



Is Honey a Reliable Substitute for Formalin in Histopathological Analysis- A Scoping Review.

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ABSTRACT:

Introduction: Formalin has long been regarded as the gold standard for tissue fixation in histopathology. However, its potential health hazards, including carcinogenicity, have prompted the exploration of safer, natural alternatives. Honey, a biocompatible and naturally available substance, exhibits antibacterial and tissue-preserving properties, making it a potential fixative.

Objectives: To assess the effectiveness of honey as a fixative in histopathological tissue processing and compare its histomorphological outcomes with formalin and other natural fixatives.

Methods: A comprehensive literature search was conducted using PubMed, Scopus, and Embase databases for studies published in English. Original research articles evaluating honey (processed or unprocessed) as a fixative in human or animal tissue samples, with formalin or other natural fixatives as comparators, were screened based on inclusion and exclusion criteria, followed by data extraction.

Results: Out of 107 identified articles, 12 met the inclusion criteria. Most studies reported that honey at low concentrations (typically 10%) and low pH (4.5–5.0) preserved the structural morphology comparable to formalin. Processed honey showed better results than unprocessed variants.

Conclusions: Honey with a low concentration of 1:10 and low pH can be used as a safe alternative to formalin. Further research with standardized protocols is needed for broader application.

1. Introduction

The fixation of tissue specimens is a crucial step in pathology to maintain the cellular components, withstand tissue processing, and avoid decomposition, putrefaction, and autolysis. Formalin, which is a commonly used fixative, has been considered the gold standard for tissue preservation in histopathology for decades.^{1,2} However, as reported by the Occupational Safety and Health Administration (OSHA), concerns about its toxic nature, potential carcinogenicity, and potential health risks have led researchers to explore alternative fixatives.³ Honey, a natural sweetener possesses dehydrating and preserving properties that qualify it to be used in pathology laboratories for fixation. This natural fixative has also been shown to possess antimicrobial, antiviral, and antimutagenic properties.⁴ Its non-toxic nature and biocompatibility make it a potentially safer option. Also,

honey is generally more affordable and readily available compared to formalin.

2. Objectives

The objective of our study was to assess the efficacy of honey as a fixative agent for preserving tissue specimens and compare its effectiveness with that of currently universally accepted formalin and other natural fixatives. To conduct this scoping review, a comprehensive search of electronic databases including PUBMED, SCOPUS, and EMBASE was performed. The search strategy incorporated keywords such as "honey," "formalin," and "fixative." Relevant studies published in English up to February 2024 were considered for inclusion. The selection criteria for eligible studies included original research articles that assessed the use of honey as a fixative in histopathology. Studies involving both animal and human tissue samples were considered. Exclusion criteria consisted of studies that did not focus on honey as



a fixative or did not evaluate histopathological outcomes. The selection process involved screening titles, abstracts, and full texts of identified articles, followed by data extraction.

3. Method

Inclusion criteria:

Original articles published in English on the utilization of honey and formalin in the histopathology laboratory till February 2024 were included.

Population: All the studies that include the natural fixative that is honey in the routine histopathological laboratory, in both males and females of all races, and animal tissues.

Intervention: Processed and unprocessed honey that has been used as a fixative for tissue sections in the histopathology laboratory.

Comparison: Processed/unprocessed honey was compared with 10% formalin and other natural fixatives such as sugar and jaggery in the histopathology laboratory.

Outcomes: Measured in terms of clarity of the and cellular and structural morphology after staining of the sections.

Type tissue sections: Smears/biopsy tissue specimens taken from humans/animals.

Exclusion criteria:

The studies that did not have the main focus on natural fixative honey in the histopathology laboratory, the literature published in languages other than English, and the studies that did not have a comparator as formalin, review, and case reports were excluded.

Focus question: The focus question was to evaluate if honey can be used as a fixative in routine histopathological procedures in the laboratory for smears taken from buccal mucosa and biopsy specimens. The main objective of this review was to answer the following questions:

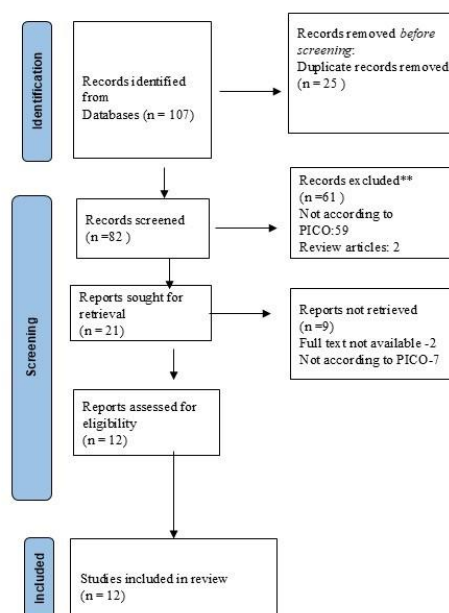
1. Can honey be a reliable fixative when compared to formalin
2. Will the cellular morphology of the tissue be preserved? Which gives better results? Honey or formalin.

