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Research Article

Efficacy of Honey and Local Anaesthetics as Tissue Fixatives

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A B S T R A C T

Introduction: Fixatives are crucial in the preparation of tissue for pathological diagnosis. The gold standard for tissue specimen fixation is formalin. Since formalin is costly, carcinogenic, and difficult to get, there is interest in finding a viable replacement. In addition to being a dehydrator, honey also contains antibacterial and antioxidant qualities. The risks associated with local anaesthetics (LA), which are accessible in any clinic, are negligible. The purpose of this study was to evaluate the efficacy of using honey and a local anaesthetic solution in place of formalin as a fixative for tissue processing.

Aim: To analyse the staining parameters of honey and local anaesthetic with formalin as tissue fixatives for traditional hematoxylin and eosin staining (H&E) procedures

Methodology: All the tissue samples were extracted from animals (goat tongue). The collected tissue samples were divided into the following three groups: Group A- tissues fixed in neutral buffered formalin (standard) (n = 20), Group B- tissues fixed in processed honey solution (n = 20), and Group C- tissues fixed in local anaesthetic solution (n = 20). Each tissue was fixed in an appropriate volume of solution for 24 hours, followed by standard procedures. After being cut into sections and coated with hematoxylin and eosin, tissues were studied to identify their quality and other characteristics.

Results: 100% of formalin-fixed, 79% of honey-fixed, and 80% of local anaesthesia-fixed tissue sections were acceptable. In all three fixatives, there were statistically significant variations in intracellular staining, cell differentiation, staining uniformity, clarity, and tissue architecture. From statistical analysis, significant differences were found in all three fixatives on cytoplasmic staining, cell morphology, clarity of staining, uniformity of staining, and tissue architecture.

Conclusion: Although there was a mild variation in staining quality, honey and local anaesthetics can be safely used as an alternate emergency fixative.

Keywords: Fixative, Formalin, Honey, Local Anaesthesia

Introduction

Fixation is the most important step in the processing of tissue for histological or microscopic study. Proper fixation should be carried out for fixing all the components of cells and as a whole for maintenance of tissue architecture, thereby making out a proper diagnosis. The gold standard fixative is formalin, which has been in use since the 19th century as a result of Ferdinand Blum's preparation and standardisation.[1] Since it is affordable, widely accessible, practicable, and delivers quick fixation with the simplicity of processing, formalin is regarded as the gold standard fixative in regular hematoxylin and eosin (H&E) treatments. Though it has several benefits, numerous health and safety threats have also been found to be associated with it. Exposure to formalin, even for a short period of time, is exceedingly irritating to the throat, nose, as well as eyes, and can cause coughing and shortness of breath. The 11th Report on carcinogen of the Environment Health and Safety Information (EHSI) "reasonably predicted it to be a human carcinogen". Also connected to lung and nasal cancer, leukaemia, brain cancer, and maybe both. Additionally, formaldehyde has also been categorised as a Group II tumour-causing agent by the International Agency for Research on Cancer (IARC) and is believed to be associated with the development of nasopharyngeal cancer. The Occupational Safety and Health Administration (OSHA) also claim that formalin is dangerous and promotes substituting less dangerous compounds for it.[2] In order to counteract these harmful consequences, the objective of the current study was to designed to find the fixative with features of biodegradable, affordable, readily available natural remedies that maintain the qualities of fixatives. The aim of the current study is to evaluate the effectiveness of honey and local anaesthetic in meeting the qualities and criteria listed above.

The first fixative we preferred was honey. It functions similarly to fixatives, which stiffen the tissues as part of their effect. Glucose molecules and other substances are combined to make honey. It includes trace levels of chrysin, pinobanksin, vitamin C, catalase, and pinocembrin among other substances. Tissues placed in honey for up to 30 days did not exhibit any signs of putrefaction or autolysis, proving that honey prevents autolysis. Honey has a number of beneficial benefits, such as antioxidant, antibacterial, and anti-autolytic actions. It has the ability to reach the deepest tissue and can halt putrefaction and autolysis.[3]

The other solution preferred was a local anaesthetic (LA) solution, which is available in every dental clinic. In 1976, a study was conducted to assess the action of LAs with the cell membrane, a membrane-associated cytoskeletal organisation in BALB/3T3 cells and also its effects on the morphology of the cells. The study result showed that LA acts with the membrane lipids and results in varied effects of altered osmotic fragility, inhibition of cell spreading, movement, adhesion and fusion. LA also raises intracellular Ca concentrations to levels (> 10–5 M) sufficient to induce microtubule depolymerisation and act with membranes by both hydrophobic and electrostatic interactions in proximity with the anionic groups of acidic phospholipids which might be the possible explanation for its mechanism of action.[4] Local anaesthesia interacts with cell membranes and based on this concept, it was here attempted for use as a fixative.

