COMPARISION OF FIXATIVE PROPERTIES OF HONEY WITH ETHANOL IN ORAL CYTOLOGICAL SMEARS

By

Dr. MAHMOOD NAWAZ KHAN

Dissertation submitted to the

B.LD.E. University, Vijayapura, Karnataka

In partial fulfilment of the requirements for the award of the degree of

DOCTOR OF MEDICINE

IN

PATHOLOGY

Under the Guidance of

DR. RATNAKAR M POTEKAR$_{M.D}$

Professor, Department of Pathology

B.L.D.E. UNIVERSITY'S, SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA, KARNATAKA.

2018

B.L.D.E. UNIVERSITY'S

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL

& RESEARCH CENTRE, VIJAYAPURA

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"COMPARISION OF FIXATIVE PROPERTIES OF HONEY WITH ETHANOL IN ORAL CYTOLOGICAL SMEARS"** is a bonafide and genuine research work carried out by me under the guidance of **DR. RATNAKAR M POTEKAR$_{M.D}$** Professor, Department of Pathology B.L.D.E.U's Shri B.M.Patil Medical College, Hospital & Research centre, Vijayapura, Karnataka.

Date: **Dr. MAHMOOD NAWAZ KHAN**

Place: Vijayapura

B.L.D.E. UNIVERSITY'S

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL

& RESEARCH CENTRE, VIJAYAPURA

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"COMPARISION OF FIXATIVE PROPERTIES OF HONEY WITH ETHANOL IN ORAL CYTOLOGICAL SMEARS"** is a bonafide research work done by **Dr. MAHMOOD NAWAZ KHAN** in partial fulfilment of the requirements for the degree of **Doctor of Medicine (Pathology).**

Date:

Place: Vijayapura

DR. RATNAKAR M POTEKAR$_{M.D}$
Professor, Department of Pathology,
B.L.D.E. University's
Shri B.M.Patil Medical College,
Hospital and Research Centre,
Vijayapura, Karnataka

B.L.D.E. UNIVERSITY'S

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL

& RESEARCH CENTRE, VIJAYAPURA

ENDORSEMENT BY HEAD OF DEPARTMENT

This is to certify that the dissertation entitled **"COMPARISION OF FIXATIVE PROPERTIES OF HONEY WITH ETHANOL IN ORAL CYTOLOGICAL SMEARS"** is a bonafide research work done by **Dr. MAHMOOD NAWAZ KHAN** under the guidance of, **DR. RATNAKAR M POTEKAR**$_{M.D}$ Professor, Department of Pathology, Shri B. M. Patil Medical College, Vijayapura, in partial fulfilment of the requirements for the degree of **Doctor of Medicine (Pathology).**

Date:

Place: Vijayapura

Dr. B.R.YELIKAR
Professor and Head
Department of Pathology,
B.L.D.E University's
Shri B.M.Patil Medical College,
Hospital & Research Centre,
Vijayapura, Karnataka

B.L.D.E. UNIVERSITY'S

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL

& RESEARCH CENTRE, VIJAYAPURA

ENDORSEMENT BY PRINCIPAL /

HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled **"COMPARISION OF FIXATIVE PROPERTIES OF HONEY WITH ETHANOL IN ORAL CYTOLOGICAL SMEARS"** is a bonafide research work done by **Dr. MAHMOOD NAWAZ KHAN** under the guidance of, **DR. RATNAKAR M POTEKAR$_{M.D}$** Professor, Department of Pathology, Shri B. M. Patil Medical College, Vijayapura, in partial fulfilment of the requirements for the degree of **Doctor of Medicine (Pathology).**

Date:

Place: Vijayapura

Dr. S. P. GUGGARIGOUDAR
Principal,
B.L.D.E University's
Shri B.M.Patil Medical College,
Hospital & Research Centre,
Vijayapura, Karnataka

B.L.D.E. UNIVERSITY'S

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL

& RESEARCH CENTRE, VIJAYAPURA

COPYRIGHT

DECLARATION BY THE CANDIDATE

I hereby declare that the B.L.D.E. University, Karnataka shall have the rights to preserve, use and disseminate this dissertation / thesis in print or electronic format for academic / research purpose.

Date: **Dr. MAHMOOD NAWAZ KHAN**

Place: Vijayapura

ACKNOWLEDGEMENT

It is most appropriate to begin by expressing my gratitude to Almighty for all his blessings.

I thank my parents **Mrs.Parveen sultana and Mr.Imam khan** for their constant support and encouragement.

I would like to express my sincere and deepest gratitude to my teacher and guide **Dr. R M Potekar, Professor, Department of Pathology**, for his encouragement and invaluable guidance throughout the course of my study. His guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my study.

I am equally grateful to **Dr. B.R. Yelikar Professor and H.O.D, Department of Pathology** for his valuable suggestions given at all the steps of the study. He has a profound influence on both my personal growth and professional pursuits.

I am also extremely fortunate to have a caring, approachable and supportive department, who have advised and mentored me and made it possible for me to expedite this dissertation. I am thankful to **Dr. S.U. Arakeri** Prof**, Dr. S.B. Hippargi** Prof, **Dr. Mahesh H. Karigoudar** Prof, **Dr. Girija Patil** Assoc Prof, **Dr. Prakash M. Patil** Assoc Prof, **Dr. Vijayalaxmi S Patil** Asst. Prof**, Dr. Anita P Javalgi** Asst prof, **Dr. Savitri M. Nerune** Asst prof, **Dr. Mamatha K.** Asst Prof and **Dr. Sneha Jawalkar** Asst Prof**.** for their supervision, assiduous concern and positive feedback at all steps of this work.

Special thanks to my dearest friends **Dr Anil K Reddy,** my seniors and juniors especially **Dr. Neha Kathpal, Dr. Ankur Kumar and Dr. V.Poojitha Ram** who have helped and encouraged me during my work.

I also thank **Mr. Mohd. Shahnawaz** Lecturer Statistics, for his guidance during my dissertation work.

My heartfelt thanks to my wife Mrs**. Nuzhat Banu,** my sisters, **Mrs. Khaja Banu** and **Mrs. Muneer Banu**, for their help, constant encouragement and moral support that led me to successfully complete this dissertation work.

I am thankful to the Technicians and non teaching staff of Department of Pathology.

Last but not the least**,** my sincere gratitude to all my study subjects for their contribution to this study.

Date: **DR. Mahmood Nawaz Khan**

Place: Vijayapur

LIST OF ABBREVATIONS USED

% – Percentage

EF – Ethanol fixed.

HF – Honey fixed.

NA – Nuclear area.

CA – Cytoplasmic area.

C:N – Cytoplasmic and Nuclear ratio.

NBF – Neutral Buffered Formalin.

FNAC – Fine needle aspiration cytology.

UMFIX – Universal molecular fixative

HOPE –Hepes-glutamic acid buffer mediated organic solvent protection effect

H & E – Hematoxylin and Eosin

IHC – Immunohistochemistry

FISH – Fluorescent In Situ Hybridization

ABSTRACT

BACKGROUND

Diagnostic accuracy of the cytological smears, histopathology slides depends mostly on quality of collection, fixation, staining and interpretation. Fixation is an important step in cytopathological diagnosis. The role of fixation is to retain cellular components in their respective compartments and to present cells with a distinct and detailed microscopical appearance.

Ever since the introduction of fixatives, there has always been an enthusiasm and quest for an ideal cytological fixative. 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.

Honey is produced from many floral sources and contains several minerals, trace elements, and vitamins, as well as carbohydrates. 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. Above said properties are being exploited for fixation of oral cytological smears, which yielded results comparable to ethanol after staining and evaluation.

OBJECTIVE

To compare fixative properties of honey in routine oral cytological smears with ethanol by studying cytomorphological features.

MATERIALS 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.

Study period: 1st December, 2015 to 30thJune, 2017.

RESULTS

In the present study, out of the 200 cases studied, 193 (96.5%) cases Ethanol fixed (EF) and 186 (93%) cases of Honey fixed (HF) smears showed acceptable nuclear staining and 7 (3.5%) cases of EF and 14 (7%) cases of HF smears showed unacceptable nuclear staining which was statistically significant with p value of 0.008. (Table.5.4) 178 (89%) cases EF and 160 (80%) cases of HF smears showed acceptable cytoplasmic staining and 22 (11%) cases of EF and 40 (20%) cases of HF smears showed unacceptable cytoplasmic staining which showed no statistical difference between both fixatives with p value of 0.821.

Out of 200 cases 181 (90.5%) cases EF and 188 (94%) cases of HF smears showed preserved cell morphology and 19 (9.5%) cases of EF and 12 (6%) cases of HF smears showed unpreserved cell morphology which showed no statistical difference between both fixatives with p value of 0.092. 190 (95%) cases EF and 176 (88%) cases of HF smears showed clarity of staining which was absent in 10 (5%) cases of EF and 24 (12%) cases of HF smears which was statistically significant with p value of 0.005

Out of 200 cases uniformity of staining was present in 191 (95.5%) cases EF and 184 (92%) cases of HF smears and uniformity of staining was absent in 9 (4.5%)

cases of EF and 16 (8%) cases of HF smears which was statistically significant with p value of 0.001

CONCLUSION

Honey fixed smears showed well preserved and acceptable cytomorphological features similar to ethanol fixed smears. The cellular features were well preserved even after revaluation after 6 months period. Introducing honey as cytological fixative will decrease cost and side effects related to ethanol usage in laboratories.

KEY WORDS:

Honey, Ethanol, Oral cytological smears.

TABLE OF CONTENTS

Sl. No.	Contents	Page No
1	INTRODUCTION	**1**
2	AIMS AND OBJECTIVES	**4**
3	REVIEW OF LITERATURE	**5**
4	MATERIALS AND METHODS	**31**
5	RESULTS	**35**
6	DISCUSSION	**50**
7	SUMMARY	**61**
8	CONCLUSION	**63**
9	BIBILIOGRAPHY	**64**
10	ANNEXURES	**73**

LIST OF TABLES

SL NO	TABLE	PAGE NO
4.1	Evaluation criteria	**33**
5.1	Distribution of cases by sex	**35**
5.2	Distribution of cases by Age and sex	**36**
5.3	Mean age of cases	**37**
5.4	Distribution of cases comparing various cytomorphological features of Ethanol fixed smears and Honey fixed smears.	**37**
5.5	Concordance between Ethanol fixed smears and Honey fixed smears in Nuclear staining	**40**
5.6	Concordance between Ethanol fixed smears and Honey fixed smears in Cytoplasmic staining	**40**
5.7	Concordance between Ethanol fixed smears and Honey fixed smears in preserving Cell morphology	**41**
5.8	Concordance between Ethanol fixed smears and Honey fixed smears in Clarity of staining	**42**
5.9	Concordance between Ethanol fixed smears and Honey fixed smears in Uniformity of staining	**42**
5.10	Correlation coefficient between Ethanol fixed smears and Honey fixed smears	**43**
6.1	Comparison of percentage of acceptable nuclear staining of ethanol and honey fixed smears in various studies.	**51**
6.2	Comparison of percentage of acceptable cytoplasmic staining of ethanol and honey fixed smears in various studies.	**53**
6.3	Comparison of percentage of preserved cell morphology of ethanol and honey fixed smears in various studies.	**54**
6.4	Comparison of percentage of clarity of staining of ethanol and honey fixed smears in various studies.	**56**
6.5	Comparison of percentage of Uniformity of Staining of ethanol and honey fixed smears in various studies.	**57**

LIST OF FIGURES

SL NO	FIGURES	PAGE NO
4.1	Commercial Honey	34
4.2	Stock solution – 50ml of 20% honey(40ml distil water and 10 ml of honey)	34
4.3	Absolute Ethanol	34
4.4	Coplin Jar	34
5.1	Distribution of cases by sex	35
5.2	Distribution of cases by Age and sex	36
5.3	Distribution of cases comparing various cytomorphological features of Ethanol fixed smears and Honey fixed smears.	38
5.4A	Ethanol fixed smear showing adequate cellularity – PAP - X100	44
5.4B	Honey fixed smear showing adequate cellularity - PAP - X100	44
5.5A	Ethanol fixed smear showing acceptable nuclear staining – PAP - X400	45
5.5B	Honey fixed smear showing acceptable nuclear staining - PAP - X400	45
5.6A	Ethanol fixed smear showing acceptable cytoplasmic staining - PAP - X400	46
5.6B	Honey fixed smear showing acceptable cytoplasmic staining - PAP - X400	46
5.7A	Ethanol fixed smear showing preserve cell morphology - PAP - X400	47
5.7B	Honey fixed smear showing preserve cell morphology - PAP - X400	47
5.8A	Ethanol fixed smear showing acceptable uniformity of staining - PAP - X100	48
5.8B	Honey fixed smear showing acceptable uniformity of staining - PAP - X100	48
5.9A	Ethanol fixed smear showing acceptable clarity of staining - PAP - X100	49
5.9B	Honey fixed smear showing acceptable clarity of staining - PAP - X100	49

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] Obtaining oral exfoliative cytology is non-invasive, simple, painless technique, providing instant results and guide patient management or referring them to higher grade of investigation when required.[2,3]

Major role of fixation in cytology and histopathology is to preserve and maintain clear and consistent morphological features. Adequate fixation is required for proper examination of tissue or cells understudy, to reach a proper diagnosis. Modern immunohistochemical and molecular techniques also require proper fixation of tissue without loss of antigens or molecules which makes tissue compatible for these techniques. Fixation prevents autolysis by preventing enzymatic destruction of cellular and extracellular molecules. They also prevent tissue from microbial contamination and destruction.[1]

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 non-toxic, cheap and easily available, should preserve tissue for long time and should be compatible with immunohistochemical and molecular techniques.

Russian chemists Alexander M Butleroy first discovered formaldehyde in 1859. Property of formaldehyde to fix tissue was discovered by Ferdinand Blum in 19th century. Since then the fixative property of formaldehyde is being exploited for fixing tissue which aid in histopathological examination and diagnosis. Though

formaldehyde provides excellent fixation, there are certain disadvantages most commonly being health hazard to laboratory personnel who are exposed to them regularly.[4] Other disadvantages are loss of tissue antigens which require additional antigen retrieval techniques for Immunohistochemistry adding to turn around time and extra cost.

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.[5]

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.[6]

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. Considering its fixation ability in histopathology it has been experimented in few cytological studies. Use of honey in funerary practices in many different cultures is well documented. A custom of preserving chief abbots in coffins full of honey by Burmese priests and mummification in honey by Egyptians is very well known.[7]

Honey is well known natural reliable sweetener. 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.[7]

Honey preserves the tissue morphology similar to formalin in histopathology and has been experimented widely. 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.[4] 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.

The antibacterial properties of honey are due to its inhibitory effect on wide varieties of aerobic, anaerobic, gram positive and gram negative bacteria.[8] Honey prevents the cells understudy from autolysis and putrefaction. Growth of moulds over a period of time is a limitation of honey which can be overcome by addition of thymol crystals. Honey when used for tissue fixation in histopathology might cause some problems such as breach in continuity of sections and intense staining with eosin.[4]

Use of natural and easily available fixatives in screening camps, doctors working in clinics in remote areas can be instant choice for immediate fixation of scrapped or biopsied tissues in honey. This can also be used as transport media under such circumstances. Implementation of eco-friendly natural fixatives in routine cytopathological diagnosis is a safety milestone in advancing the field of cytology. Honey as a fixative, is still at experimental levels and is yet to be implemented as a routine fixative in the long run.[9]

AIMS AND OBJECTIVES

To compare fixative properties of honey in routine oral cytological smears with ethanol by studying cytomorphological features.