Search Strategy and keywords: The following keywords were used: "Honey" AND "Fixative" in PubMed, Scopus,

and Embase (dentistry and open access), databases updated till February 2024. The references in the included studies were checked for additional records. The details of the search strategy are given in Table 1.

Table 1: Search strategy

Identification of studies via databases (PUBMED, SCOPUS, EMBASE)



4. Results

Reviewers independently performed the searches in different databases and collected all the articles. The initial check for the title and abstract screening followed by the removal of duplicates in the Excel sheet (version 1.19.4) was based on the inclusion and exclusion criteria. The title and abstract screening and full-text screening were done manually using a Microsoft Excel spreadsheet by 4 reviewers. Any disagreements were mutually discussed between the reviewers, and a consensus was reached through arbitration by the expert. Our study involved a thorough screening process of scholarly articles, where we assessed a total of 107 articles. During the screening process, we identified 25 duplicates, which were removed from consideration. After eliminating the duplicates, we were left with 82 unique articles that underwent a detailed assessment to determine their eligibility. Upon further analysis, we found that only 12 articles met our strict inclusion criteria and were deemed



relevant to our research. These 12 articles were carefully reviewed and analyzed to extract valuable insights and information for our study.

Data Extraction

This was followed by full-text screening against the predefined inclusion criteria. The reasons for the exclusion of studies were also noted. The data extraction sheet comprised the data such as details of publication, study design, and details regarding the preservation of the cellular morphology, outcome, and drawbacks. The detailed characteristics of the included studies are summarized in Table 2.

4. Discussion

The final data was extracted from 12 articles. Table 2 describes the characteristics of the included studies. The study conducted by Ozkan et al⁴, on the tissues obtained from the endometrium, breast, placenta, uterus, omentum, suprarenal, stomach, and lung, wherein the histomorphology of the stained sections (staining done with Hematoxylin and Eosin, Gomori's one-step trichrome stain for muscle and collagen) were assessed based on the cellular outline, cytoplasmic and nuclear detail, erythrocyte integrity, overall morphology, and staining. Immunohistochemistry was also done with the tissue sections fixed in honey and 10% Neutral Buffered Formalin (NBF) and alcoholic formalin. The cellular morphology was well preserved with 10% Honey-fixed tissues (HFT), whereas the nuclear and cytoplasmic details were well preserved with tissues fixed with NBF ($p < 0.05$). Minor discrepancies were observed concerning the special stains that did not hinder the diagnosis. Immunohistochemical staining (Ki67 and Vimentin) showed no statistical significance in the sections stained with NBF, Honey Fixed tissues (HFT), and alcoholic formalin. Thus, their study indicates that the staining properties of the tissues fixed with Honey, 10% NBF, and alcoholic formalin fixation in both histochemical and immunohistochemical studies are similar, and low concentrations of pine honey can be used as an alternative to formaldehyde for standard histopathology.

A similar study conducted by Srii et al⁵, on the tissues obtained from the gingiva, found that the general stain uptake and the preservation of tissue architecture, including the nuclear and cytoplasm sizes, were similar to that of the formalin-fixed tissues ($p < 0.05$). One of the

observations was a more hyalinised appearance of the collagen fibers in both H & E staining and special stains when compared to that of formalin, which was seen in high concentrations of HFT. Thus, low concentrations of honey in the ratio 1:10 at room temperature with pH maintained at 4.5-5.0 gave comparable results to the tissues fixed in formalin.