With this background, the present study intended to find natural, non-toxic, easily available, economical substitutes for formalin to minimise its hazardous effects and to make clinics as well as histopathology laboratories a bio-friendly environment.

Materials and Methodology

The study was planned and conducted as a cross-sectional (analytical) study. Animal tissue (goat tongue) was used for the study. The total sample, (i.e. sample size) was determined using the software application of G-Power. The tissues were fixed in three fixative media and so it was constructed as three groups:

Group- I- Tissues fixed in formalin solution (n = 20)

Group- II- Tissues fixed in honey solution (20% honey) (n = 20)

Group- III- Tissues fixed in local anaesthetic solution (n = 20)

Sixty sections, each 1 cm in size, were sectioned from goat tongue. Twenty sections were kept in each of the three groups. All the tissues were fixed for the same time of 24 hours. After fixation, all the tissues were processed by usual standard procedures using increasing grades of alcohol and sectioned with four-micron thicknesses. All the sections were stained using H&E stain and were examined by microscope (Figures 1–3). All the slides were examined as per the criteria mentioned in Table 1 and were scored individually.

Table 1.Evaluation Criteria[3]

Features	Scores & Criteria	Result
Nuclear staining	Acceptable = 1 Round, smooth & clear nuclear membrane	Unacceptable = 0 Granular, disintegrated and out of focus
Cytoplasmic staining	Acceptable = 1 Intact Cytoplasmic membrane and transparent cytoplasm	Unacceptable = 0 Disintegrated cytoplasmic membrane, granular cytoplasm & out of focus

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Cell morphology	Preserved = 1 Absence of folds, no overlap & maintained N:C ratio	Unpreserved = 0 Overlapping cells, folded and disintegrated cells
Clarity of staining	Present = 1 Crispness in staining and transparency	Absent = 0 Obliterates 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
Tissue architecture	Acceptable = 1 All the structures in connective tissue and epithelial layers are viewed clearly	Unacceptable = 0 All the structures in connective tissue and epithelial layers are not viewed clearly

Results

All the slides were examined microscopically with the aforementioned criteria by two examiners individually and the values were presented as tables. Further analysis was done (statistical test of chi-square) using SPSS software.

Tables 2 and 3 show the analysis of the results and the scores given by two examiners.

Figures 4–10 represent the results of three fixative evaluation criteria, i.e. the scoring and evaluation done by two examiners. They also represent a comparison of the results of three fixatives.

Table 2.Evaluation of Tissue Sections Based on Criteria

Evaluation Criteria	10% Neutral Buffered Formalin	Honey	Local Anaesthesia
Nuclear staining	Acceptable - 20 Unacceptable - 0	Acceptable - 18 Unacceptable - 2	Acceptable - 16 Unacceptable - 4
Cytoplasmic staining	Acceptable - 20 Unacceptable - 0	Acceptable - 16 Unacceptable - 4	Acceptable - 16 Unacceptable - 4
Cellular morphology	Preserved - 20 Unpreserved -0	Preserved - 16 Unpreserved - 4	Preserved - 19 Unpreserved - 1
Nuclear morphology	Preserved - 20 Unpreserved - 0	Preserved - 16 Unpreserved - 4	Preserved - 14 Unpreserved - 6
Clarity of staining	Present - 20 Absent - 0	Present - 14 Absent - 6	Present - 16 Absent - 4
Uniformity of staining	Present - 20 Absent - 0	Present - 15 Absent - 5	Present -15 Absent - 5
Tissue architecture	Present - 20 Absent - 0	Present - 15 Absent - 5	Present - 15 Absent - 5

Table 3.Statistical Analysis for the Evaluated Tissue Sections

Variables	10% NBF Fixed (n = 20) (%)	Honey Fixed (n = 20) (%)	Local Anaesthetic Fixed (n = 20) (%)	p Value
Cytoplasmic staining	100	90	80	0.000
Nuclear staining	100	80	80	0.012
Cell morphology	100	80	95	0.001
Nuclear morphology	100	80	70	0.000
Clarity of staining	100	70	80	0.000
Uniformity of staining	100	75	75	0.000
Tissue architecture	100	75	75	0.000