REVIEW OF LITERATURE

Exfoliative Oral Cytology

Cytology provides rapid diagnosis by minimally invasive technique. Exfoliative cytology as a method of diagnosis was first introduced by Papanicolaou in 1943. Exfoliative cells from oral epithelium have been widely used in cytology to detect abnormal nuclear and cellular morphology depicting precancerous and cancerous changes. Buccal mucosa due to more surface area is widely affected when exposed to insults in oral cavity resulting in epithelial changes.[10]

Exfoliative oral cytology has undergone significant advances and sequential improvisation related to screening of oral cancers and evaluation of oral precursor lesion. Gold standard in diagnosing oral lesions is histopathological examination of the excised biopsy tissue but exfoliative cytology technique provides a range of diagnosis of preneoplastic, cancerous, infective and inflammatory disorders. Exfoliative oral cytological smears can play an important role in diagnosing lesions which are clinically not obvious or suspicious for malignancy and might obviate the need of invasive biopsy procedure.[11]

Heterogenous oral mucosa can be separated into masticatory mucosa, lining and specialized types. Masticatory type of mucosa covers the hard palate and gingivae and in places is bound directly to bone forming a mucoperiosteum. Pink colour is due to keratin layer. Lining mucosa covers the ventral tongue, floor of mouth, soft palate, buccal, labial and vestibular surface of oral cavity. Transparent lining epithelium is non-keratinizing, appearing red in colour due to underlining blood vessels in lamina propria. Specialized mucosa lines the dorsum of the tongue covered by filiform papillae at specific locations.[11]

Evolution of oral cytology

Oral exfoliative cytology modifications have started with Gladstone in year 1951 who has used sponge technique in improving the quantity of cell obtained by oral exfoliative cytology. Schneider (1952) and Cawson (1960) have modified staining methods. King (1963) has used frosted glass slides. Staats and Goldsby in 1963 have compared metal and wooden spatulas for obtaining oral exfoliative material. Sandler in 1964 has improvised the technique further and used sharp curette to remove keratotic layer. Dumbach *et al* (1981) included deeper layers by use of curette.[12]

Oral exfoliative cytology involves scraping of the oral cavity randomly or from visible lesions of oral cavity. Collection devices such as Cytobrush, Orca-brush etc are used to procure cells from superficial and intermediate layers. Scrapped material is spread over the slide followed by immediate fixation. These fixed smears are evaluated for cellular abnormalities after staining.[13]

Buccal smears are also used in Forensic Medicine and Criminology and Civil Law in cases of legitimacy, divorce, paternity, affiliation, marriage, education, impotence, right to disposal of property, in intersex condition, in cases of concealed sex and identification of the sex of individual whether living or dead. Sex determination can be determined by just using a single specimen of buccal smear.[14]

Buccal mucosa is simplest one, easily approachable and most widely used for sex determination and clinical studies.[15]Buccal smears are also exploited to confirm the expected performance of a new lot of stain. Buccal smears after fixation are stained separately with haematoxylin and combination of OG and EA stains followed by drying and mounting. These sets of Buccal smears are evaluated separately for

nuclear staining by haematoxylin and cytoplasmic differentiation by OG and EA. Preparing buccal smears in this way provide the true colour of each major dyes without any possible interference.[16]

Buccal smears can be obtained by simple procedure as follows[13]

1. Explain the procedure in brief to the client and take an informed consent.
2. Instruct the client to vigorously rinse mouth with water several times before the test. It cleanses the area of excessive organism
3. Scrape the client's oral cavity or oral lesion with a spatula. If the scrape is for genetic assessment it is taken from lateral Buccal mucosa just above the dentate line along the anterior two-thirds of the Buccal mucosa. If the scrape is for pemphigus, the lesion should be scrapped, where the normal and the affected mucosa meet.
4. First scrape material is discarded.
5. Repeated scrapping of mucosa gently from deeper layers will obtain healthy epithelial cells.
6. Scrapped material is gently spread over the labelled slide in single layer and fixed immediately with spray or liquid fixative. It ensures accurate results
7. Fixed smears are stained and evaluated.
8. Instruct the client to rinse mouth after scrapping. Promote good oral hygiene.

Karthik KR *et al*[17] have compared glycosylated haemoglobin obtained from 40 known cases of diabetes mellitus with cytomorpholgically evaluated nuclear area (NA) cytoplasmic area (CA) and C:N (cytoplasmic and nuclear) ratio as a new parameter to assess its usefulness and reliability in glycemic control of diabetes mellitus. CA was normal and NA was found to increased in diabetic patients when

compared to healthy controls, as a result C:N ratio was decreased in diabetic individual correlating with poor glycemic control and higher percentage of HBA1c. This is one of other advantage of buccal smear which can be used in remote areas to roughly analyze glycemic control in individuals where well equipped laboratories are lacking. Honey can be a good alternative in such areas as ethanol is obtained only by license laboratories.

Diagnostic accuracy of oral exfoliative cytological samples depends upon the technique of procuring samples, fixation, staining, screening, interpretation of the smears and quality control. Fixation is most important step in maintaining intact cellular details, useful for proper evaluation and increasing diagnostic accuracy.

Fixation

Fixation is an important step in histopathology and cytology without which interpretation of cellular details are difficult and provides no pathological information. Fixation prevents autolysis by stabilizing the hydrolytic enzymes which are released from non viable tissue. Apart from fixation it also prevents tissue from microbial contamination and tissue damage.[1]

Cellular material should be spread uniformly on slide and immediately transferred to fixative for appropriate fixation. Marked distortion of cells occurs if smears are allowed to be air dried. In past fixative of choice were equal parts of ethanol and ether.[18] But this has been discontinued because ether is highly inflammable. Presently most commonly used fixative in cytology is 95% ethanol which provides excellent results. This method of fixation may be used for all smears prepared bedside, such as fine needle aspiration smears, pap smears and buccal

smears, but alternate easily available fixatives are required in health camps and rural areas where free availability of ethanol is limited. [19]

95% Ethanol is also used as a final fixative for all smears prepared in the laboratory from fresh fluids or those collected in other fixatives or 50% alcohol. For ideal results smears should remain in fixative for atleast 15min prior to staining. However, prolonged fixation of several days or even weeks will not materially alter the appearance of the smear.[19]

License is required to obtain ethanol for its use in laboratory. This is one of the major limitations of ethanol being not freely available.[20] 100% Methanol, 95% denatured alcohol, 80% Propanol, 80% Isopropanol are all used as alternative to 95% ethanol which gives similar results. To yield good results alcohol fixatives should be discarded or filtered after each use with a good-grade, medium-speed filter, such as Whatman No.1, and the concentration should be tested with a hydrometer before reuse.[19]

Certain advantages and disadvantages are always associated with various fixatives used in histopathology and cytology. Most common disadvantages are cellular loss, swelling and shrinkage of the cells and tissue during processing and antigen loss which affect results of Immunohistochemistry and biochemical analysis. However, this limitation has been overcome by heat-induced epitope retrieval methods to a large extent but unfortunately this technique will increase cost and turnaround time.[21]

Considering advantages over disadvantages, different fixatives are used appropriately as and when indicated.[1]

Ideal Fixative

Ideal fixative must have following characteristics[1]

1. An ideal fixative must provide consistent and good quality of staining with routine and special stains. Consistency should be maintained even after long period of storage.
2. It should preserve tissue for longer time and prevent autolysis.
3. It should maintain tissue and cellular integrity.
4. An ideal fixative must be non-toxic and non-flammable.
5. It should preserve tissue antigens and molecules which are required for Immunohistochemistry and molecular analysis.
6. It must be able to fix various tissue including neural, muscle and fat.
7. It should be feasible for small and large tissue specimens and must be able to provide constant and proper results when tissues are subjected to Immunohistochemistry and special procedures such as in situ hybridization.
8. It should fix tissue rapidly and must provide desirable tissue sections for analysis with various modern instruments.
9. It should be reusable and upon prolonged storage its inhernt properties should be maintained.
10. It should be easily available and cost-effective.

Various methods of fixation

Tissues can be fixed by two basic methods such as chemical and physical fixation. Some of the physical methods include freeze-drying, microwave fixation and freeze substitution. Organic and non organic chemicals are used as chemical fixation.

Various common types of chemical fixatives are aldehyde fixatives, compound and coagulant fixatives.[22]

Types of fixative

Aldehyde fixative

Gold standard and most commonly used fixative in histopathology is Neutral buffered formalin (NBF). It cross-links and adds active hydroxymethyl groups to amines, amides, some reactive alcohols, and sulfydryl groups. It penetrates between the proteins and nucleic acid and cross-links sulfydryl side chains.

Huang BQ *et al*[22] have described various types (Physical and Chemical fixation) and importance of fixation. Chemical fixation is preferred over physical fixation for preservation and appropriate hardening of the specimen received for histopathological examination. Histopathological specimens must be properly immersed in suitable chemical fixative for adequate period of time for complete fixations of cells in the tissue by stabilizing the cell contents.[22]

In physical fixation, cells in the tissue are inactivated using cryopreservation and microwaving. Basic mechanism of most of the fixatives is to inactivate the biochemical and proteolytic processes which immobilize structures and tissues which are locked in space.[22]

Kiernan JA *et al*[23] has described fixation and preparatory methods of various aldehyde fixatives such as formaldehyde, formalin, glutaraldehyde and paraformaldehyde. Among the formaldehyde fixatives, formalin (liquid state) contains around 60% of water by weight. Fixation of the tissue due to formalin is entirely because of interaction of these molecules with proteins present within the tissue.

These aldehyde group form methylene bridges by cross linking leading to fixation of the tissues. Other substances such as carbohydrates, nucleic acid and lipids are insolubilized by trapping these substances in matrix formed by cross-linking of protein molecules.

Fox HC *et al*[24] has described the mechanism of fixation by formalin, its advantages and disadvantages. They also highlighted the formic acid contamination during fixation, effect of temperature on formaldehyde fixation and shrinkage of cells and tissue by formaldehyde.[24] Formalin, when stored for longer periods, gets oxidized to form formic acid. Hence, in stored formaldehyde, presence of unknown formic acid (also reacts with blood to form a birefringent crystal called formalin pigments) is expected. Formic acid formation is usually confused with melanin and other pigments. This can be overcome by use of natural fixatives.[25]

Srinivasan M *et al*[26] have described the disadvantages of the aldehyde and coagulant fixatives related to maintenance of nucleic acid integrity. When compared to the DNA isolated from frozen tissues, formalin-fixed tissues exhibit a high frequency of non reproducible sequence alteration. Coagulant fixatives preserve tissue antigens and nucleic acid pretty well when compared to aldehyde fixatives but upon chemical measurement coagulant fixatives have shown collapsed DNA. These collapsed DNA show reversion to original form on rehydration.

Formalin solutions are typically used for fixation of histological samples, where as alcohol-based fixatives are typically used in cytological smears. Formalin works by covalently cross-linking proteins and nucleic acid. Alcohol fixatives remove the stabilizing water molecules around proteins and nucleic acid and cause these

biomolecules to unfold and then rapidly aggregate within the cells. This precipitation of biomolecules in the cells is partially reversible.[27]

The duration required for fixation of cells in formalin is more when compared to alcohol fixatives. Formalin penetrates immediately within the cells, but duration required fixing cellular structures are much less in alcohol fixatives. Most of the time formalin fixed tissue leads to loss of proper nuclear details resulting in artefacts commonly known as nuclear budding/soapsuds and washed out nuclei/blue halo.[28]

Formalin though most widely used fixative in histopathology, it carries many health hazards. It is corrosive to most metals. It causes severe eye and skin irritation. It is toxic when inhaled or ingested. It is a proven carcinogen which has been linked as an etiological factor in many tumours especially nasopharyngeal carcinoma. All the above health hazards are directly proportional to duration of exposure and concentration of formalin. Due to its carcinogen properties proper precautions and guidelines should be made to monitor exposure level.[1]

Fritzsche FR *et al*[29] have done a nationwide online questionnaire with regard to occupational health hazard among pathologists in Switzerland. Around one-third of them have reported exposure to formalin has caused intolerance, severe eye, mucosal and skin irritation. Apart from this formalin is also a well known carcinogen causing nasopharyngeal carcinoma upon prolong exposure. Technical staff working in histopathology laboratories are more prone to develop side effects from prolong exposure of formalin which warrants monitoring of exposure level.

Buesa RJ *et al*[30] have stressed upon the use of formalin substitution with newer fixatives such as BOON-Fix, Fix All and UNI-Fix considering the health issues related to exposure of formalin. Loss of tissue antigens making it useless for

Immunohistochemistry and in situ-hybridization warrants need of formalin substitutes which are compatible with modern methods of pathological analysis at antigen and molecular levels.

Coagulant fixatives

Dehydrant coagulant fixatives include acetone and alcohol fixatives such as ethanol and methanol. Ethanol is gold standard fixative in cytology. Ethanol cause rapid fixation of cells within the tissue, prevents autolysis and preserves cell morphology for long period of time. Ethanol is very well compatible with routine cytological staining and gives excellent results. Alcohols are also been used widely for fixing histopathological specimen alone or in combination with other fixatives.

Kumarasinghe MP *et al*[31] used methanol as an alternative to ethanol in 108 cases of fine needle aspiration cytology (FNAC). Minimum of two slides were obtained from each FNAC. One was fixed immediately in routine 95% ethanol and other was fixed in 99% methanol. After fixing for 15 min they were subjected to routine haematoxylin and eosin staining. Both slides of all cases were blindly evaluated by separate pathologist and scoring was allotted. No difference was observed in both fixatives. Hence methanol can used as an alternative to ethanol, which is a cheap, free available and certain issues with misuse of ethanol can be overcome.

Ethanol is expensive, flammable, carcinogenic and not freely available. Laboratories should produce license to obtain ethanol for specific purpose of laboratory. Considering the limitations of above said gold standard fixatives in histopathology and cytology, there exists a quench to search a new natural alternate fixative which should meet the criteria of an ideal fixative. During this enthusiastic

search, honey has been experimented as fixative in various histopathological, cytological and immunohistochemical evaluation and diagnosis. Honey has proved to be cost effective, eco-friendly and on-par with gold standard fixatives.

Ananthalakshmi Ramamoorthy SR *et al*[32] have highlighted the health hazards of commonly used chemicals in histopathology and suggested their safe and natural alternatives. Among natural sweetener honey has come up with inherent fixative properties due to its low pH. It also has anti-bacterial properties which prevents cellular and tissue contamination. Apart from above said properties it also prevents autolysis. Considering this, honey has been intensely investigated to evaluate its fixative properties in histopathological specimens. Tissue fixed in honey when subjected to tissue processing and staining has shown acceptable results.

Nathan NA *et al*[33] recommends the use of an ethanol formalin fixative (1:9) solution of 40% formaldehyde and 100% ethanol for processing of cell blocks. This fixative results in excellent cytomorphologic features that closely resemble the cytologic detail seen in Papanicolaou-stained smears. The histochemical and immunocytochemical properties are also maintained. The fixative must be prepared fresh and used immediately because formalin is capable of oxidizing to formic acid.

Coating Fixatives

Coating Fixatives which can be sprayed or applied with a dropper to freshly prepared smears are on high demand in market today, eliminating use of bottles and fixing solutions. High content of alcohol in hair spray was at one time as effective as fixative, but it is no longer considered suitable for this purpose.[34] Currently numerous cost-effective Pap smear collection kits and aerosol sprays are available, that gives excellent results. These spray fixatives not only fix the smears but also provide

protective coating over smears. Spray fixative comes into picture when these unstained smears are required to be transported or mailed to distant cytology laboratory for evaluation.