Bhattacharyya A et al⁶ conducted a single-blinded study on the tissues obtained from the fresh goat (tongue), the comparators for the HFT were sugar syrup (100%), jaggery syrup (100%), and distilled water (negative control), 10% formalin (positive control) and they were assessed for the nuclear and cytoplasmic staining. The fixation was done at 24hrs, 48hrs and 72 hrs. The tissue sections had good overall morphology and also the nuclear, cytoplasmic details and staining quality with all the fixatives, but jaggery gave better results compared to the other fixatives used in the study. They found a statistically significant difference between jaggery and other fixatives ($P \leq 0.001$). Thus, their study concluded that the cytoprotective and antioxidant activity in jaggery makes it superior to honey and sugar syrup and thus can be used as a substitute for formalin in the histopathology lab. One of the limitations of their study was the smaller sample size.

Lalwani V et al⁷ conducted a study on 36 human tissues including oral epithelium, lymphoid, salivary gland, fat, muscle, and skin. The comparison was done between 10% unprocessed honey (pH: 3.6), 10% processed honey (pH: 5.05), and 10% NBF (pH 7.2-7.4) for 24 hours at room temperature. The comparison between the 3 groups was done using the Kruskal-Wallis test, post hoc Dunn's multiple comparisons test, and Chi-square test or Fisher's exact test. The histomorphologic analysis of their study revealed 92% of adequate staining patterns in processed and unprocessed honey as compared to NBF in the cytoplasm. There was 75% adequacy for tissue morphology in processed and unprocessed honey as compared to NBF, which showed 92%. There was no statistically significant difference in clarity and uniformity of the staining pattern among all three fixatives. On the assessment of artifacts, unprocessed honey showed more artifacts compared to processed honey; a statistically significant difference was seen between unprocessed honey and processed honey when compared with formalin ($P = 0.004$). A few of the sections fixed with unprocessed honey showed



overall compromised morphology and architecture for the cellular details when considering NBF. There was no statistically significant difference between the clarity and the uniformity of the staining patterns, unprocessed and processed honey were equivalent to NBF, and the collagen fibers appeared to be more hyalinized, one of the reasons they quoted for this appearance was the low pH of unprocessed (pH-3.6) and processed honey (pH-5.05). Similar findings were noted in the study done by Srii et al⁵. On analysis for the adequacy of diagnosis, there was no statistically significant difference among all three fixatives, indicating that the tissue architecture and morphology were very well preserved in all three groups without hampering the appreciation of the tissue section. However, they found that the positive predictive value was better in processed honey compared to unprocessed honey.

Patil S et al⁸ in their longitudinal study on goat buccal mucosa, preserved the tissues in 30% jaggery, 20% honey, and 10% buffered formalin (control) over 6 months at intervals, the slides were evaluated at 48 hours, 1 week, 3months, and 6 months. Their study revealed that jaggery gave better results than honey, but formalin was superior to honey and jaggery; cellular and nuclear shrinkage was observed with jaggery and honey as compared to formalin, towards the end of 6 months. The authors suggested that the slightly inferior results with jaggery and honey could be due to altered cross-binding with the tissue as compared to formalin. Certain problems encountered with the use of honey included sectioning difficulties due to breaches in continuity caused by fragility and intense staining with eosin. Additionally, the growth of molds over time was another shortcoming, which was addressed by using thymol crystals. In a study conducted by Narwal A et al⁹ several natural fixatives were compared to formalin (10%) for their effectiveness. These included aloe vera (20%), sugar solution (20%), honey (20%), and jaggery (30%). The results showed that, while aloe vera had better fixative properties compared to other natural fixatives, it was not as effective as 10% formalin. HFT and JFT showed poor cellular morphology, and foldings in the sections were observed.

Gunter M et al¹⁰ conducted an immunohistochemical study on mastectomy specimens fixed with honey. 10% Formalin was taken as a positive control, 10% manuka honey, 10% commercial honey, and 10% local honey

were used for the study. The antibodies used for the immunohistochemical study were Common Leukocyte Antigen (RP2/18, Clone RP2/22), Cytokeratin 5 (XM26), Cytokeratin AE1/AE3 (AE1, Clone AE3), Epithelial membrane antigen (Clone GP1.4). They found that the histomorphology was well preserved in sections where the antigen retrieval was not done, which is an important step for the binding of antibodies to specific antigens in the tissues. Overall, the honey-preserved tissues showed compromised morphology compared to control tissues fixed in formalin.