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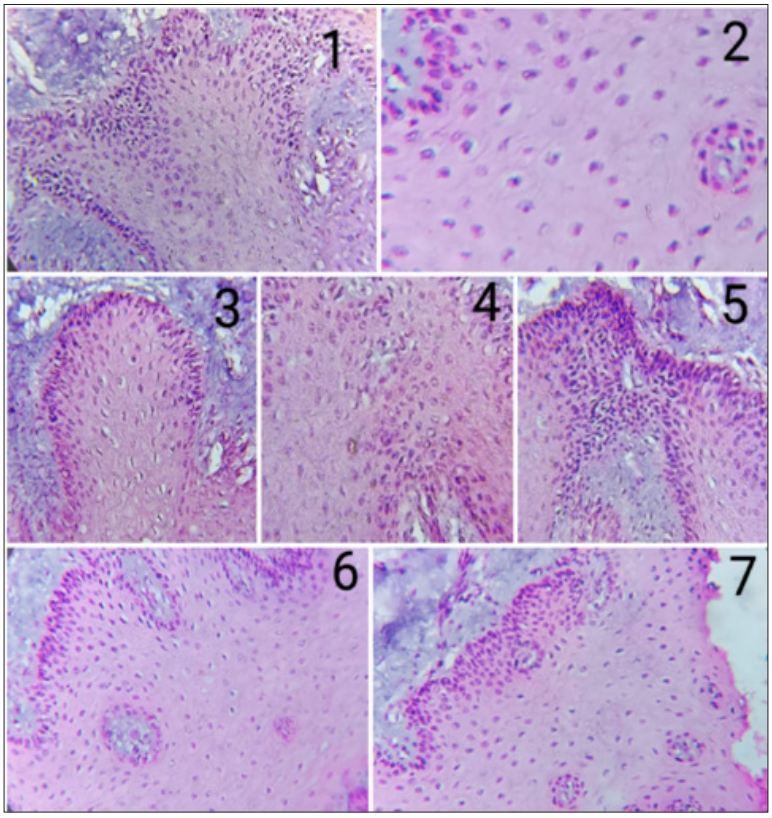

Figure 1.H&E Stained Tissue Section with Formalin Fixation 40x (1- Cytoplasmic Staining; 2- Nuclear Staining; 3- Cell Morphology; 4- Nuclear Morphology; 5- Clarity of Staining; 6- Uniformity of Staining; 7- Tissue Architecture)

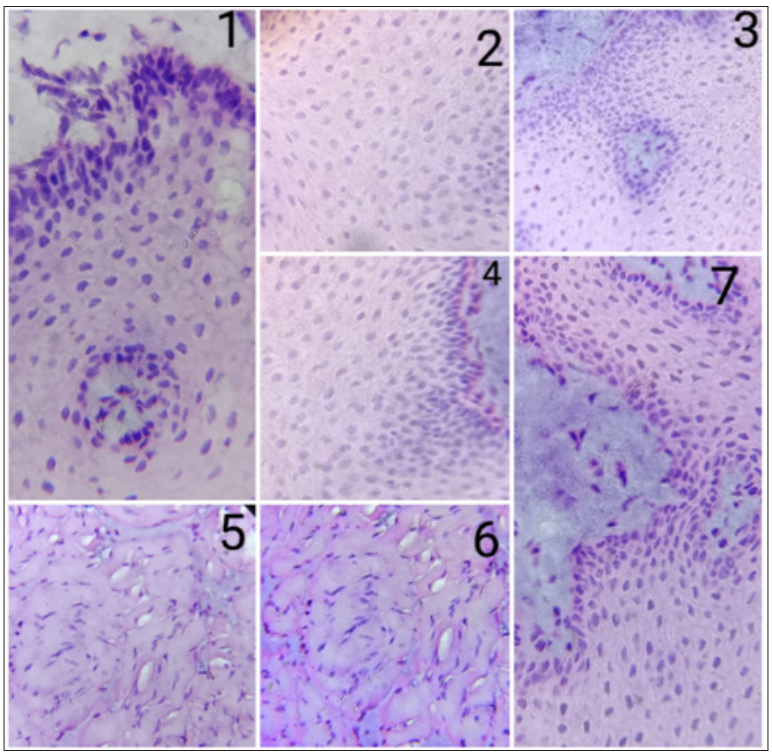

Figure 2.H&E Stained Tissue Section with Honey Fixation 40x (1- Cytoplasmic Staining; 2- Nuclear Staining; 3- Cell Morphology; 4- Nuclear Morphology; 5- Clarity of Staining; 6- Uniformity of Staining; 7- Tissue Architecture)