Instruction provided by the manufacturer for the use of coating fixatives should be followed strictly. Cans should be shaken well prior to each use to ensure optimal dispersal and adequate fixation. Coating fixatives should be applied to fresh smears immediately. Quality of cytological details depends on the distance from which spray fixatives are sprayed on the smears. The optimal distance differs with the brand of fixative used. Danos-Holmquist tested several spray fixatives and found that the distance of 10–12 inches was optimal.[35]

Aerosol spray should be avoided in bloody smears because they cause clumping of RBCs. Coating fixatives which can prepared in the laboratory are Polyethylene Glycol (Carbowax) fixative and Diaphane Fixative. Coating fixatives must be removed prior to staining to avoid contamination, by washing in 95% ethyl alcohol.[36]

Rehydration of gyenocological smears i.e air dried smears are done by placing in 50% aqueous solution of glycerine for 3 min followed by two rinses in 95% ethyl alcohol prior to routine staining. Non gynaecological smears which are air dried prior to fixation provide superior cytological details when compared to rapidly fixed smears. Other advantages include lesser risk of cell loss and ease of collection by untrained personnel.[37]

Some of the alternative methods that can be used instead of coating fixatives for mailing smear to distant centres. Glycerine method:- Smears are first fixed in 95% ethyl alcohol for a minimum of 15 minutes. The slides are then removed and one or

two drops of glycerine are placed on the smear and covered with a clean glass slide. The slides may now be wrapped in wax paper and mailed to the laboratory in a suitable container.[19]

Honey in field of medicine

Dated back to 1250 BC, honey has been well known for its medicinal uses. Honey is supersaturated solution of sugars, acids, vitamins, minerals and other minor components. The main sugar components in honey are fructose and glucose. Honey has been documented to have medicinal properties, used in wound dressing due to its antiseptic properties since ancient times. Sumerian clay tablets dated from 1900 to 1250 BC containing honey 30% was used as medicinal drug in ancient times. It has also been used as ointment for treating various diseases of skin and eyes by Egyptians in ancient times.[38]

It has been documented that Hippocrates was a great believer in Honey. He considered it as a good cough expectorant, which bring up phlegm from the lungs. He also added that honey as ability to cause heat which heals ulcers, sore tissue and carbuncles. In last 10-15 years honey has gained substantial recognition for its antibacterial and wound healing properties.[38]

Constituents in Honey

Floral sources provide honey. Different floral sources produce honey which differ in their constituents.

It contains

1. Lysozymes (hydrolytic enzymes active at acid pH is responsible for anti-bacterial property.

2. Several minerals and trace elements such as potassium, sodium, chlorine, calcium, magnesium, magnesium, iron, manganese, copper, magnesium, sulfur and silicon (as SiO2) and many other elements.
3. Vitamins such as B1 (Thiamine), Riboflavin, Niacin, B6 (Pyridoxine), Pantothenic acid, vitamin B12 and vitamin C (Ascorbic acid) are also found in honey.[39]
4. It also contains tetracycline derivatives, fatty acids, amylases, lipids, hydrogen peroxide and ascorbic acid which prevent autolysis and putrefaction.[40]
5. Small amount of enzymes which are present in honey makes it unique, when compared with other sweeteners.

Enzymes in honey

Various enzymes present in honey

1. Diastase and amylase, catalyses reaction which converts starch to other carbohydrates.
2. Invertase, saccharase, hydrolase and Sucrase catalyses reaction which converts sucrose to fructose and glucose.
3. Other enzymes such as glucose oxidase converts glucose to gluconolactone, which in turn yields gluconic acid and hydrogen peroxide

Other enzymes present in honey are catalase, beta-glucosidase, Esterase, Acid Phosphatase and Protease.

Antibacterial property of honey

Antibacterial property of honey was first reported by Van ketel (1892). Dold *et al* (1937) used the term "inhibine" for the antibacterial activity for honey.[41]

Inhibition of growth of Staphylococcus aureus was noticed when honey was pipetted into the wells of agar plate which was impreganated with Staphylococcus aureus. The clear zone created on agar plate is the measure of potency of honey. Honey has no effect on fungi beyond its osmotic action hence, growth of moulds was observed when honey was diluted. Growth of moulds was noted in present study when processed honey was diluted 1:4 ratio with distilled water for a period of 8 to 12 hours. Simple addition of a pinch of thymol was enough to prevent moulds formation.[42]

Sugar molecules present in honey react strongly with water molecules which leave behind scant free water to be utilized by micro-organisms. This free water is measured as the water activity (a_w). Mean value of honey water activity is 0.562. These water activity in range of 0.94 – 0.99 prevents growth of many bacteria (better with natural honey) which form the basis behind antibacterial properties of honey. This water activity is not so powerful to affect fungal organism.[42]

Acidic property of honey (pH between3.2 to 4.5) is due to presence of gluconolactone/gluconic acid and hydrogen peroxide which are formed as a result of enzyme action in nectar ripening. This gluconic acid is also responsible for honey antibacterial property. Potency of honey in performing anti-bacterial property can be measured by quantity of hydrogen peroxide which was reported by Adcock in 1962. Addition of catalsae neutralizes the antibacterial property of honey making it ineffective against fighting bacteria.[43,44,45]

Almasaudi BS *et al*[46] have compared antibacterial properties of various concentrations of different types of honey against Staphylococcus aureus. They observed that at concentration of 20% and 10% (V/V), the antibacterial properties

were best. Among the different types of honey Manuka honey showed powerful antibacterial activity against Staphylococcus aureus.

Molan CP *et al*[40] listed the various bacteria which are sensitive to antibacterial action of honey. Some of the bacteria which are sensitive to antibacterial action of honey include Salmonella, Mycobacterium tuberculosis, Listeria moncytogenes, Corynebacterium diptheriae, Haemophilius influenza, Vibro cholera, Pasturella multocida etc. Growth of this bacteria is prevented due to which honey provide contamination free fixation of cells and tissue.

Afroz R *et al*[47] have described various other properties of honey such as anti-hyperglycemic, and its utilization for digestive problems by inhibiting microbial growth due to its antibacterial property. Honey plays a role in maintaining balance of good and harmful bacteria inside the gut. Therapeutic properties of honey have been attributed to the presence of Phenolic acids and flavinoids. All these properties depend upon various types of honey and floral sources from which they are obtained.

Honey because of medicinal properties has been regarded as a wonderful gift of nature. Popular medicine has used it in treatment of many diseases since time immemorial. Honey is not only a tasteful and nourishing food but due its complex chemical and physical composition, it can be successfully used as a medicine against various diseases.[48]

Moore OA *et al*[49] have highlighted the antibacterial properties of honey by comparing them with various antiseptics and antibiotics. These antiseptics and antibiotics were compared with honey in wound dressing which showed that honey was superior to other wound healing agents in terms of efficacy and duration requirement. Features of honey such as low pH, high osmolarity and presence of

contents like hydrogen peroxide prevents the growth of bacteria and ensure rapid healing. Antibacterial activity of honey was potent even when the dilution was fourteen fold beyond the point where it is normally used for wound dressing agent. Similar finding has been observed in present study where 1 in 5 dilution was done for fixing buccal smears, which show well preserved cell morphology and growth of bacteria was inhibited which was confirmed by reviewing the slides after 6 months.

Avwioro G *et al*[50] preserved various organs enblocked after sacrificing rat in undiluted pure honey and formalin. The tissues where then subjected to routine processing and staining. After evaluation results showed that honey fixed tissues showed well preserved nuclear and cytoplasmic details similar to formalin. Some of the tissue bits stored for a period of 30 days before processing in undiluted pure honey also showed similar quality of tissue and cellular morphology as compared to formalin. These preserved tissue showed adequate hardening without evidence of autolysis and putrefaction.

Wahba NM *et al*[51] studied effect of honey in treatment of subclinical mastitis. A total of 20 cases of cows with subclinical mastitis were taken from two farms (10 cases of subclinicalmastitis from each farm) One group received infusion of honey solution and other group received antihistaminic drug intramuscularly for three consecutive days. Both groups showed decrease in total bacterial count in milk at 3rd and 10th day after administration. This was a supporting evidence of antibacterial and anti-inflammatory properties of honey which have potency equivalent to routinely prescribed drugs.

Ahmed S *et al*[52] have highlighted that inherent properties of honey are not only limited to its antibacterial property or tissue preservation but they also have potential

anti-cancer effect, Apoptotic activity, Antiproliferative activity, Effect on Tumor Necrosis Factor, Anti-Inflammatory and Immunomodulatory activities, Antioxidant activity, Antimutagenic activity, estrogenic modulatory activity and act as an also anti-cancer agent.

Rashad U *et al*[53] have used honey as an alternative in treatment of mucositis which were caused by chem-radiotherapy in head and neck cancer patients. A total of 40 cases on chemo-radiotherapy treatment were randomized into two groups. One group received chemo-radiotherapy along with topical application of honey to oropharyngeal mucosa and other group were subjected to chemo-radiotherapy without application of honey. From all patients oral swabs were collected before and after chemo-radiotherapy. After microbial culture results it was concluded that prophylactic use of pure natural honey could prevent radiochemotherapy induced mucositis in patients with head and neck tumours.

Olaitan PB *et al*[54] discussed the antibacterial and antifugal properties of honey. This property has provided an alternate pathway for preventing emergence of drug resistance. Over 60 species of bacteria, dermatophytes and fungus like aspergillus and penicillium has shown sensitivity to honey treatment.

Honey as fixative

Lalwani V *et al*[55] in their study evaluated fixative ability of processed and unprocessed honey in which 36 human tissues including oral epithelium, lymphoid, salivary gland, fat, muscle and skin that were taken from the Department of Oral Pathology. Twelve different tissues were cut into 3 bits and were immediately fixed in a 10% unprocessed honey (10%), processed honey (10%) and NBF (10%) for 24 h at room temperature. A total score of 3–5 was considered adequate for diagnosis and

score of ≤2 was considered inadequate for diagnosis. These tissues were then subjected to routine processing and staining. Sections were evaluated for the nuclear staining, cytoplasmic staining, clarity and uniformity of staining pattern. Upon evaluation they concluded that processed honey has better fixative properties compared to unprocessed honey. In present study processed honey was used as a fixative by diluting with distilled water in 1: 4 ratio.

Sabarinath B *et al*[56] conducted a study on oral tissue biopsy in patients (n=13) with pericoronitis and pericoronal abscess. One tissue was fixed in formalin and other was fixed in honey for a period of 24 hours. Both the tissues were subjected to routine tissue processing and staining. Both honey fixed and formalin fixed tissue slides were interpreted by simple scoring system such as poor, satisfactory, good and excellent ranging from 1 to 4 for each slide. The tissue fixed in both formalin and honey showed similar cytomorphological architecture. Considering above results, honey can be introduced as an alternate fixative in histopathology, which is natural eco-friendly and less hazardous compared to formalin.

Muddana K *et al*[57] conducted a study in which honey was compared with formalin and olive oil in place of xylene in routine histopathology samples. A total of thirty routine biopsy tissue of 1-2cm were taken and divided into Group A and Group B. Group A was fixed in formalin and Group B was fixed in honey for one day. Both these fixed tissue were followed by routine processing and staining. Group B biopsy tissues were immersed in olive oil instead of xylene. Sections from both groups were evaluated. Honey gave superior results when compared to formalin and olive oil was found to be an effective clearing agent compared to xylene.

Singh, *et al*[7] conducted a pilot study for comparing ethanol and honey in oral cytological smears. Two buccal smears from each individual were fixed separately in honey and ethanol and their cytomorphological features were compared. After routine staining, the results showed that the maximum number of honey fixed smears had acceptable nuclear and cytoplasmic staining as compared with ethanol fixed smears, in which 97% of honey fixed smears showed acceptable nuclear and cytoplasmic staining as compared to 90% and 93% of ethanol fixed. Considering this, 20% of honey can be adequately and efficiently utilized in cytological smear fixation for preservation of cellular details.

Ishaq R *et al*[58] conducted a study in which they compared honey with alcohol fixatives in samples of fine needle aspiration cytology. A total of 30 cytological smears were selected randomly after performing FNAC. Smear were fixed separately in 95% ethanol and 20% honey for minimum of 10 min. Post fixation they were subjected to routine staining with Hematoxylin and Eosin stain (H & E). Smears were examined blindly by two reviewers and score were allotted for nuclear staining, cytoplasmic staining, cell morphology, clarity of staining and uniformity of staining. Evaluation of results showed no significant difference in the fixative properties of alcohol and honey.

Sona M *et al*[59] conducted a study in which they compared fixatives properties of honey with ethanol on exfoliated oral cytological smears. Two buccal smears were collected from each individual using wooden spatula/blunt end of the Ayers spatula. One smear was fixed in ethanol (95%) and the other smear was fixed in honey (20%). The smears were subjected to routine Papanicolaou staining. Smears were evaluated based on the assessment parameters: nuclear staining, cytoplasmic staining, cell

morphology, clarity of staining and uniformity of staining. Based on the results, they concluded that honey has got acceptable inherent fixative property comparable to ethanol which can be implemented in the routine cytological samples.

Ozkan N *et al*[5] has compared formalin with honey as fixative in various histopathology samples such as breast, placenta, endometrium, suprarenal, adrenals, uterus, omentum etc. These tissues were cut into small bits and transferred immediately into 3 containers (honey (10%), neutral buffered formalin (10%) alcohol formalin) All these tissue were fixed for 1 day. After fixation these tissues were subjected to routine tissue processing, embedding, sectioning and staining. Stained sections were evaluated for nuclear, cytoplasmic details and over all morphology. Tissue fixed in honey showed well preserved cell morphology, nuclear and cellular details which provide further evidence and support to the inherent property of honey as fixative which can replace routine histological fixative.

Patil *et al*[60] compared various natural Indian sweeteners such as honey and jaggery with 10% neutral buffered formalin on samples received in histopathological laboratory. They compared concentrations of 20% honey and 30% jaggery with 10% formalin. All the three reagents were subjected to testing for their efficacy using Hematoxylin and Eosin, Periodic acid Schiff, and Masson–Trichrome over a period of 6 months. The overall morphology of the tissues fixed in jaggery and honey was relatively intact even at the end of 6 months. Hence they concluded that natural substitutes have better scope due to their desirable results in which honey was the first proven natural fixative.

Dhengar YS *et al*[8] conducted similar study where they compared 10% neutral buffer formalin with naturally available sweeteners such as 20% sugar syrup, 30%

jaggery syrup and distilled water on gingivectomy samples. A total of 50 gingival samples were collected which were separated into four bits and fixed immediately in each of the above said fixatives for comparison. All these tissue were fixed for one day, after which they were subjected to routine tissue processing, embedding, sectioning and staining. These stained sections were assessed for predetermined criteria such as quality of staining, nuclear and cytoplasmic details. Among all the fixatives tissues fixed in jaggery showed better results when compared to sugar syrup and distilled water and quality was almost comparable to formalin.

Rajanikanth M *et al*[61] highlighted the use of natural fixative as transport media where routine fixatives were not available. In rural health medical camps, public health service hospital and clinics located in remote area these fixatives can be utilised as alternative to routine fixative as they are easily available and cost effective. In this study they compared commonly available solutions like Spirit, Saline, Betadine solution, Hydrogen peroxide (H_2O_2), Local anesthesia (L.A), Rose water, Coconut oil, Coconut water, Ice cold water, Honey and Milk while keeping formalin as control. Biopsied tissue was cut into multiple bits and fixed immediately into above said fixatives under study and kept of fixation for a period of eight hours. After which they were transferred to formalin and followed by routine histopatholgical procedure and evaluation. Results showed that all the transit fixatives were able to preserve the tissue over a period of 8 h comparable to formalin.

Patil S *et al*[4] compared various natural sweeteners with formalin which is considered to be the gold standard in histology. Excised goat buccal mucosal biopsy tissue was fixed immediately in 10% NBF, Honey (20%), Sugar syrup (20%), and jaggery (30%) for minimum of 24 hours. This was followed by routine processing and

staining. The tissue sections were assessed for cytoplasmic, nuclear details & staining quality under light microscopy. Each criteria was rated on a scale of 1- 4 (1 for poor & 4 for excellent) & the whole procedure was blinded. After evaluation it was concluded that, the preservation of tissue by honey, sugar & jaggery syrup was comparable to that of formalin. Among the three natural fixatives, jaggery syrup excelled.

Majumdar B *et al*[62] further took natural fixative to evaluate on higher level by comparing immunohistochemical staining in formalin fixed tissue and tissue fixed in natural fixatives such as 20% honey, and 30% jaggery solution. A total of 30 cases of oral mucosal biopsy of goat were taken and these tissues were cut into small bits and fixed immediately in all fixatives under study. A minimum of 24 hour fixation time was allotted before tissues were subjected to routine histopathological processing. Immunohistochemical stains pan-cytokeratin and desmin were performed on all tissues obtained from above fixatives and compared. Tissue fixed in jaggery and honey showed results comparable to formalin. This further highlights the antigen preserving ability of honey which could be lost with formalin. An ideal fixative should be compatible with immnuohistochemical staining, this property has been very well shown by honey when used as fixative in various studies. But this property needs to be further evaluated with more number of stains and various tissues.