Rahma Al-Maaini et al¹¹ analyzed the effectiveness of honey (1%-20% diluted with distilled water) as a fixative in the demonstration of the connective tissue components in the fresh goat tissues using special stains such as Van Gieson stain, Gordon & Sweet's reticulin, Miller's elastic stain, and Masson trichrome. The efficacy of honey was checked at dilutions of 1%, 5%,10%, and 20%. HFT at 1% dilution demonstrated poor collagen fibers compared to HFT at a dilution of 10% which was comparable to 10% Formalin fixed tissues. Also, the reticulin fibers and the elastic fibers were well demonstrated at a greater dilution of honey (10 %) compared to 1 % honey. The staining of collagen and keratin with 1% honey in Massons Trichrome method was better, but 10% HFT gave superior results compared to 1 % honey.

Sabrinath B et al¹² conducted a double-blind pilot study on patients with pericoronitis and pericoronal abscess. They found that the nuclear details were well preserved compared to cytoplasmic details with honey and were similar to those of formalin-fixed tissues, but they found intense background staining and hyalinization of the collagen fibers, which were some of the drawbacks.

Majumdar B et al,¹³ in their cross-sectional study on fresh goat oral mucosa, compared the effectiveness of 20% honey and 30% jaggery with 10% formalin for immunohistochemical analysis, where Pan-cytokeratin and desmin markers were used. Immunohistochemical analysis of pan-cytokeratin revealed that staining with jaggery-fixed tissues (JFT) was intense, which was one of the important findings compared to HFT and formalin ($p < 0.001$), whereas staining with the desmin marker was significantly inferior for HFT compared to JFT and formalin-fixed tissues.

Another study done by Rahma Al-Maaini et al¹⁴ on rat liver and kidney tissues showed that tissues fixed in low



concentrations of honey at room temperature gave results similar to those of formalin-fixed control tissues. However, fixation in all honey concentrations at 37°C and concentrations of honey of 30% or greater at room temperature resulted in poor tissue preservation with some tissue shrinkage and was not recommended for routine histological fixation. After their experiment, they concluded that using 10% honey was a safer option than formalin and provided better results.

Honey is a natural substance that possesses antibacterial properties. This is due to its high sugar and low water content, acidity, and the enzymatic reaction that forms hydrogen peroxide. When honey is diluted, the glucose oxidase enzyme is activated, which converts glucose to gluconic acid and hydrogen peroxide, which has antibacterial effects.⁷

The main objective of our study was to check the effectiveness of honey as a fixative in the histopathology laboratory and whether it can replace formalin which is considered to be the 'gold standard' for the fixation of tissues in the diagnostic pathology. The selection of an ideal fixative is a crucial step and necessitates careful consideration, as it is the most imperative step in tissue processing. Ideal fixative imparts mechanical rigidity to the tissues to prevent decomposition, putrefaction, and autolysis as the tissue passes through various steps during processing. Though formalin is routinely used in the laboratory due to its fixative abilities, there are certain concerns related to the deleterious effects of formalin on the technicians working in the histopathology laboratory. Studies have reported that exposure to formalin can lead to health hazards such as respiratory tract and skin irritation. Long-term exposure to formaldehyde has also been linked to an increased risk of nasopharyngeal cancer, cancer of the nasal sinus, and myeloid leukemia.¹⁵ The International Agency for Research on Cancer (IARC) has classified formaldehyde as "carcinogenic to humans" and therefore represents a risk to anyone handling the solution.¹⁶ Considering these hazardous effects of formalin, there is a quest for a safer alternative for the fixation of tissues, which can preserve the histomorphology of the sections without hindering the diagnosis.

Honey being a naturally available, eco-friendly product has been shown to have fixative properties as formalin. Based on this review, we put forward that honey at low

concentrations can be used as an alternative to 10% neutral buffered formalin in the histopathology laboratory. In almost all the studies, the parameters such as nuclear staining, cytoplasmic staining, tissue morphology, clarity, and uniformity of staining were assessed. Nuclear details were better preserved than the cytoplasmic staining and were found to be comparable to 10% NBF.^{4,7,12} At low concentrations of honey (10% dilution), the connective tissue components were well demonstrated using special stains.¹¹ A few drawbacks related to the utilization of honey as a fixative could be alteration in the tissue morphology due to the fragility of the sections, leading to the breach, intense background staining, and the compromised morphology of the tissue sections that was seen in immunohistochemical studies.^{10,13} The clarity and the uniformity of the sections stained with honey were comparable to that of 10% NBF which did not hinder the diagnosis.^{4,7} Processed honey had better staining properties compared to that of unprocessed honey which exhibited slit-like spaces at the basement membrane of epithelium and homogeneous eosinophilic stroma in a few areas of connective tissue could be due to the shrinkage of tissue during fixation.⁷