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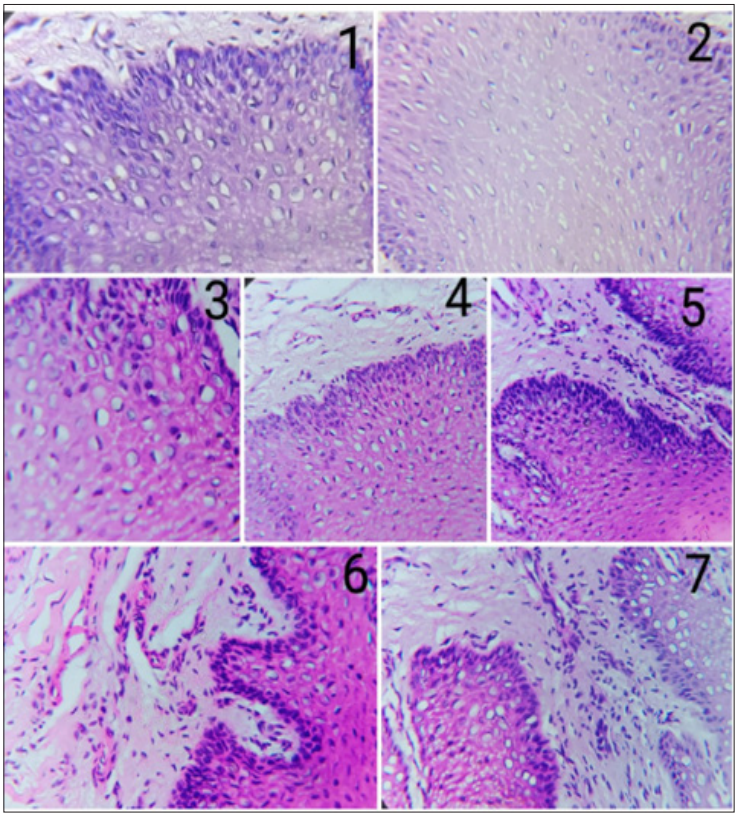

Figure 3.H&E Stained Tissue Section with Local Anesthetic Fixation 40x (1- Cytoplasmic Staining; 2- Nuclear Staining; 3- Cell Morphology; 4- Nuclear Morphology; 5- Clarity of Staining; 6- Uniformity of Staining; 7- Tissue Architecture)

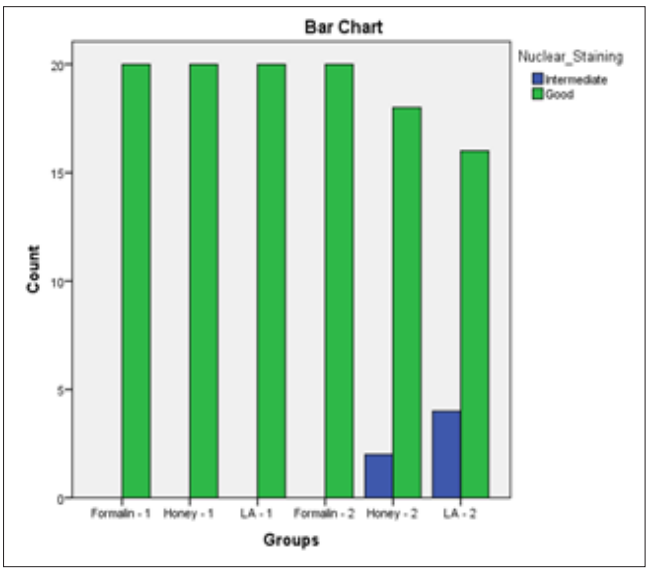

Figure 4.Nuclear Staining (Formalin- 100%; Honey- 90%; LA- 80%)

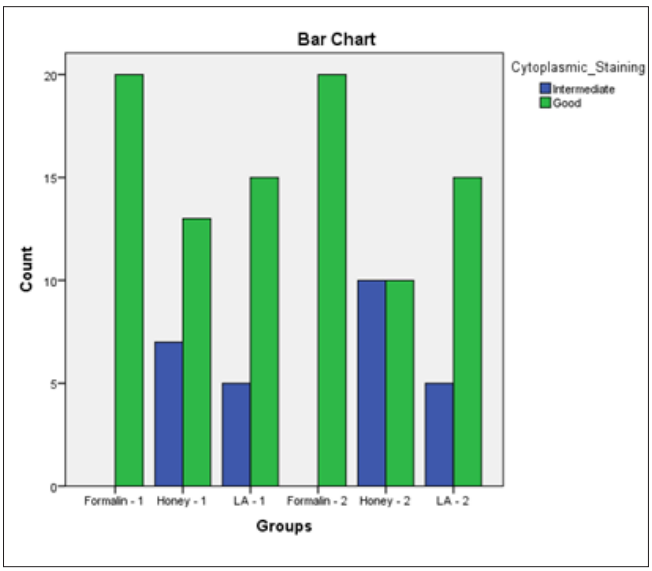

Figure 5.Cytoplasmic Staining (Formalin- 100%; Honey- 80%; LA- 80%)

