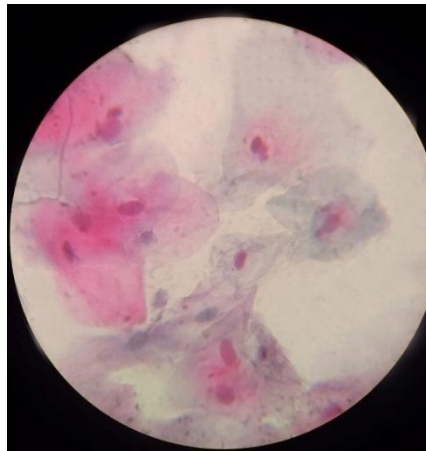


Fig 7. Honey used as Fixative Agent

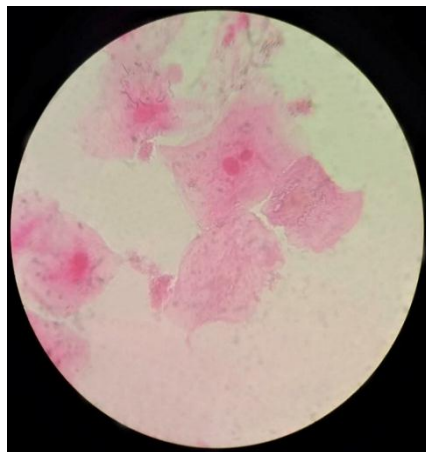


Fig 8. Jaggery used as Fixative Agent

Cell morphology

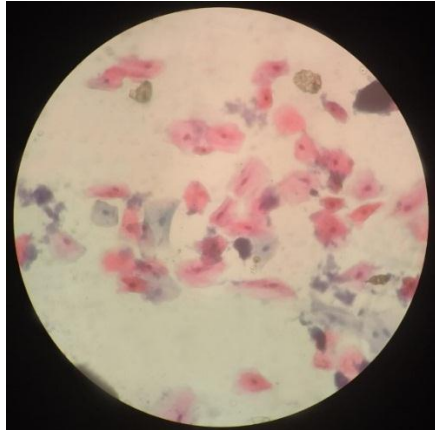


Fig 9. Ethanol used as Fixative Agent

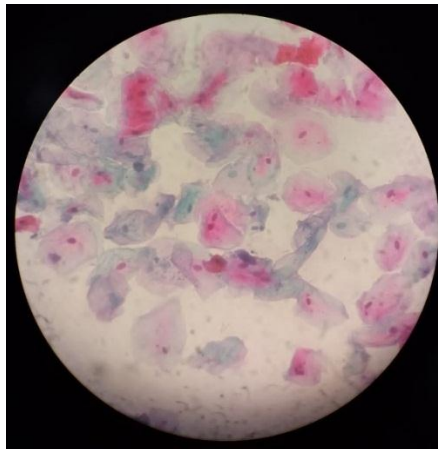


Fig 10. Honey used as Fixative Agent

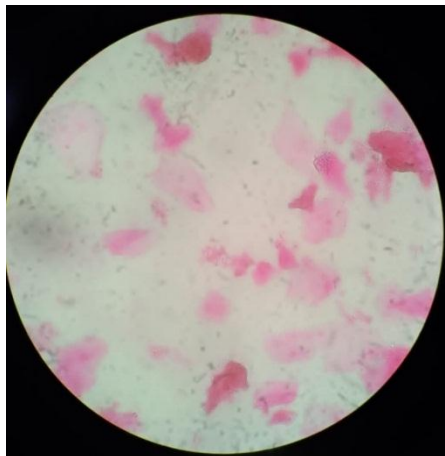


Fig 11. Jaggery used as Fixative Agent

Uniformity of stain

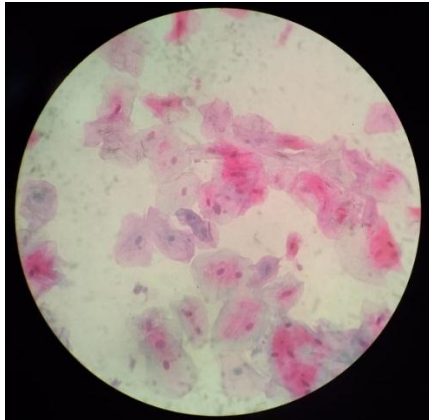