Gunter M *et al*[63] compared common leucocyte antigen, cytokeration AE1/AE3 and epithelial membrane antigen immunohistochemical stains in breast tumour tissues which were fixed separately in formalin and honey. Evaluation of immuonohistochemical stains showed that honey has acceptable staining and antigen retrieval steps can be skipped when honey is used, as they were able to preserve tissue

antigens. This property will decrease turnaround time and cost involved in immunohistochemical staining.

Newer Fixatives

Lily P *et al*[64] has described the use of new commercial fixatives such as Fine-Fix, universal molecular fixative (UMFIX), and RCL2. These newer fixatives not only provide acceptable cellular and nuclear details for diagnosis but also are compatible with higher studies such as molecular analysis. Other new fixative HOPE (Hepes-glutamic acid buffer mediated organic solvent protection effect) has the ability to provide complete pathological analysis such as routine histopathology, Immunohistochemistry and molecular analysis. This fixative has overcome the disadvantages of formalin where antigen loss was major concern and step involved in antigen retrieval were time consuming and costlier.

Olert J *et al*[65] further evaluated HOPE fixative which was constantly proving better results in all fields of pathological analysis. HOPE-technique (Hepes-Glutamic acid buffer mediated Organic solvent Protection Effect) comprises of protection-solution with an organic buffer, acetone as the only dehydrating agent, and pure paraffin of 52-54^0 C melting temperature. When they evaluated tissue fixation by comparing HOPE fixative with formalin in various techniques, HOPE fixative showed excellent preservation of proteins and antigenic structures for differential analysis by immunohistochemical and/or enzyme histochemical techniques.

Delfour C *et al*[66] compared Methacarn and RCL2 new cross linking fixatives with formalin fixed or frozen tissue samples of invasive breast carcinoma. These tissues were subject to various techniques such as routine histopatholgical staining, Immunohistochemistry using various antibodies such as estrogen receptors (ER),

progesterone receptors (PR). Evaluation of these tissues revealed that evaluation and results of histomorphology and immunohistochemistry of methacarn- or RCL2-fixed paraffin-embedded tumours were similar to that of formalin-fixed tissues.

Titford ME *et al*[67] compared formalin with various new substitutes such as Histochoice, Glyo-Fixx, Omnifix II and Histofix for fixing various tissue specimens. Gross and microscopic examination was done for all fixatives and compared with formalin. They concluded that nuclear features and lymphocyte appearance were rated higher with Glyo-Fixx and cytoplasmic details were excellent with Omnifix II. Their idea of ideal fixatives is one which should preserve tissue in lifelike state, prepare tissues for subsequent processing, prevent autolysis, prevent osmotic damage, prepare tissues for subsequent staining, prevent shrinkage or swelling and harden tissue to facilitate easy sectioning, alter refractive index of tissue and render tissue components resistant to extraction by water and organic solvents.

Vacuum sealed devices

New technique of Vacuum sealed device serves as an ideal alternative to transport larger specimens from the surgical theatre to the pathology laboratory for histological evaluation without requirement of formalin or other tissue fixatives. This technique can also be used to store and transport specimens for transplantation and tissue banking.[68]

Advantages of vacuum sealed bags are[68]

(a) Prevents drying and slows down autolysis.
(b) Provides faster cooling and original colour of the specimen is maintained which provides clue to the diagnosis.
(c) Long period of preservation (3 to 9 days)

(d) Compatible with various pathological analysis techniques such as , IHC and FISH technique with superior quality of nuclear staining.

(e) Can be used in tissue banking.

(f) Eco-friendly.

(g) Tissue can be transported to far off places.

Novi CD *et al*[69] evaluated tissue morphology of tissues which are received in histopathology laboratories by vacuum based preservation. Tissues received is vacuum were transferred to formalin and subjected to routine histopatholgical processing, embedding, processing and staining. Tissue received in vacuum showed well preserved overall morphological details in comparison to formalin. This tissue has shown promising results and can be a better and eco-friendly alternative for transporting tissue specimen.

MATERIALS AND METHODS

SOURCE OF DATA

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.

Study period: 1st December 2015 to 30th June, 2017.

METHODS OF COLLECTION OF DATA:

- 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 distal 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.

PAPANICOLAOU STAIN:

Reagents required:

1. Harris Hematoxylin
2. 95% Alcohol
3. 70% Alcohol
4. 50% Alcohol
5. OG 6
6. EA 36
7. 1% Acid alcohol

Technique:

- Fix smear in 95% alcohol – 15 min
- Wash with water.
- Stain with Harris Hematoxylin – 5 minutes.
- Wash with water.
- Dip in 1% Acid alcohol.
- Wash in running tap water until bluing.
- Dehydration in 70% alcohol 2 min
- Dehydration in 95% alcohol 2 min
- Dehydration in 95% alcohol 2 min
- Stain in OG 6, for 2 min.
- Rinse in 95% alcohol, 2 min
- Rinse in 95% alcohol, 2 min
- Stain in EA 36, 3 min
- Rinse in 95% alcohol, 1 min
- Drying
- Clearing in Xylene
- Mounting

- The slides thus fixed and stained were evaluated separately for ethanol and honey.
- The cytoplasmic and nuclear details will be scored for 50 cells in each slide.

Table4.1: 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 colour in an individual cell

Statistical analysis:

Data was analyzed using

1. Mean ± S.D
2. Diagrams
2 Chi square test

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

Exclusion criteria: Nil

Figure 4.1: Commercial Honey

Figure 4.2 : Stock solution 50ml of 20% HONEY (40ml distil water and 10 ml of honey)

Figure 4.3: Absolute Ethanol

Figure 4.4: Coplin Jar

RESULTS

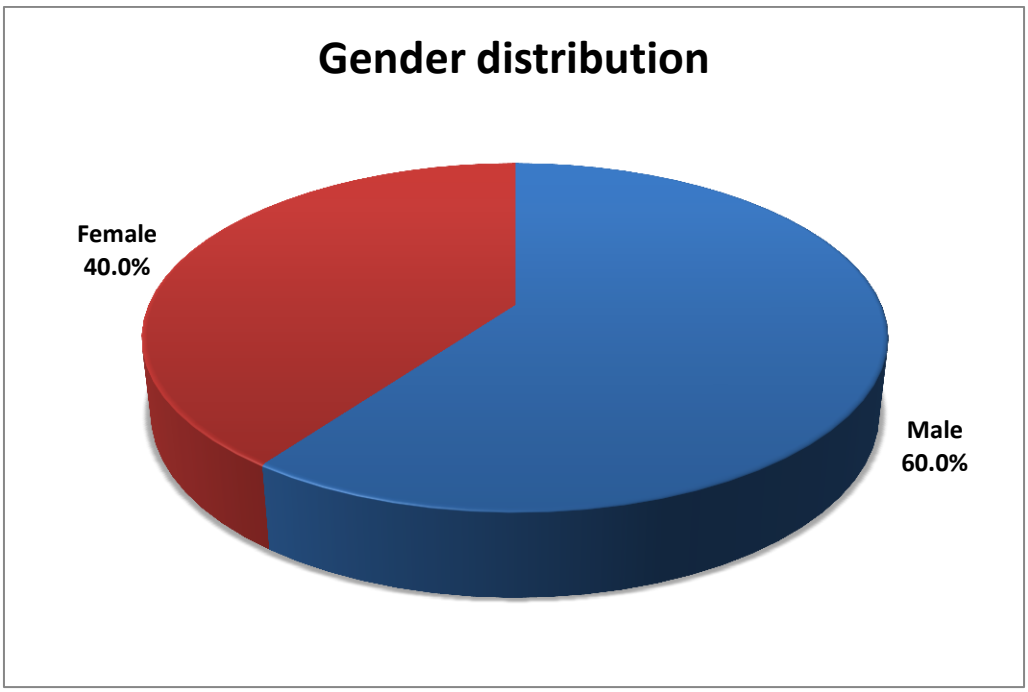
DISTRIBUTION OF CASES ACCORDING TO SEX

A total of 200 cases were collected out of which 120 cases (60%) were male and 80 cases (40%) were female. (Table. 5.1 & Figure 5.1)

Table 5.1: Distribution of cases by sex

Gender	N	Percent
Male	120	60
Female	80	40
Total	200	100

Figure 5.1: Distribution of cases by sex



DISTRIBUTION OF CASES ACCORDING TO AGE

Out of 200 cases, maximum numbers of cases (100) were in age group of 20 - 30 years and minimum number of cases (6) were from age group of above 50 years. There is significant association between distribution of age and sex. (p value = 0.001) (Table 5.2 & Figure 5.2)

Table 5.2: Distribution of cases by Age and sex

Age groups (Yrs)	Male		Female		Total		p value
	N	%	N	%	N	%	
10-20	32	26.7	37	46.3	69	34.5	0.001*
20-30	74	61.7	26	32.5	100	50.0	
30-40	3	2.5	6	7.5	9	4.5	
40-50	7	5.8	9	11.3	16	8.0	
>50	4	3.3	2	2.5	6	3.0	
Total	120	100.0	80	100.0	200	100.0	

Note:*significantly distributed at 5% level of significance

Figure 5.2 : Distribution of cases by Age and sex

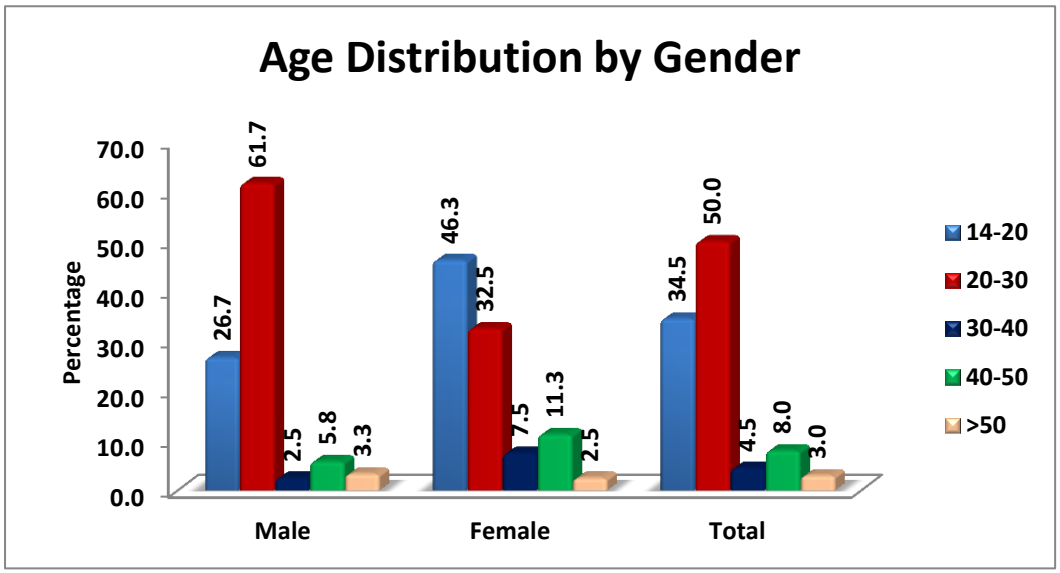


Table 5.3: Mean age of cases

In present study among 200 cases, minimum age was 14 years and maximum was 65 years with mean age of 24 years.

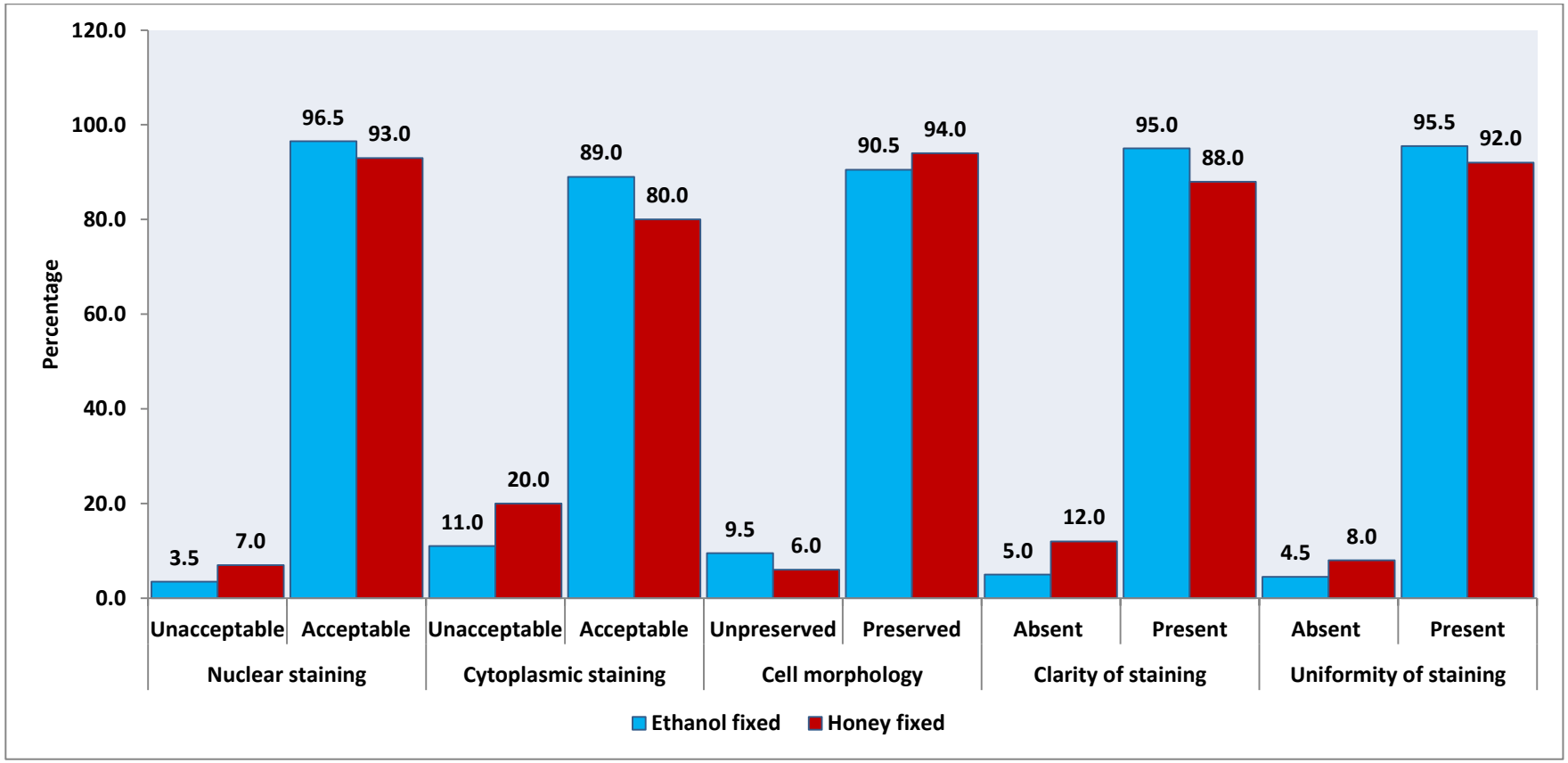
Age (yrs)	Minimum	Maximum	Mean	SD
	14	65	24.4	9.2

Table5.4: 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	
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

Figure 5.3: Distribution of cases comparing various cytomorphological features of Ethanol fixed smears and Honey fixed smears.



Out of 200 cases 193 (96.5%) cases of EF smears and 186 (93%) cases of HF smears showed acceptable nuclear staining, 7 (3.5%) cases of EF smears and 14 (7%) cases of HF smears showed unacceptable nuclear staining which was statistically significant with p value of 0.008

Out of 200 cases 178 (89%) cases EF smears and 160 (80%) cases of HF smears showed acceptable cytoplasmic staining and 22 (11%) cases of EF smears, 40 (20%) cases of HF smears showed unacceptable cytoplasmic staining with no statistical difference between both fixatives with p value of 0.821.