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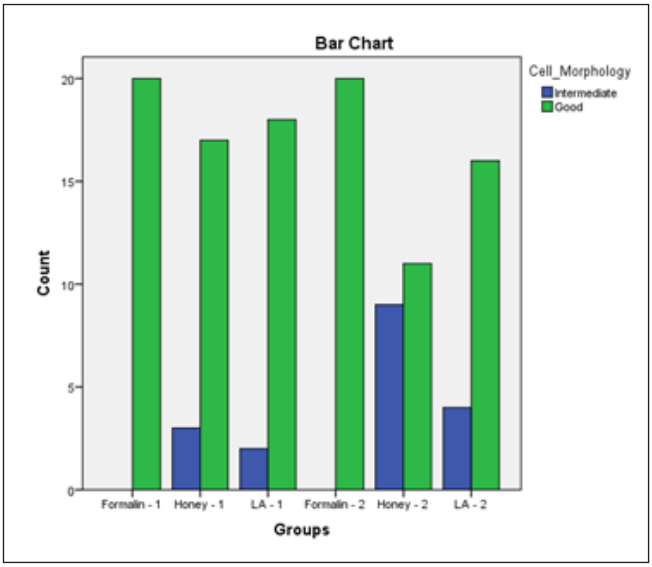

Figure 6.Cell Morphology (Formalin- 100%; Honey- 80%; LA- 95%)

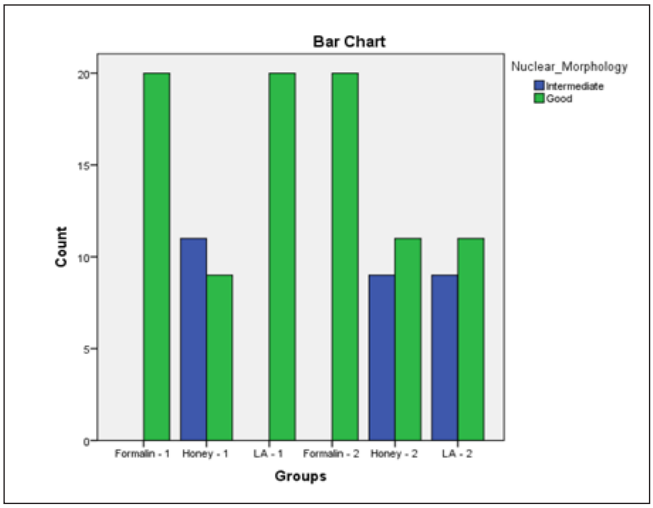

Figure 7.Nuclear Morphology (Formalin- 100%; Honey- 80%; LA- 70%)

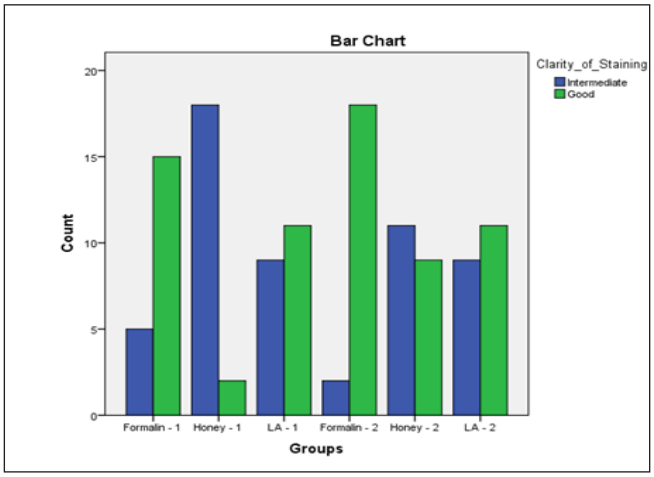

Figure 8.Clarity of Staining (Formalin- 100%; Honey- 70%; LA- 80%)

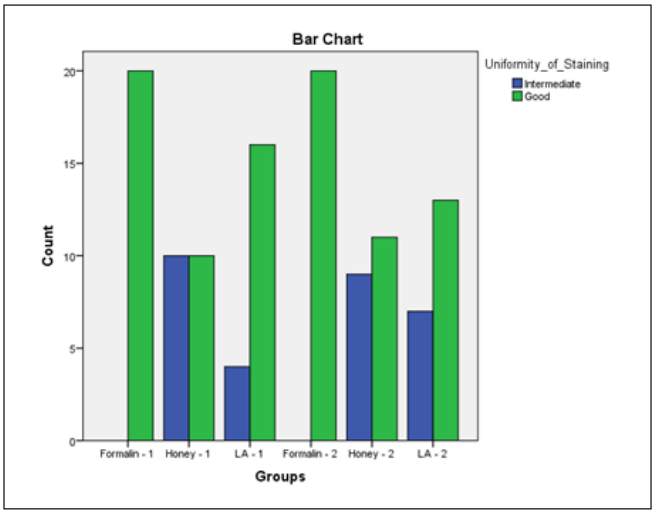

Figure 9.Uniformity of Staining (Formalin- 100%; Honey- 75%; LA- 75%)