Fig 12. Ethanol used as Fixative Agent

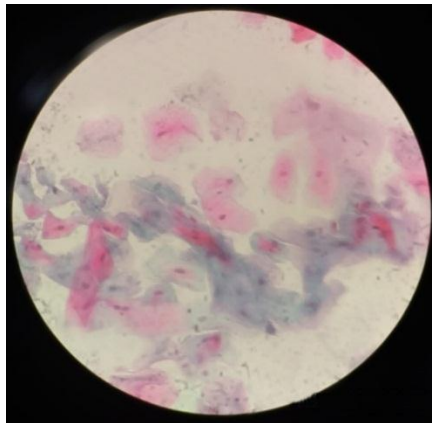


Fig 13. Honey used as Fixative Agent

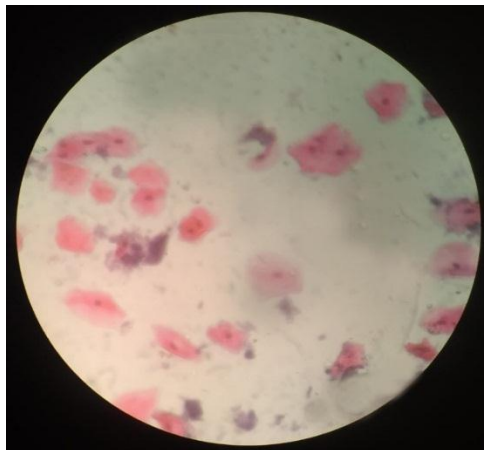


Fig 14. Jaggery used as Fixative Agent

Clarity of stain

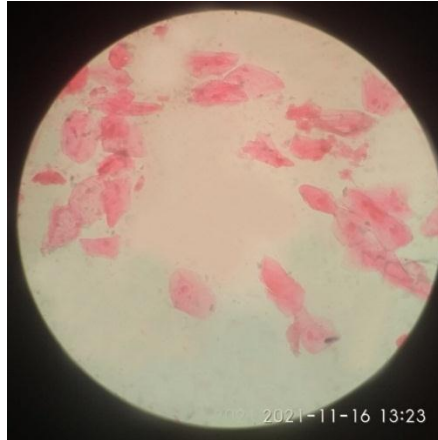


Fig 15. Ethanol used as Fixative Agent

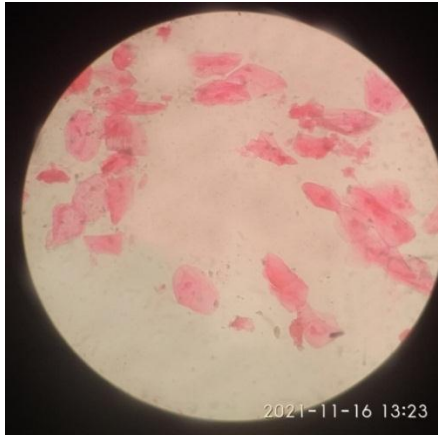


Fig 16. Honey used as Fixative Agent

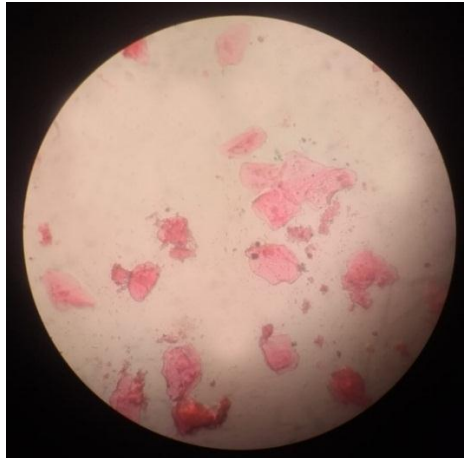


Fig 17. Honey used as Fixative Agent