Out of 200 cases 181 (90.5%) cases EF smears and 188 (94%) cases of HF smears showed preserved cell morphology and 19 (9.5%) cases of EF smears and 12 (6%) cases of HF smears showed unpreserved cell morphology with no statistical difference between both fixatives with p value of 0.092

Out of 200 cases clarity of staining was present in 190 (95%) cases EF smears and 176 (88%) cases of HF smears, clarity of staining was absent in 10 (5%) cases of EF smears and 24 (12%) cases of HF slides which was statistically significant with p value of 0.005

Out of 200 cases uniformity of staining was present in 191 (95.5%) cases EF smears and 184 (92%) cases of HF smears, uniformity of staining was absent in 9 (4.5%) cases of EF smears and 16 (8%) cases of HF smears which was statistically significant with p value of 0.001

Out of 200 cases 1.5% cases of both Ethanol fixed and Honey fixed smears show unacceptable nuclear staining where as 91% cases of both Ethanol fixed and Honey fixed smears show acceptable nuclear staining. Concordance between Ethanol

fixed slides and Honey fixed smears in Nuclear staining is statically significant (p value = 0.008)

Table 5.5: Concordance between Ethanol fixed smears and Honey fixed smears in Nuclear staining

Nuclear staining		**Ethanol**		**P value**
		Unacceptable	Acceptable	
Honey	Unacceptable	**3 (1.5%)**	**11 (5.5)**	**0.008**
	Acceptable	**4 (2%)**	**182 (91%)**	

Note:*significantly associated at 5% level of significance

Out of 200 cases 2% cases of both Ethanol fixed and Honey fixed smears show unacceptable cytoplasmic staining where as 71% cases of both Ethanol fixed and Honey fixed smears show cytoplasmic nuclear staining. Concordance between Ethanol fixed smears and Honey fixed smears smears in cytoplasmic staining showed no statistical difference between both fixatives (p value = 0.821)

Table 5.6: Concordance between Ethanol fixed smears and Honey fixed smears in Cytoplasmic staining

Cytoplasmic staining		**Ethanol**		**P value**
		Unacceptable	Acceptable	
Honey	Unacceptable	**4 (2%)**	**36(18%)**	0.821
	Acceptable	**18 (9%)**	**142 (71%)**	

Note:*significantly associated at 5% level of significance

Out of 200 cases 1.5% cases of both Ethanol fixed and Honey fixed smears show unpreserved cell morphology where as 86% cases of both Ethanol fixed and Honey fixed smears show well preserved cell morphology. Concordance between Ethanol fixed smears and Honey fixed smears in preserving cell morphology showed no statistical difference between both fixatives (p value = 0.092)

Table 5.7: Concordance between Ethanol fixed smears and Honey fixed smears in preserving Cell morphology

Cell morphology		**Ethanol**		**P value**
		Unacceptable	Acceptable	
Honey	Unacceptable	**3(1.5%)**	**9(4.5%)**	0.092
	Acceptable	**16 (8%)**	**172(86%)**	

Note:*significantly associated at 5% level of significance

Out of 200 cases 2% cases of both Ethanol fixed and Honey fixed smears show unacceptable clarity of staining where as 85% cases of both Ethanol fixed and Honey fixed smears show well acceptable clarity of staining. Concordance between Ethanol fixed smears and Honey fixed smears in clarity of staining is statically significant (p value = 0.005)

Table 5.8: Concordance between Ethanol fixed smears and Honey fixed smears in Clarity of staining

Clarity of staining		Ethanol		P value
		Unacceptable	Acceptable	
Honey	Unacceptable	**4 (2%)**	**20 (10%)**	0.005*
	Acceptable	**6 (3%)**	**170 (85%)**	

Note:*significantly associated at 5% level of significance

Out of 200 cases 2% cases of both Ethanol fixed and Honey fixed smears show unacceptable uniformity of staining where as 89.5% cases of both Ethanol fixed and Honey fixed smears show well acceptable uniformity of staining. Concordance between Ethanol fixed smears and Honey fixed smears in uniformity of staining is statically significant (p value = <0.001)

Table 5.9: Concordance between Ethanol fixed smears and Honey fixed smears in Uniformity of staining

Uniformity of staining		Ethanol		P value
		Unacceptable	Acceptable	
Honey	Unacceptable	**4 (2%)**	**12 (6%)**	<0.001*
	Acceptable	**5 (2.5%)**	**179 (89.5%)**	

Note:*significantly associated at 5% level of significance

Among 5 parameters under evaluation, Nuclear staining, Clarity of staining and Uniformity of staining there is direct correlation between ethanol and honey fixed smears These cytological parameters are statistically significant (p value = <0.005)

Among 5 parameters under evaluation, Cytoplasmic staining and preservation of cell morphology there is indirect correlation between Ethanol and Honey with no statistical difference (p value = >0.005)

Table 5.10: Correlation coefficient between Ethanol fixed smears and Honey fixed smears

Staining	**Spearman's rho correlation coeff**	**p value**
Nuclear staining	0.268	<0.001*
Cytoplasmic staining	-0.016	0.822
Cell morphology	0.134	0.059
Clarity of staining	0.198	0.005*
Uniformity of staining	0.292	<0.001*