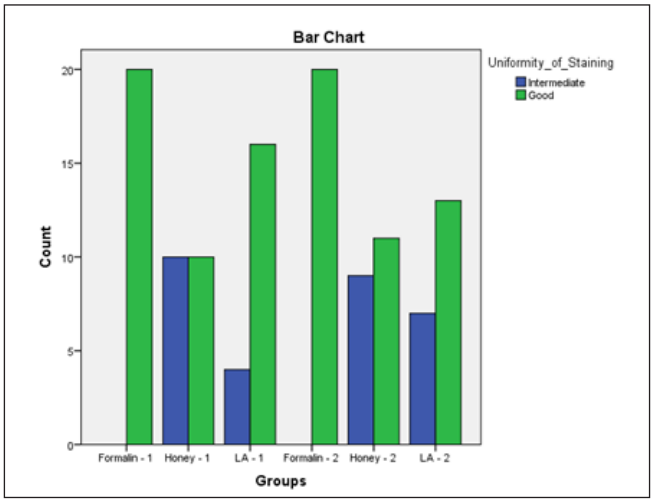

Figure 10.Tissue Architecture (Formalin- 100%; Honey- 75%; LA- 75%)

Nuclear staining was acceptable in 20 formalin-fixed, 18 honey-fixed and 16 local anaesthetic-fixed tissues. The p value obtained after chi-square analysis was 0.012 which indicated a non-significant association.

Cytoplasmic staining was acceptable in 20 formalin-fixed, 16 honey-fixed and 16 local anaesthetic-fixed tissues. The chi-square test performed between the groups showed a p value of 0.000 which was significant.

Cellular morphology was preserved in 20 formalin-fixed, 16 honey-fixed and 19 local anaesthetic-fixed tissues. The chi-square analysis between the groups showed a p value of 0.001 which was significant.

Nuclear morphology was preserved in 20 formalin-fixed, 16 honey-fixed and 14 local anaesthetic-fixed tissues. Chi-square tests between these groups showed a p value of 0.000 which was significant.

Clarity of staining was present in 20 formalin-fixed, 14

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honey-fixed and 16 local anaesthetic-fixed tissues. A chi-square analysis was done between these groups and the p value obtained was 0.000 which was significant.

Uniformity of staining was present in 20 formalin-fixed, 15 honey-fixed and 15 local anaesthetic-fixed tissues. A chi-square analysis between these groups showed a p value of 0.000 which was significant.

Tissue architecture was preserved in 20 formalin-fixed, 15 honey-fixed and 15 local anaesthetic-fixed tissues. A chi-square analysis between these groups showed $p < 0.000$ (significant).

Chi-square analysis on three fixatives showed that nuclear staining was only not significant and all other criteria were significant. From statistical analysis, we found that significant differences were found in all three fixatives on the staining of the cytoplasm, cell morphology of the cell, clarity and uniformity of the stain, and tissue architecture.

From the above analysis, we recommend the usage of honey and local anaesthetic solution as an indispensable fixative medium when formalin is not available or can not be transported soon in cases of emergency.

Discussion

Fixation is a necessary step prior to histopathological tissue processing for all forms of microscopic evaluation. The specimen is initially preserved with a chemical, formaldehyde, which inhibits degeneration and disintegration of the tissue specimen (autolysis). Russian chemist Alexander M Butlerov (1859) discovered formaldehyde. Because it is readily available and inexpensive, formaldehyde continues to be the gold standard fixative for preserving tissue specimens. Formaldehyde is categorised as "carcinogenic to humans" by the International Agency for Research on Cancer (IARC). According to the US Occupational Safety and Health Administration, the allowable exposure limit is 0.75 ppm on average over an 8-hour time period. Exposure to more than this level of formalin is harmful to health. It can irritate the eyes, nose, and throat, and can create an allergic skin reaction.[3]

Honey is a naturally occurring sweetener, which honeybees make from plant nectar. In addition to trace levels of additional substances including pinobanksin, chrysin, catalase, vitamin C, and pinocembrin, honey also contains a complex blend of carbohydrates. Much research conducted in the past demonstrated that honey has dehydrating and preserving capabilities like formaldehyde, making it the ideal choice for usage as an eco-friendly natural fixative in pathology labs. Due to its antioxidant, antibacterial, anti-inflammatory, and antimutagenic properties, it has a significant medical value. There are several research studies proving that honey is more successful in healing wounds.[3]

Using H&E stain to determine the findings, Patil et al. carried out research to assess the fixative efficacy of honey and jaggery, for more than 6-month period. They also examined the tissues' suitability for Periodic Acid Schiff (PAS) and Masson-Trichrome special stains (MT). After analysing 42 specimens of tissue, they concluded that after six months, H and E, special stains- formalin, jaggery, and honey all produced good results. They suggested using eco-friendly jaggery and honey in place of formalin for long-term tissue preservation.[1]

Rajanikanth et al. in their work, used frequently accessible solutions such as betadine solution, spirit, saline, hydrogen peroxide (H_2O_2), milk, and local anaesthesia (LA), as fixatives, and formalin serving as the control. They found that they could be used as fixatives for a minimum of 8 hours without causing any harm or distortion before being treated in formalin solution and termed them "transient fixatives".[2]

Thamilselvan et al., in their systematic review, extracted 9 articles using keyphrases like honey, natural alternative, natural substitute, neutral buffered formalin, etc. from Google search, PubMed, and manual search between 2009 and 2019. The final nine papers compared the effectiveness of honey as a natural substitute for formalin as a tissue fixative. They used many criteria to examine the tissues, including connective tissue stability, connective tissue staining, and epithelial preservation and staining for 24 hours, 48 hours, and 72 hours. They confirmed that formalin produced equivalent or better outcomes in fixing than honey. Also, their research demonstrated that there were statistically significant variations in nuclear features and cytoplasmic staining between honey and formalin fixative (p value 0.01).[3]

They also stated that formalin-fixed tissues show a preferred outcome over honey in all aspects and stained consistently in all the research works. Similarly, honey has shown results comparable to that of formalin in histopathological tissue processing and suggested using honey as a natural fixative which is cost-effective, non-toxic and non-allergenic and can be used as efficiently in all histopathological laboratories.[3]

Bhattacharyya et al. in their study used formalin as positive control and, honey, jaggery, sugar syrup and distilled water as negative control in forty samples of animal goat tissues. They classified five tissue sections in each group. Jaggery was found better than all the natural fixatives in nuclear details and cytoplasmic staining. From the results, they concluded that jaggery can be used as an effective fixative similar to formalin.[4]

Kasetty et al. conducted a study in which formalin, local anaesthesia, distilled water, and normal saline solution were used as fixatives for 12 h and 24 h each utilised in 40 soft tissue specimens obtained from 2 goat tongues. All

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the tissues were directly immersed in local anaesthesia, Distilled Water, Normal Saline solution and formalin for 12 and 24 h each. There was a significant difference in staining characteristics as well as the efficacy of all these three fixatives. They suggested using LA solution as the only emergency fixative in case of unavailability of formalin or other solution.[5]

Lam-Ubol et al. used oral tissues which were obtained during impaction or tooth extraction procedures in their study. The tissues were fixed in 4 varied fixatives for 24 and 72 hours in the solutions of 30% jaggery, 70% ethanol, 2% mepivacaine with 1:100000 epinephrine, and formalin. They found that the fixative efficacy of formalin was not statistically significant to 70% ethanol and 30% jaggery at 24 and 72 hours. 70% ethanol and 30% jaggery gave better results as fixative at 24 hours. And best fixative effect of 30% jaggery was given at 72 hours.[6]

Muddana et al. in their study evaluated the effectiveness and comparison of honey as an alternative fixative to formalin and Olive oil as an alternative to xylene in thirty tissue sections. Their study results showed honey as a better alternative to formalin and it showed properties of good preservation, good staining quality, good cellular architecture and maintained good cyto/nuclear-morphometrics.[7]

Patil et al. carried out a preliminary study using the buccal mucosa of a goat which was sectioned into five and all sections were placed in containers with 10% buffered formalin, distilled water, 20% honey, 20% sugar syrup & 30% jaggery syrup with formalin as positive control & distilled water as a negative control. Their study result inferred that jaggery syrup fixed tissues gave the best results in staining and all qualities. Thus, they concluded that cost-effective and easily available jaggery can be used as tissue fixatives in screening camps.[8]

Sinha et al., in their study, analysed the fixative property of jaggery and neutral buffered formalin in sixty-five pathological tissues. They evaluated with various criteria and found that jaggery fixed tissues showed better staining quality than NBF. From this study, it is proven that natural fixatives can be used as a substitute.[9]

Chittemsetti et al., in their study, analysed the fixative property of jaggery and khandsari in 90 tissue specimens. All the samples were assessed with features of "cellular outline, cytoplasmic details, nuclear details, staining quality and overall morphology". It was found that khandsari gave better results than formalin and also jaggery gave better results between the two solutions.[10]

Majumdar et al. did a cross-sectional study in thirty goat oral mucosal tissue and fixed tissues in formalin, honey and jaggery. He evaluated with certain criteria and concluded that jaggery was the best fixative when compared with honey and was on par with formalin.[11]

Sabarinath et al., in their study, evaluated honey as a fixative in 30 specimens with a fixation duration of 24 hours followed by routine processing. They found tissues fixed in honey gave staining features as well as nuclear size, cell size, overall tissue architecture, as well as general connective tissue staining were the same as those of formalin-fixed sections.[12]

Lalwani et al.'s study doesn't show any statistical difference between processed and unprocessed honey compared to formalin-fixed tissues, thus stating both honey and formalin show equal staining effects.[13]