Note:*significantly correlated at 5% level of significance

CELLULARITY

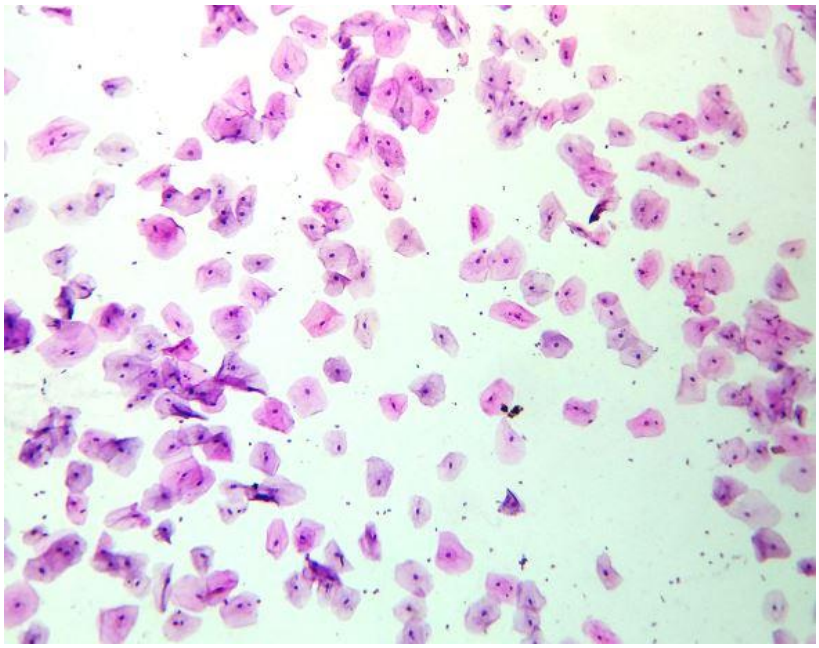


FIGURE 5.4A

Ethanol fixed smear showing adequate cellularity – PAP-X100

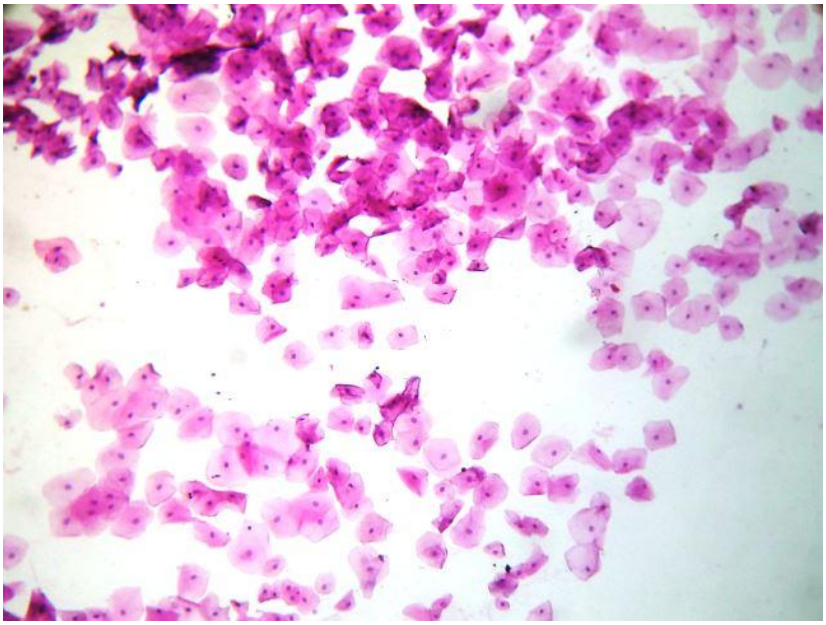


FIGURE 5.4B

Honey fixed smear showing adequate cellularity – PAP – X100

NUCLEAR STAINING

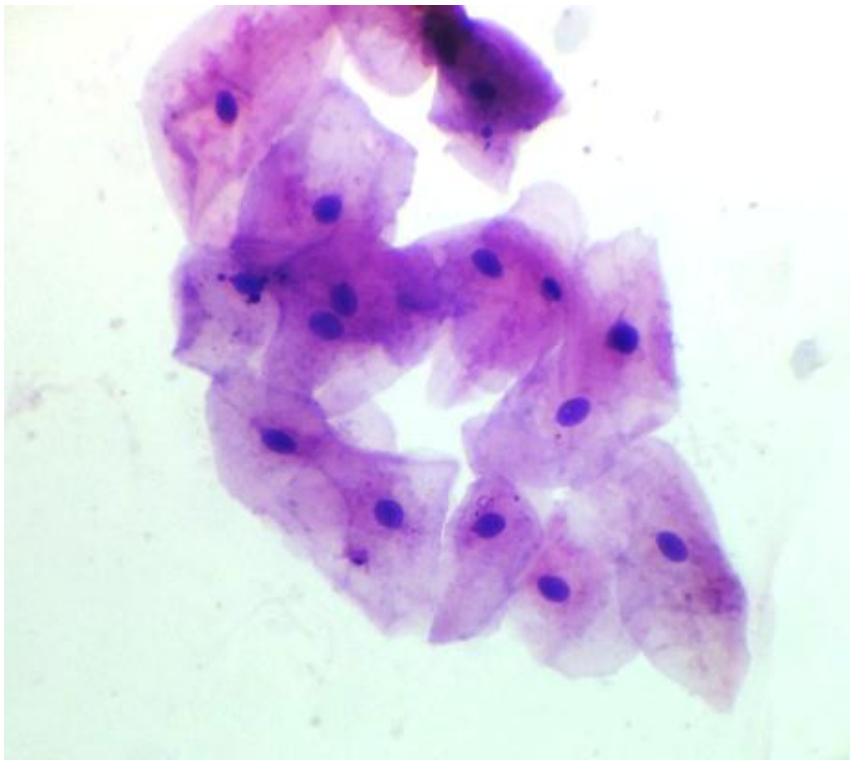


FIGURE 5.5A

Ethanol fixed smear showing acceptable nuclear staining – PAP - X400

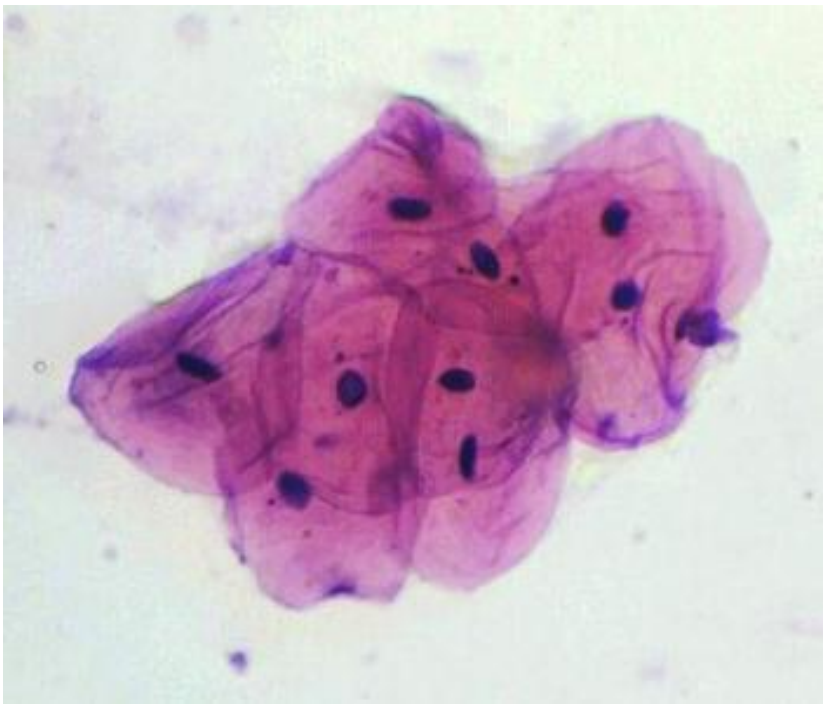


FIGURE 5.5B

Honey fixed smear showing acceptable nuclear staining - PAP - X400

CYTOPLASMIC STAINING

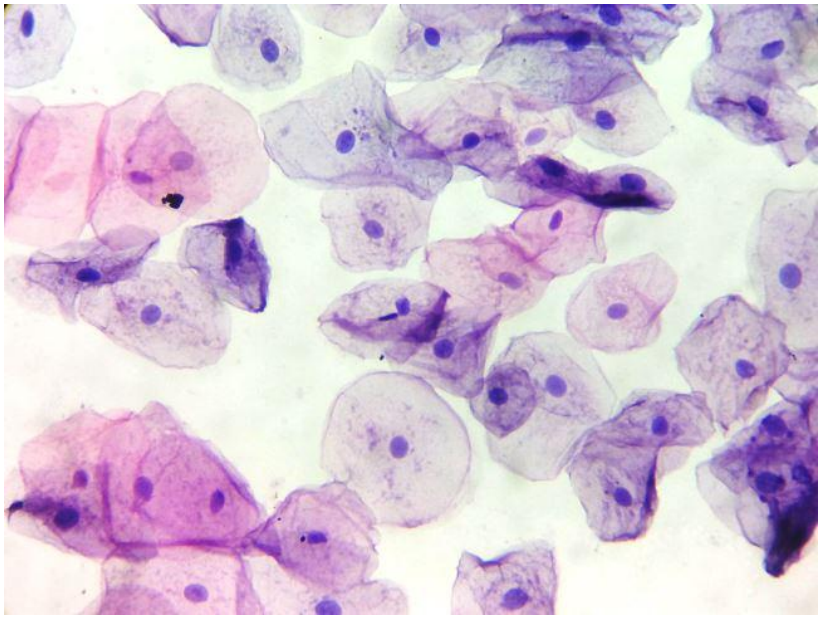


FIGURE 5.6A

Ethanol fixed smear showing acceptable cytoplasmic staining - PAP - X400

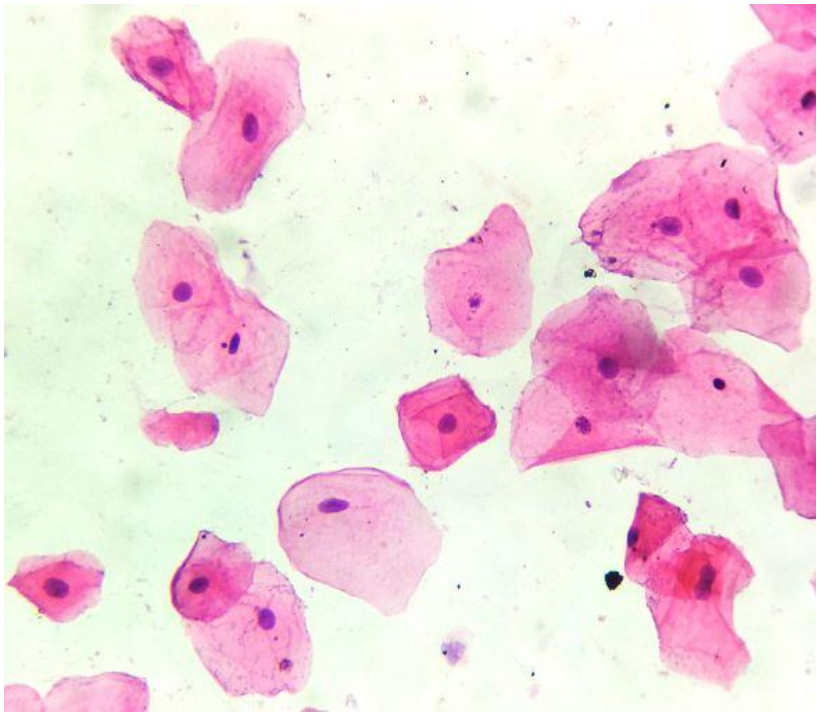


FIGURE 5.6B

Honey fixed smear showing acceptable cytoplasmic staining - PAP - X400

CELL MORPHOLOGY

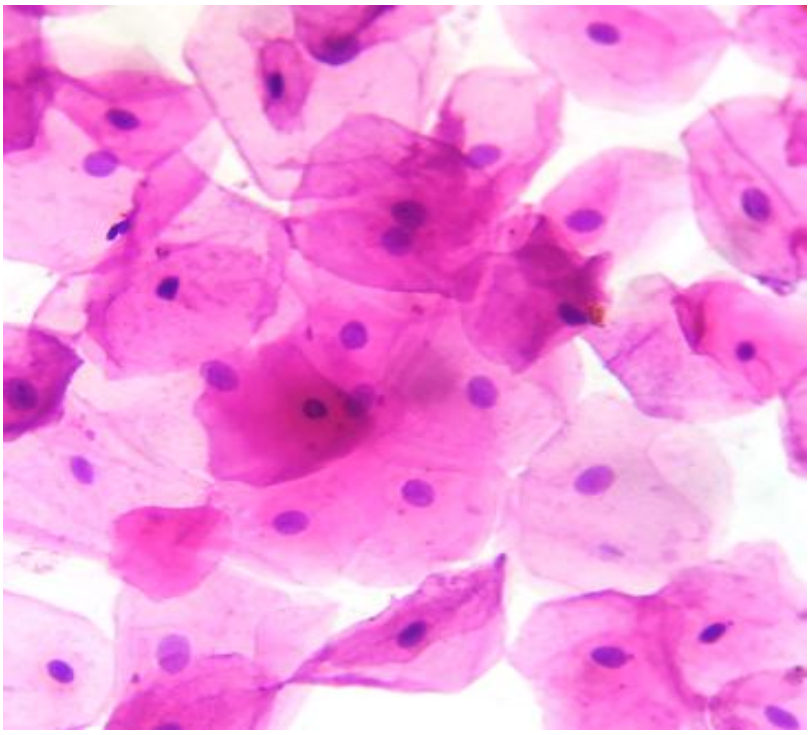


FIGURE 5.7A

Ethanol fixed smear showing well preserve cell morphology - PAP - X400

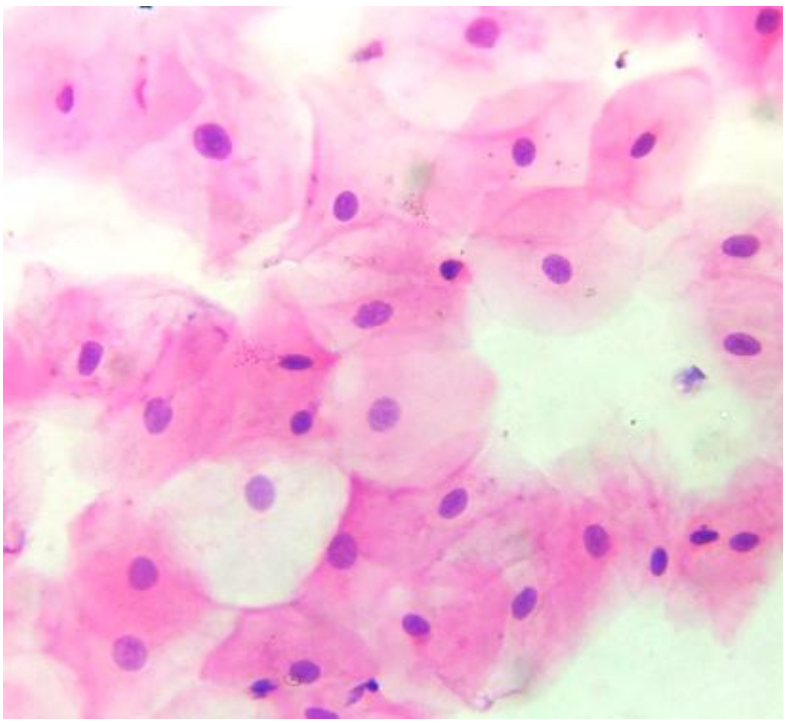


FIGURE 5.7B

Honey fixed smear showing well preserve cell morphology - PAP - X400

UNIFORMITY OF STAINING

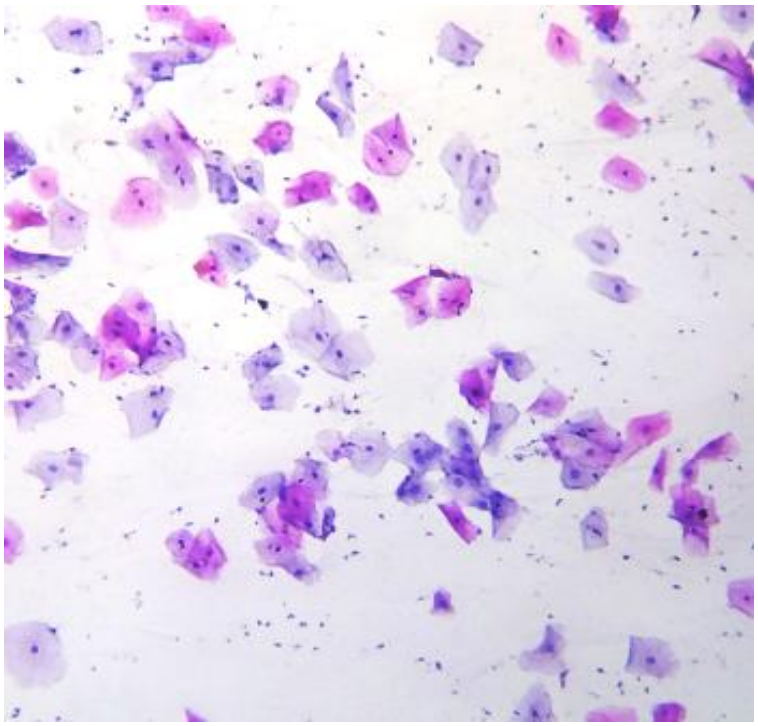


FIGURE 5.8A

Ethanol fixed smear showing acceptable uniformity of staining - PAP - X100

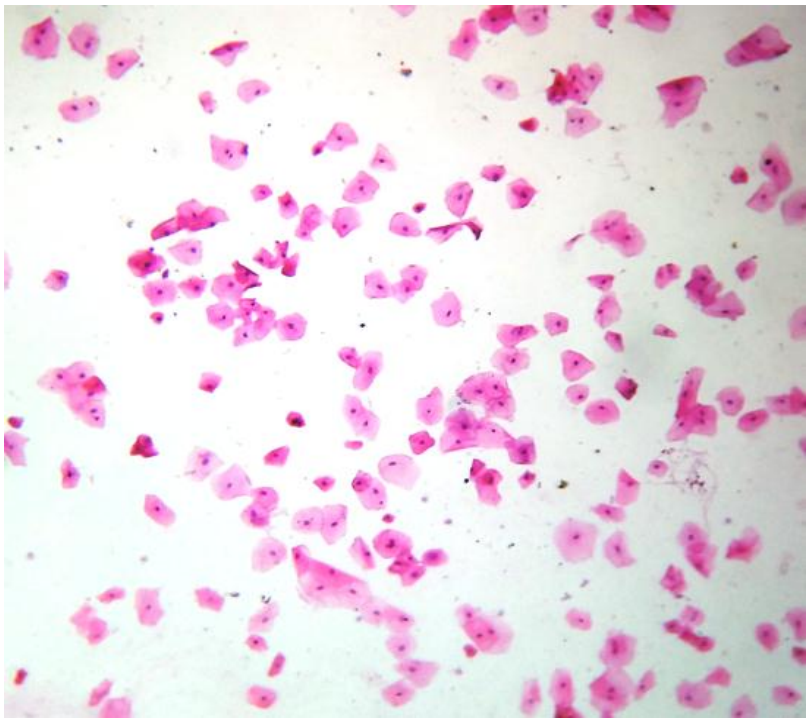


FIGURE 5.8B

Honey fixed smear showing acceptable uniformity of staining - PAP - X100

CLARITY OF STAINING

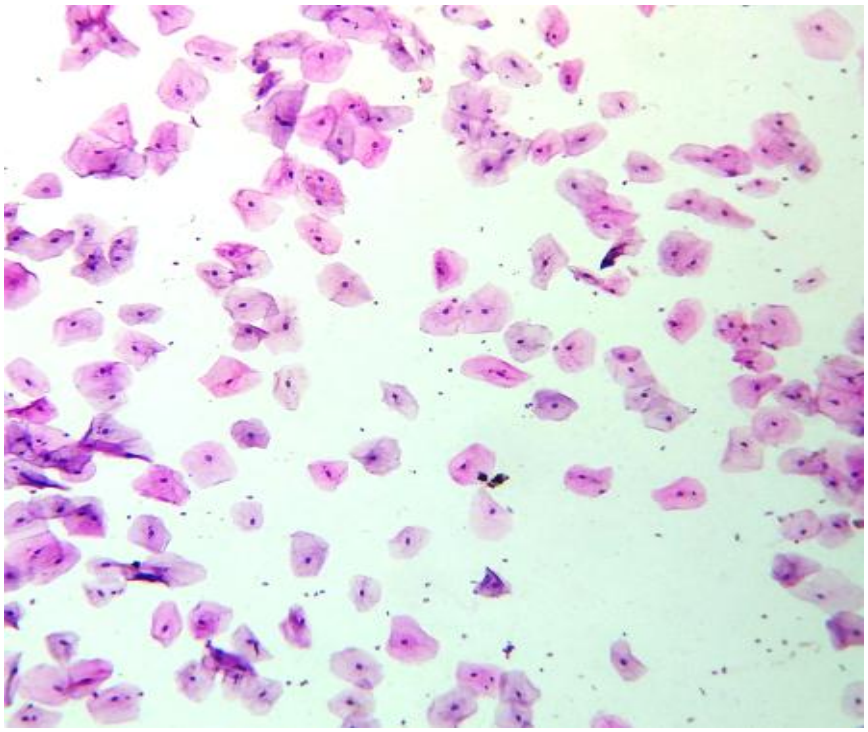


FIGURE 5.9A

Ethanol fixed smear showing acceptable clarity of staining - PAP - X100

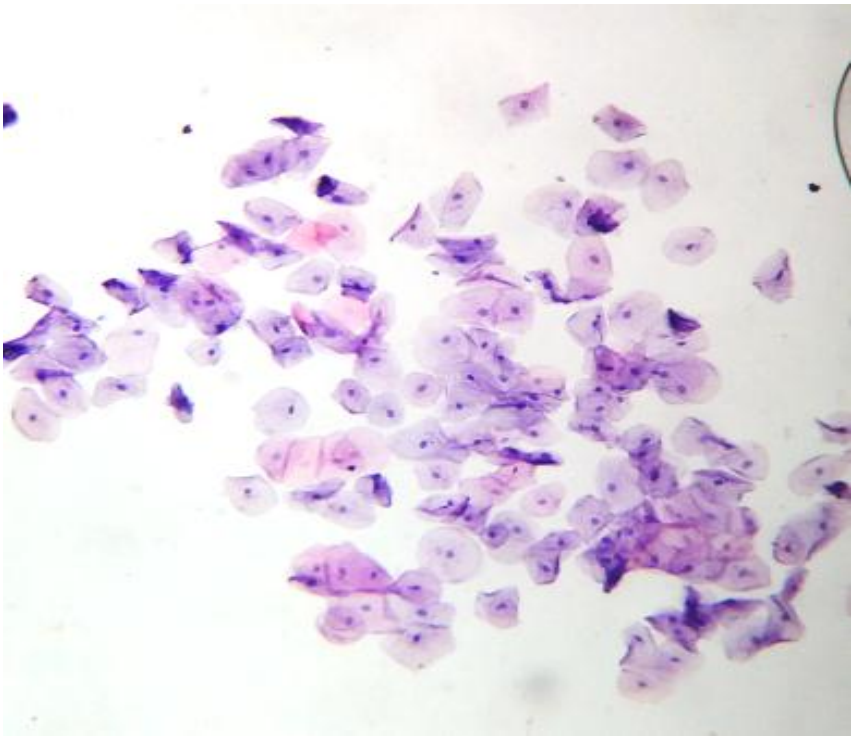


FIGURE 5.9B

Honey fixed smear showing acceptable clarity of staining - PAP - X100

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. For appropriate cytological evaluation proper collection, fixation, staining and evaluation are required. Each of these steps play a vital role in cytological diagnosis.

Fixation preserves the cells similar to living state and 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. Formaldehyde is well known fixative used most commonly in histopathology. Though widely used and impressive performance it has certain disadvantages which necessitates for search of better alternative. Formalin causes irritation to eyes and nasal passage and it is a proven carcinogen.

Ethanol is a gold standard widely used cytological fixative in many laboratories. Advantages are rapid fixation, antibacterial properties and acceptable preservation of cytological details. Disadvantages such as not being freely available, costly and inflammable prevent it from being an ideal fixative. 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.

In search of ideal, natural non-toxic alternate fixative this study has been carries out, to introduce honey as cytological fixative. 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.

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*[7] showed that cellularity and cell morphology were well preserved in honey which provides adequate cytological material for diagnosis.

Table 6.1: Comparison of percentage of acceptable nuclear staining of ethanol and honey fixed smears in various studies.

Studied by	Percentage of Ethanol fixed smears showing acceptable nuclear features	Percentage of Honey fixed smears showing acceptable nuclear features	P value
Singh A *et al*[7]	90	97	0.61
Sona M *et al*[59]	97.4	97	---
Ishaq R *et al*[58]	100	100	0.66
Present study	96.5	93	0.008

In the present study, out of the 200 samples evaluated, 96.5% of ethanol-fixed (EF) smears showed acceptable nuclear staining as compared with 93% of the honey fixed (HF) smears with significant p value (p value = 0.008) where as in Singh A *et al*[7] out of the 30 samples studied, 90% and of ethanol-fixed (EF) smears showed

acceptable nuclear staining as compared with 97% of the honey fixed (HF) smears with no statistical difference between both fixatives. (p value = 0.61).

Similar findings were observed on Sona M *et al*[59] study in which out of 194 samples evaluated 97.4% and 97% of cases showed adequate nuclear staining with EF and HF smears respectively. The measure of agreement kappa was 0.719, indicating strong agreement between the two methods- Honey and Ethanol fixation for Nuclear staining

Similar study done by Ishaq R *et al*[58] in which they compared cytological details of honey fixed smears with ethanol in smears made by fine needle aspiration cytology of various lesions. Both EF and HF fixed smears showed 100% acceptable nuclear staining with no statistical difference between both fixatives. (p value = 0.66)

In present study ethanol fixed smears showed slightly better nuclear staining than honey fixed smears where as in Singh A *et al*[7], Sona M *et al*[59] honey fixed smears showed slightly better nuclear staining than ethanol fixed slides.

Table 6.2: Comparison of percentage of acceptable cytoplasmic staining of ethanol and honey fixed smears in various studies.

Studied by	Percentage of Ethanol fixed smears showing acceptable cytoplasmic features	Percentage of Honey fixed smears showing acceptable cytoplasmic features	P value
Singh A *et al*[7]	93	97	0.99
Sona M *et al*[59]	94.3	94.8	---
Ishaq R *et al*[58]	100	93	0.16
Present study	89	80	0.821

In the present study, out of the 200 samples evaluated, 89% of ethanol-fixed (EF) smears showed acceptable cytoplasmic staining as compared with 80% of the honey fixed (HF) smears with no statistical difference between both fixatives. (p value = 0.821) where as in Singh A *et al*[7] out of the 30 samples studied, 93% and of ethanol-fixed (EF) smears showed acceptable cytoplasmic staining as compared with 97% of the honey fixed (HF) smears with no statistical difference between both fixatives. (p value = 0.99). Similar findings were observed on Sona M *et al*[59] study in which out of 194 samples evaluated 94.3% and 94.8% of cases showed acceptable cytoplasmic staining with EF and HF smears respectively. The measure of agreement kappa was 0.748, indicating strong agreement between the two methods- Honey and Ethanol fixation for cytoplasmic staining

Similar study done by Ishaq R *et al*[58] in which they compared cytological details of honey fixed smears with ethanol fixed smears made by fine needle aspiration cytology of various lesions. Acceptable cytoplasmic staining was observed in 100% and 93% of EF and HF fixed smears respectively with no statistical difference between both fixatives. (p value = 0.16)

In present study ethanol fixed smears showed slightly better cytoplasmic staining than honey fixed smears in concordance with Ishaq R *et al*[58] *et al* study. Where as in Singh A *et al*[7] study honey fixed smears showed slightly better cytoplasmic staining when compared to ethanol fixed slides.

Table 6.3: Comparison of percentage of preserved cell morphology of ethanol and honey fixed smears in various studies.

Studied by	Percentage of Ethanol fixed smears showing preserved cell morphology	Percentage of Honey fixed smears showing preserved cell morphology	P value
Singh A *et al*[7]	93	97	0.99
Sona M *et al*[59]	73	72	---
Ishaq R *et al*[58]	100	93	0.16
Present study	90.5	94	0.092

In the present study, out of the 200 samples evaluated, 90.5% of ethanol-fixed (EF) smears showed preserved cell morphology as compared with 94% of the honey fixed (HF) smears with no statistical difference between both fixatives. (p value =

0.092) where as in Singh A *et al*[7] out of the 30 samples studied, 93% and of ethanol-fixed (EF) smears showed preserved cell morphology as compared with 97% of the honey fixed (HF) smears with no statistical difference between both fixatives. (p value = 0.61). Similar findings were observed on Sona M *et al*[59] study in which out of 194 samples evaluated 73% and 72% of cases showed preserved cell morphology of EF and HF smears respectively. The measure of agreement kappa is 0.961 indicating strong agreement between the two methods- Honey and Ethanol fixation for cell morphology.

Similar study done by Ishaq R *et al*[58] in which they compared cytological details of honey fixed smears with ethanol in smears made by fine needle aspiration cytology of various lesions. Well preserved cell morphology was observed in 100% and 93% of EF and HF fixed smears respectively with no statistical difference between both fixatives. (p value = 0.16)

In present study honey fixed smears showed slightly better preservation of cell morphology when compared to ethanol fixed smears which was in accordance with Singh A *et al*[7] both ethanol and honey fixed smears showed much better preservation of cell morphology in present study when compared to Sona M *et al*[59] study.

Table 6.4: Comparison of percentage of clarity of staining of ethanol and honey fixed smears in various studies.

Studied by	Percentage of Ethanol fixed smears showing clarity of staining	Percentage of Honey fixed smears showing clarity of staining	P value
Singh A *et al*[7]	83	83	0.4
Sona M *et al*[59]	62	61	---
Ishaq R *et al*[58]	90	77	0.006
Present study	95	88	0.005

In the present study, out of the 200 samples evaluated, 95% of ethanol-fixed (EF) smears showed clarity of staining as compared with 94% of the honey fixed (HF) smears with significant p value (p value = 0.005) where as in Singh A *et al*[7] out of the 30 samples studied, 83% and of ethanol-fixed (EF) smears showed clarity of staining as compared with 83% of the honey fixed (HF) smears with no statistical difference between both fixatives. (p value = 0.4). Similar findings were observed on Sona M *et al*[59] study in which out of 194 samples evaluated 62% and 61% of cases showed clarity of staining in EF and HF fixed smears respectively. The measure of agreement kappa is 0.967 indicating strong agreement between the two methods- Honey and Ethanol fixation for clarity of staining.

Similar study done by Ishaq R *et al*[58] in which they compared cytological details of honey fixed smears with ethanol in smears made by fine needle aspiration cytology of various lesions. Clarity of staining was noticed in 90% of ethanol fixed

smears and 77% in honey fixed smears with no statistical difference between both fixatives. (p value = 0.006)

In present study ethanol fixed smears showed better clarity of staining when compared to honey fixed smears which is in accordance with Ishaq R *et al*[58]study. Both ethanol and honey fixed smears showed much better clarity of staining in present study when compared to Sona M *et al*[59]study.

Table 6.5: Comparison of percentage of Uniformity of Staining of ethanol and honey fixed smears in various studies.

Studied by	Percentage of Ethanol fixed smears showing Uniformity Of Staining	Percentage of Honey fixed smears showing Uniformity Of Staining	P value
Singh A *et al*[7]	90	90	0.99
Sona M *et al*[59]	66.5	64	---
Ishaq R *et al*[58]	100	100	0.66
Present study	95.5	92	0.001

In the present study, out of the 200 samples evaluated, 95.5% of ethanol-fixed (EF) smears showed Uniformity of Staining as compared with 92% of the honey fixed (HF) smears with significant p value (p value = 0.001) where as in Singh A *et al*[7] out of the 30 samples studied, 90% of ethanol-fixed (EF) smears showed Uniformity of Staining as compared with 90% of the honey fixed (HF) smears with no statistical difference between both fixatives. (p value = 0.99). Similar findings were observed on

Sona M *et al*[59]study in which out of 194 samples evaluated 66.5% and 64% cases showed uniformity of staining in EF and HF smears respectively. The measure of agreement kappa is 0.942 indicating strong agreement between the two methods

Similar study done by Ishaq R *et al*[58] in which they compared cytological details of honey fixed smears with ethanol in smears made by fine needle aspiration cytology of various lesions. 100% Uniformity of Staining was observed in both EF and HF smears with no statistical difference between both fixatives. (p value = 0.66)

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*[7] and Ishaq R *et al*[58] 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*[7] and Ishaq R *et al*[58] study in which nuclear staining, clarity of staining, uniformity of staining also showed no statistical difference between both fixatives.

Similar studies have also been done to compare fixative ability of honey in comparison to formalin in histopathology. Ozakan N *et al*[5] 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*[5] 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.($p > 0.05$)

Study conducted by Patil S *et al*[4] where natural sweeteners such as honey and jaggery were compared with formalin in histopathology samples showed preserved cell morphology and staining even at the end of six months. Similar observation was noted in present study in which honey fixed smear on cytological examination showed well preserved cell morphology and staining after a period of 6 months.

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.

In Rajnikanth M *et al*[61] Patil S *et al*[60] studies where they compared honey with formalin in histopathology showed convincing results which further strengthen its inherent fixatives properties and signify that honey can even be a better alternative to formalin in histopathological examination. Honey is natural, cheap and easily available which make it a near ideal fixative. Even its antibacterial and non toxic properties add a flavor and support its race to win a place as fixative in routine histology and cytology. Therefore this finding provides strong evidence of fixative property of honey which can be utilized in cytology.

Fructose present in honey breaks down into aldehydes at low pH. These aldehydes cross-link with amino acids resulting in fixation of tissue is one of the possible mechanisms of fixation.[60] Most unprocessed honeys, when diluted slowly, generate hydrogen peroxide owing to activation of the enzyme i.e, glucose oxidase, which oxidizes glucose to gluconic acid and hydrogen peroxide.[5]

Use of natural alternatives can be attempted in screening camps, as an instant choice for biopsied tissues in private clinics and as a transporting media as ethanol is not freely available. This idea can equally be used for preservation of museum specimens, in the forensic field wherein stored tissue has to be occasionally retrieved for histological examination. Implementing eco-friendly fixatives in routine histopathology is necessary.[60]

SUMMARY

In present study 200 samples were collected and evaluated from 1st December 2015 to 30th June 2017 in Department of Pathology in BLDEU'S Shri B. M. Patil Medical College, Hospital and Research centre, Vijayapur.

1. From all 200 cases two buccal smear samples were collected one was fixed n ethanol and one in honey, after minimum of 15 min both were subjected to routine Papanicolaou staining and evaluation.
2. Buccal smears were evaluated for 5 parameters such as nuclear staining, cytoplasmic staining, preserve cell morphology, uniformity and clarity of staining, and score of 0 or 1 was allotted based on prefixed criteria.
3. Out of 200 cases 193 (96.5%) cases EF and 186 (93%) cases of HF smears showed acceptable nuclear staining and 7 (3.5%) cases of EF and 14 (7%) cases of HF smears showed unacceptable nuclear staining which was statistically significant with p value of 0.008
4. Out of 200 cases 178 (89%) cases EF and 160 (80%) cases of HF smears showed acceptable cytoplasmic staining and 22 (11%) cases of EF and 40 (20%) cases of HF smears showed unacceptable cytoplasmic staining which showed no statistical difference between both fixatives with p value of 0.821.
5. Out of 200 cases 181 (90.5%) cases EF and 188 (94%) cases of HF smears showed preserved cell morphology and 19 (9.5%) cases of EF and 12 (6%) cases of HF smears showed unpreserved cell morphology which showed no statistical difference between both fixatives with p value of 0.092
6. Out of 200 cases clarity of staining was present in 190 (95%) cases EF and 176 (88%) cases of HF smears and clarity of staining was absent in 10 (5%)

cases of EF and 24 (12%) cases of HF smears, which was statistically significant with p value of 0.005

7. Out of 200 cases uniformity of staining was present in 191 (95.5%) cases EF and 184 (92%) cases of HF smears and uniformity of staining was absent in 9 (4.5%) cases of EF and 16 (8%) cases of HF smears, which was statistically significant with p value of 0.001

CONCLUSION

- To conclude, 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.
- Our procedure will improve the safety and work environment in cytology.
- Tissue fixation of honey is as efficient as ethanol. The components of honey which serves as a fixative is still a matter of further research.
- In rural areas health camps, public health service centres and in absence of alcohol fixatives, honey can be used as a successful alternative.
- Much proven antibacterial properties of honey, ongoing establishment of its fixative ability both in histopathology and cytology provides a hope of exploration of an ideal fixative.
- Honey is cheap, pleasant smelling, easily available, non toxic and antibacterial. This novel properties full fills the minor limitations of ethanol.
- Honey has shown favourable results during Immunostaining with a reduction of turnaround time for certain antigens by the omission of antigen retrieval. Further studies using various tissue selection and broader spectrum of antibodies will provide data regarding benefits and pitfalls of using honey in this way.

BIBLIOGRAPHY

1. Rhodes A. Fixation of tissues. In: Suvarna KS, Layton C, Bancroft DJ editors. Bancroft's Theory and Practice of Histological Techniques. 7th ed. Churchill Livingstone: Elsevier; 2013.p69-93.
2. Mulki S, Shetty P, Pai P. Oral rinse as a simpler approach to exfoliative cytology: A comparative study. Journal of clinical and diagnostic research: JCDR. 2013 Dec;7(12):3036-3038
3. Kabiraj A, Khaitan T, Bhowmick D, Ginjupally U, Bir A, Chatterjee K. Screening of Oral Potentially Malignant Disorders Using Exfoliative Cytology: A Diagnostic Modality. Journal of cancer epidemiology. 2016 Sep 18;2016:1-4
4. Patil S, Premalatha BR, Rao RS, Ganavi BS. Revelation in the field of tissue preservation–A preliminary study on natural formalin substitutes. Journal of international oral health: JIOH. 2013 Feb;5(1):31
5. Özkan N, Şalva E, Cakalağaoğlu F, Tüzüner B. Honey as a substitute for formalin?. Biotechnic & Histochemistry. 2012 Feb 1;87(2):148-53
6. Culling CFA, Allison RT, Barr WT. Fixation, processing, microtomy. In: Culling CFA Editors. Cellular Pathology Technique. 4th ed. London:Butterworths;p35
7. 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. Journal of cytology.2015 Apr;32(2):113.

8. Dhengar YS, Palve D, Thakur M, Bhagwatkar T, Bhondey A, Chaturvedi S. Natural substitutes for formalin-chemical versus natural: a comparative study. Annals Of Dental Specialty. 2016 Jan 1;4(1):1-5.
9. Patil S, Rao RS, Ganavi BS, Majumdar B. Natural sweeteners as fixatives in histopathology: A longitudinal study. Journal of natural science, biology, and medicine. 2015 Jan;6(1):67.
10. Jindal S, Chauhan I, Grewal HK. Alteration in buccal mucosal cells due to the effect of tobacco and alcohol by assessing the silver-stained nucleolar organiser regions and micronuclei. Journal of Cytology. 2013 Jul;30(3):174
11. Sloan P, Bocking A. Oral cavity. In Gray W, Kocjan G. Diagnostic Cytopathology E-Book: Expert Consult: Online and Print. Elsevier Health Sciences; 2010 May 24:253-4.
12. Mehrotra R, Hullmann M, Smeets R, Reichert TE, Driemel O. Oral cytology revisited. Journal of Oral Pathology & Medicine. 2009 Feb 1;38(2):161-6
13. Daniels R. Delmar's guide to laboratory and diagnostic tests. Cengage Learning; 2014 Feb 26:584.
14. Shankar R, Master PB, Obulesu LC, Rangiah YK. A study of sex chromatin form buccal smear. Journal of Evolution of Medical and Dental Sciences. 2015 Oct 15;4(83):14497-503.
15. Sharma AK, Sharma A. Chromosome techniques: theory and practice. Butterworth-Heinemann; 2014 Apr 24:148.
16. Gill G. Cytopreparation: principles & practice. Springer Science & Business Media; 2012 Oct 19:178-81.
17. Karthik KR, Malathi N, Poornima K, Prakash S, Kadhiresan R, Arunmozhi U. Evaluation of Glycemic Control in Type 2 Diabetes Mellitus using

Cytomorphometry of Buccal Cells and Correlation with Glycosylated Hemoglobin. Journal of international oral health. 2015 Feb;7(2):20.

18. Malik K. Laboratory Techniques in Diagnostic Exfoliative cytology. In Mukherjee KL. Medical Laboratory Technology Volume 3. Tata McGraw-Hill Education; 2013 Feb 1:1058.
19. Bales CE. Laboratory Techniques. In Koss L, Melamed M. Koss' Diagnostic Cytology and Its Histopathologic Bases. Philadelphia: Wolters Kluwer Health; 2011:p1570.
20. RoyBiswas R, Paral CC, Dey R, Biswas SC. Rapid Economic, Acetic Acid, Papanicolaou Stain (REAP)-Is it suitable alternative to standard PAP stain?. Al Ameen J Med Sci. 2008;1:99-103.
21. Syrbu SI, Cohen MB. An enhanced antigen-retrieval protocol for immunohistochemical staining of formalin-fixed, paraffin-embedded tissues. Signal Transduction Immunohistochemistry: Methods and Protocols. 2011:101-10.
22. Huang B, Yeung E. Chemical and Physical Fixation of Cells and Tissues: An Overview. Plant Microtechniques and Protocols. 2015:23-43.
23. Kiernan JA. Formaldehyde, formalin, paraformaldehyde and glutaraldehyde: what they are and what they do. Microscopy today. 2000 Dec 1;1(5):8-12
24. Fox CH, Johnson FB, Whiting J, Roller PP. Formaldehyde fixation. Journal of Histochemistry & Cytochemistry. 1985 Aug;33(8):845-53.
25. Thavarajah R, Mudimbaimannar VK, Elizabeth J, Rao UK, Ranganathan K. Chemical and physical basics of routine formaldehyde fixation. Journal of oral and maxillofacial pathology. 2012 Sep;16(3):400.