Pandiar et al. evaluated the fixative properties of ethanol &20% aqueous honey solution and 30% aqueous jaggery. They also evaluated the fixatives for special stains - Periodic acid Schiff (PAS). There was no statistical difference between ethanol, honey and jaggery. They concluded that honey and jaggery solutions have equal effectiveness as that of ethanol and can be used in unavailable/ emergency conditions.[14]

Our study findings showed a significant difference in the evaluation criteria of cytoplasmic staining, cell morphology, uniformity of staining, clarity of staining, and tissue architecture. The nuclear staining difference was only not significant and was noted to be good or acceptable in all three fixative media. Our study results were similar to Bhattacharyya et al., Lam-Ubol et al., Kasetty et al., Rajanikanth et al., and Thamilselvan et al.[2–6]

Conclusion

Fixation is an important procedure in the process of tissue processing. It provides clear vision including cellular details, nuclear details and tissue architecture, thereby helping in prompt diagnosis. Formalin, which is mostly used in the form of neutral buffered formalin (10%) in most histopathological laboratories, imposes danger as it has numerous side effects on the respiratory system mainly and also affects the central nervous system and skin. Various studies are being conducted by researchers to find an economical and effective alternative. We also attempted a study here using local anaesthesia and honey as an alternative to formalin and recommend that it can be used as an indispensable/ essential fixative at times or in cases when formalin is not available or can be used as a transport medium until transfer.

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Conflict of Interest: None

References

1. Patil S, Rao RS, Ganavi BS, Majumdar B. Natural sweeteners as fixatives in histopathology: a longitudinal

study. J Nat Sci Biol Med. 2015;6(1):67-70. [PubMed] [Google Scholar]
2. Rajanikanth M, Ravi PA, Sreenath G, Jk SB, Ndvn S. Transit fixatives: an innovative study. J Clin Diagn Res. 2015 Mar;9(3):ZM01-3. [PubMed] [Google Scholar]
3. Thamilselvan S, Don KR, Sherlin HJ, Jayaraj G, Santhanam A. Honey as a natural alternative for formalin fixative - a systematic review. J Evol Med Dent Sci. 2021;10(6):369-74. [Google Scholar]
4. Bhattacharyya A, Gupta B, Singh A, Sah K, Gupta V. Probing natural substitute for formalin: comparing honey, sugar, and jaggery syrup as fixatives. Natl J Maxillofac Surg. 2018;9(1):14-21. [PubMed] [Google Scholar]
5. Kasetty S, Dwivedi D, Ragavendra TR, Kallianpur S, Gupta S, Prabhakar N. Comparison of efficacy of local anesthetic solution, distilled water and normal saline as emergency fixatives. J Oral Maxillofac Pathol. 2018;22(2):283. [PubMed] [Google Scholar]
6. Lam-Ubol A, Kitrueangphatchara K, Putthanuparp T, Arayakhun R, Kwanthong R, Choonhawarakorn K. Nonformalin fixative agents: a comparative study of fixative efficacy and histomorphology. Int J Surg Pathol. 2018 Dec;26(8):701-6. [PubMed] [Google Scholar]
7. 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 J Dent Res. 2017;28(3):286-90. [PubMed] [Google Scholar]
8. Patil S, Premalatha BR, Rao RS, Ganavi BS. Revelation in the field of tissue preservation – a preliminary study on natural formalin substitutes. J Int Oral Health. 2013;5(1):31-8. [PubMed] [Google Scholar]
9. Sinha N, Nayak MT, Sunitha JD, Dawar G, Rallan N, Gupta S. Comparitive efficacies of a natural fixative with a conventional fixative. J Oral Maxillofac Pathol. 2017;21(3):458. [PubMed] [Google Scholar]
10. Chittemsetti S, Nallamala S, Sravya T, Guttikonda VR, Manchikatla PK, Kondamari S. Natural substitutes for formalin: a boon to histopathology!! J Oral Maxillofac Pathol. 2018;22(1):143. [PubMed] [Google Scholar]
11. Majumdar B, Rao RS, Patil S. Tissue preservation with natural fixatives: an immunohistochemical evaluation. World J Dent. 2016;7(2): 87-91. [Google Scholar]
12. Sabarinath B, Sivapathasundharam B, Sathyakumar M. Fixative properties of honey in comparison with formalin. J Histotechnol. 2014;37(1):21-5. [Google Scholar]
13. Lalwani V, Surekha R, Vanishree M, Koneru A, Hunasgi S, Ravikumar S. Honey as an alternative fixative for oral tissue: an evaluation of processed and unprocessed honey. J Oral Maxillofac Pathol. 2015;19(3):342-7. [PubMed] [Google Scholar]
14. Pandiar D, Baranwal HC, Kumar S, Ganesan V, Sonkar PK, Chattopadhyay K. Use of jaggery and honey as adjunctive cytological fixatives to ethanol for oral smears. J Oral Maxillofac Pathol. 2017;21(2):317. [PubMed] [Google Scholar]

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