26. Srinivasan M, Sedmak D, Jewell S. Effect of fixatives and tissue processing on the content and integrity of nucleic acids. The American journal of pathology. 2002 Dec 31;161(6):1961-71.
27. McManus LM, Mitchell RN. Pathobiology of human disease: a dynamic encyclopedia of disease mechanisms. Elsevier; 2014 Aug 1:3315.
28. Feldman AT, Wolfe D. Tissue Processing and Hematoxylin and Eosin Staining. In Day CE. Histopathology: Methods and Protocols. Human Press;2014:33.
29. Fritzsche FR, Ramach C, Soldini D, Caduff R, Tinguely M, Cassoly E, Moch H, Stewart A. Occupational health risks of pathologists-results from a nationwide online questionnaire in Switzerland. BMC public health. 2012 Dec 6;12(1):1054.
30. Buesa RJ. Histology without formalin?. Annals of diagnostic pathology. 2008 Dec 31;12(6):387-96.
31. Kumarasinghe MP, Constantine SR, Hemamali RL. Methanol as an alternative fixative for cytological smears. The Malaysian journal of pathology. 1997 Dec;19(2):137-40.
32. Ananthalakshmi Ramamoorthy SR, Jeddy N, Thangavelu R, Janardhanan S. Natural Alternatives for Chemicals Used in Histopathology Lab-A Literature Review. Journal of clinical and diagnostic research. 2016 Nov;10(11):1.
33. Nathan NA, Narayan E, Smith MM, Horn MJ. Cell block cytology: improved preparation and its efficacy in diagnostic cytology. American journal of clinical pathology. 2000 Oct 1;114(4):599-606.

34. Holm K, Grinsted P, Poulsen EF, Fenger C. Can hairspray be used as a smear fixative? A comparison between two types of coating fixatives. Acta cytologica. 1988;32(3):422-4.
35. Ryan WL, inventor; Streck Laboratories, Inc., assignee. Fixative system, method and composition for biological testing. United States patent US 6,337,189. 2002 Jan 8.
36. Mielzynska I, Payne J, Lazar J, inventors; Digene Corporation, assignee. Device and method for cytology slide preparation. United States patent US 6,436,662. 2002 Aug 20.
37. DasDores GB, Mielzynska-Lohnas I, Taromaru E, Payne WJ, Slattery JP, Lazar JG, inventors; Digene Corporation, assignee. Device and method for cytology slide preparation. United States patent US 7,001,776. 2006 Feb 21.
38. Chepulis L. Healing Honey: A Natural Remedy for Better Health and Wellness. Universal-Publishers; 2008:27-36.
39. McCarthy J. antibacterial effects of honey: medical fact or fiction?. American Bee Journal. 1995:171-2.
40. Molan PC. Why honey is effective as a medicine. 1. Its use in modern medicine. Bee world. 1999 Jan 1;80(2):80-92.
41. Dold D, Du DH, Dziao ST. The antibacterial, heat-and light-sensitive inhibitory substance, inhibine, in natural honey. Z Hyg Infektionskrankh. 1937;121:333.
42. Cooper RA, Molan PC, Harding KG. Antibacterial activity of honey against strains of Staphylococcus aureus from infected wounds. Journal of the royal society of medicine. 1999 Jun;92(6):283-5.

43. White JW, Subers MH, Schepartz AI. The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system. Biochimica et Biophysica Acta (BBA)-Specialized Section on Enzymological Subjects. 1963 May 7;73(1):57-70.
44. Bizerra FC, Da Silva Jr PI, Hayashi MA. Exploring the antibacterial properties of honey and its potential. Frontiers in microbiology. 2012:3:398.
45. White JW, Subers MH, Schepartz AI. The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system. Biochimica et Biophysica Acta (BBA)-Specialized Section on Enzymological Subjects. 1963 May 7;73(1):57-70.
46. Almasaudi SB, Al-Nahari AA, El Sayed M, Barbour E, Al Muhayawi SM, Al-Jaouni S, Azhar E, Qari M, Qari YA, Harakeh S. Antimicrobial effect of different types of honey on Staphylococcus aureus. Saudi Journal of Biological Sciences. 2017;24(6):1255-1261.
47. Afroz R, Tanvir E, Zheng W, Little P. Molecular Pharmacology of Honey. Journal of Clininal Experimental Pharmacology. 2016;6(3).
48. Yoirish N. Curative properties of honey and bee venom. Honolulu: University Press of the Pacific; 200:p.9-11.
49. Moore OA, Smith LA, Campbell F, Seers K, McQuay HJ, Moore RA. Systematic review of the use of honey as a wound dressing. BMC complementary and alternative medicine. 2001 Jun 4;1(1):2.
50. Avwioro G, Bankole J, Iyiola S, Avwioro T, Akinola G. One of the properties of honey in wound healing is prevention of autolysis. Der Pharmacia Lettre. 2010;2(3):321-5.

51. Wahba NM, El-Nisr NA, Sayed SM, Abdallah MR. Intramammary honey infusion: a new trend in the management of bovine subclinical mastitis. Journal of Animal and Veterinary Advances. 2011 Jan 1;10(20):2740-4.
52. Ahmed S, Othman NH. Honey as a potential natural anticancer agent: a review of its mechanisms. Evidence-Based Complementary and Alternative Medicine. 2013 Dec 2;2013.
53. Rashad U, Al-Gezawy S, El-Gezawy E, Azzaz A. Honey as topical prophylaxis against radiochemotherapy-induced mucositis in head and neck cancer. The Journal of Laryngology & Otology. 2008;123(02):223.
54. Olaitan PB, Adeleke OE, Ola IO. Honey: a reservoir for microorganisms and an inhibitory agent for microbes. African health sciences. 2007;7(3):159-165
55. 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. Journal of oral and maxillofacial pathology. 2015 Sep;19(3):342.
56. Sabarinath B, Sivapathasundharam B, Sathyakumar M. Fixative properties of honey in comparison with formalin. Journal of Histotechnology. 2014 Mar 1;37(1):21-5.
57. Muddana K, Muppala JN, Dorankula SP, Maloth AK, Kulkarni PG, Thadudari D. Honey and olive oil as bio-friendly substitutes for formalin and xylene in routine histopathology. Indian Journal of Dental Research. 2017 May 1;28(3):286.
58. Ishaq R, Azmat H, Omair M, Sheikh AK, Tanwani AK. Comparison Of Honey With Alcohol As A Fixative In Fine Needle Aspiration Cytology. International Journal of Pathology. 2017;15(1):15-8.

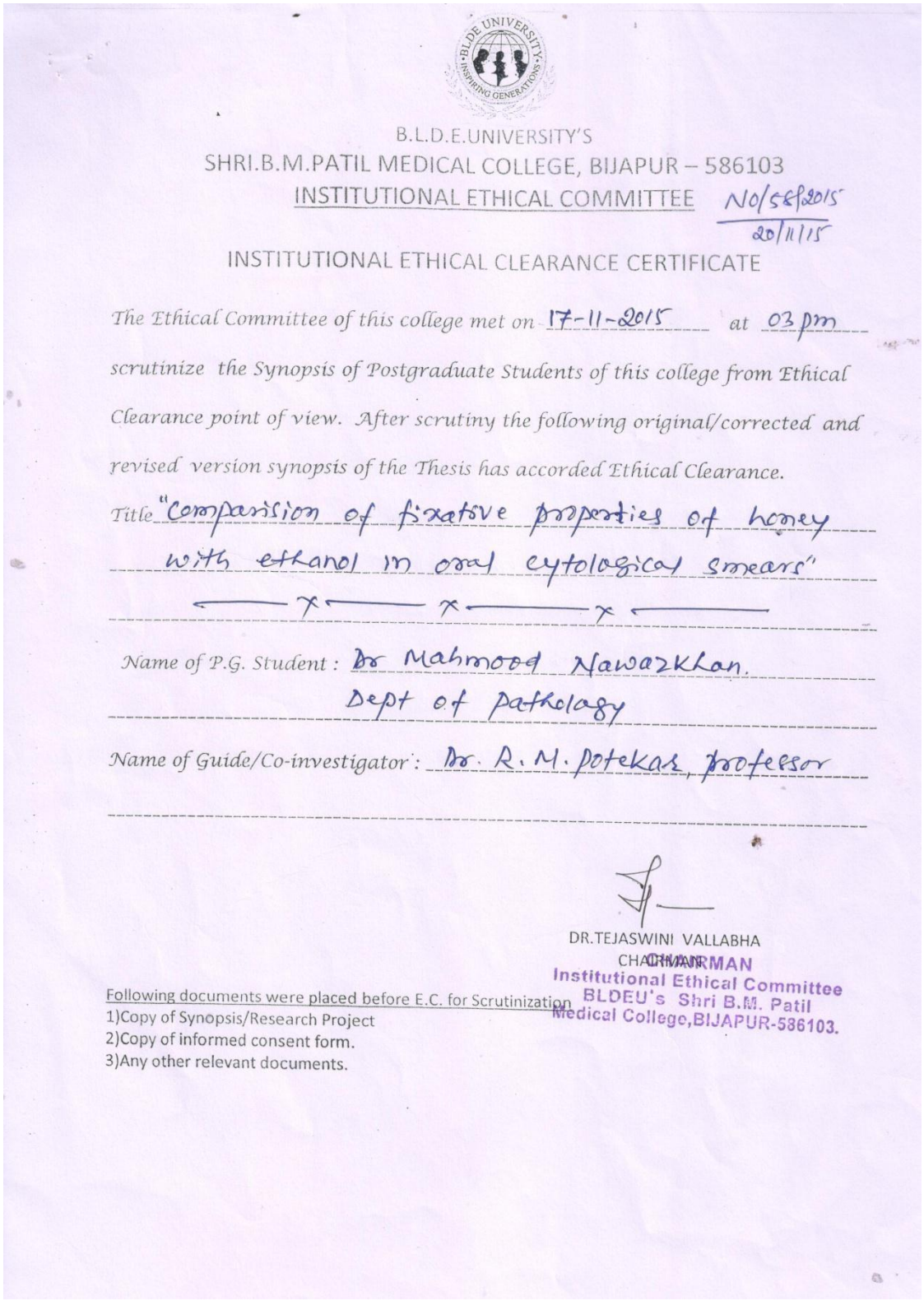
59. Sona M, Preethamol S. A comparative study on the efficacy of honey and ethanol as cytological fixatives. International Journal of Advances in Medicine. 2017 May 23;4(3):762-7.
60. Patil S, Rao RS, Ganavi BS, Majumdar B. Natural sweeteners as fixatives in histopathology: A longitudinal study. Journal of natural science, biology, and medicine. 2015 Jan;6(1):67.
61. Rajanikanth M, Ravi Prakash A, Sreenath G, Sonia Bai JK, Shyam ND. Transit Fixatives: An Innovative Study. Journal of clinical and diagnostic research. 2015 Mar;9(3):01.
62. Majumdar B, Rao R, Patil S. Tissue Preservation with Natural Fixatives: An Immunohistochemical Evaluation. World Journal of Dentistry. 2016;7:87-91
63. Gunter M, Bryant P. Immunocytochemical evaluation of ductal carcinoma in breast after preservation in honey. Journal of Histotechnology. 2009 Jun 1;32(2):54-9.
64. Pal L. Pathology laboratory in Formalin free system [Internet]. 2017 [cited 12 October 2017]. Available from: http://www.pathoindia.com/articles/ formalin_free.pdf
65. Olert J, Wiedorn KH, Goldmann T, Kühl H, Mehraein Y, Scherthan H, Niketeghad F, Vollmer E, Müller AM, Müller-Navia J. HOPE Fixation: A Novel Fixing Method and Paraffin-embedding Technique for Human Soft Tissues11Dedicated to Prof. Dr. H. Müntefering on the occasion of his 65th birthday. Pathology-Research and Practice. 2001 Jan 1;197(12):823-6.
66. Delfour C, Roger P, Bret C, Berthe ML, Rochaix P, Kalfa N, Raynaud P, Bibeau F, Maudelonde T, Boulle N. RCL2, a new fixative, preserves morphology and nucleic acid integrity in paraffin-embedded breast carcinoma

and microdissected breast tumor cells. The Journal of Molecular Diagnostics. 2006 May 31;8(2):157-69.

67. Titford ME, Horenstein MG. Histomorphologic assessment of formalin substitute fixatives for diagnostic surgical pathology. Archives of pathology & laboratory medicine. 2005 Apr;129(4):502-6.
68. Bussolati G, Chiusa L, Cimino A, D'Armento G. Tissue transfer to pathology labs: under vacuum is the safe alternative to formalin. Virchows Archiv. 2008 Feb 1;452(2):229-31.
69. Di Novi C, Minniti D, Barbaro S, Zampirolo MG, Cimino A, Bussolati G. Vacuum-based preservation of surgical specimens: an environmentally-safe step towards a formalin-free hospital. Science of the total environment. 2010 Jul 15;408(16):3092-5.

ANNEXURES

ETHICAL CLEARANCE



B.L.D.E.UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR – 586103
INSTITUTIONAL ETHICAL COMMITTEE No/58/2015
20/11/15

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 17-11-2015 *at* 03 pm *scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has accorded Ethical Clearance.*

Title "Comparision of fixative properties of honey with ethanol in oral cytological smears"

Name of P.G. Student : Dr Mahmood Nawazkhan. Dept of pathology

Name of Guide/Co-investigator : Dr. R.M. Patekar, professor

DR.TEJASWINI VALLABHA
CHAIRMAN
CHAIRMAN
Institutional Ethical Committee
BLDEU's Shri B.M. Patil
Medical College,BIJAPUR-586103.

Following documents were placed before E.C. for Scrutinization
1)Copy of Synopsis/Research Project
2)Copy of informed consent form.
3)Any other relevant documents.

BLDE University's

Shri B M Patil Medical College, Hospital & R.C

Vijayapur, Karnataka

INFORMED CONSENT FOR PARTICIPATION IN

DISSERTATION/RESEARCH

I, the undersigned,_____ , S/O D/O W/O _____, aged ____years, ordinarily resident of _____ do hereby state/declare that Dr Mahmood Nawaz khan of Shri B.M Patil medical college Hospital has examined me thoroughly on _____ at _____ (place) and informed me that he/she is conducting dissertation/research titled "comparison of fixative properties of honey with ethanol in oral cytological smear" under the guidance of Dr Ratnakar M Potekar, requesting my participation in the study. Doctor has also informed that the observation/results of test will be utilized for the study as reference data.

Doctor has also informed me that during conduct of this procedure like adverse results may be encountered. Among the procedure related complications most of them are treatable but are not anticipated. Further Doctor has informed me that my participation in this study help in evaluation of the results of the study which is useful reference to treatment of other similar cases in near future, and also I may be benefited in getting relieved of suffering or cure of the disease I am suffering.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept secret and not assessed by the person other than me or my legal hirer except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on information given by me, I can ask any clarification during the course of

treatment / study related to diagnosis, procedure of treatment, result of treatment or prognosis. At the same time I have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can terminate me from the study at any time from the study but not the procedure of treatment and follow-up unless I request to be discharged.

After understanding the nature of dissertation or research, diagnosis made, mode of treatment, I the undersigned Shri/Smt _____ under my full conscious state of mind agree to participate in the said research/dissertation.

Signature of patient:

Signature of doctor:

Witness: 1.

2.

Date:

Place

PROFORMA

NAME : OP/IP No. :

AGE :

SEX :

RELIGION :

OCCUPATION :

RESIDENCE :

VITALS: PR: RR:

BP: TEMPERATURE:

WEIGHT:

Evaluation of slides:

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

Score 1.Ethanol fixed slides ___ 2. Honey fixed slides ____

KEY TO MASTER CHART

Sl.no	– Serial Number
OP	– Out Patient
IP	– In patient
OBG	– Obstetric and Gynecology
Dept	– Department
BLDEA	– Bijapur liberal district education association
ENT	– Ear Nose and Throat
NA	– Not applicable.

MASTER CHART