The conversion of carbohydrates to gluconic acid is said to be one of the mechanisms in the process of fixation for honey. The gluconic acid produced by the dehydrogenation reaction is catalyzed by gluconic oxidase.¹⁷ The other hypothesis that is thought to play a role in the process of fixation is due to the presence of fructose/glucose in honey which at low pH breaks down to form aldehydes. These aldehydes cross-link with amino acids present in the tissue (similar to the action of formaldehyde), resulting in tissue fixation. Using honey as a fixative can preserve the structural components of tissue similar to NBF, but it is not suitable for long-term storage. Bhattacharya A et al,⁶ and Patil S¹⁸ in their comparative study found that the fixative properties of 100% jaggery syrup and 10% jaggery could be used as a natural fixative, and the staining properties were superior to honey fixed tissues.

Though all the studies indicate that the tissues fixed with honey at a low concentration, at a low pH (4-5), at room temperature for 24 hours show similar results as that of 10% NBF, there are certain limitations in the literature that needs to be taken into considerations such as smaller sample size, subjective nature of the criteria for



assessing the quality by scores. One of the potential issues with using honey in the diagnostic laboratory is that it has a high sugar content, which can lead to fermentation and crystallization. This can also cause difficulty in sectioning the tissues without causing them to break. Additionally, over time, molds may grow on the honey-fixed tissues, and its shelf life can be quite short. Hence, it should be stored in a cool, dry place, and diluted solutions should be freshly prepared as and when required in the laboratory. Also concentrated honey causes tissue dehydration and shrinkage, therefore dilution of honey is an important step, it should be sufficiently diluted, as it becomes less hypertonic on dilution with minimal or no tissue changes.¹⁴

5. Conclusion:

Evidence suggests that natural substitutes like honey can be used in the histopathology laboratory as formalin can be toxic to health. Honey is non-toxic and has similar fixative properties as formalin, preserving the histomorphologic details better when processed at low pH and low concentration. However, the use of honey in immunohistochemistry requires further exploration with studies on larger sample sizes for more conclusive results.

Table 2: Characteristics of included studies

S.No	Author/country/year	Study type	Sample size	Type of specimen	comparator	pH of the solution and time taken to fix	Stain used	Preservation of cellular morphology and outcome	Drawback
1	Özkan N et al ⁴ 2012, Turkey	Observational	7	Endometrium, breast, placenta, uterus, omentum, suprarenal, stomach, and lung.	10% NBF, alcoholic formalin	pH-4.8-5, Fixation time:24 hrs	H & E, Special stains: Gomori's one-step trichrome stain for muscle and collagen, IHC-Ki67, Vimentin	H & E and special stains: No significant difference between HFT and NBF. IHC Ki67: Mild to moderate staining with NBF Negative for HFT Vimentin:staining intensity was similar to Ki67 in all the sections. Outcome: A low concentration of pine honey can be used.	Subjective nature of the criteria for assessing the quality by scores.
2	Sirii et al ⁵ 2017, India	Observational	10	Gingiva	10% formalin	pH:4.5-5. Fixation time:24hrs	H&E, Masson's trichrome, and Van Gieson.	Nuclear staining-similar to the tissues fixed with formalin, Connective tissue : Hyalinized appearance seen in	The smaller sample size and the biopsy specimens were limited to only gingival tissues.



							IHC: Pancytokeratin, Vimentin	both H & E and special stains. IHC - similar results Outcome: Honey at low concentrations (1:10) and pH 4.5-5.0, at room temperature, fixes tissues comparably to formalin.	
3	Bhattacharyya A et al ⁶ 2018, India	Observational	40	Tongue (goat meat)	sugar syrup, jaggery syrup, and distilled water (negative control), 10% formalin (positive control)	24 hrs, 48 hrs, 72 hrs	H & E	The tissue sections displayed clear cellular outlines and excellent morphology, nuclear, cytoplasmic details, and staining quality with all three 3 fixatives. Outcome: Though honey and jaggery gave comparable results as formalin, Jaggery gave better results than honey.	Brownish discoloration (jaggery fixed tissues) and breach in the continuity of the section, intense staining with eosin, and folding of tissue sections
4	Lalwani V et al ⁷ 2015, India	Observational	36	oral epithelium, lymphoid, salivary gland, fat, muscle, and skin	10% unprocessed honey, 10% processed honey, and 10% NBF	10% unprocessed honey (pH: 3.6); 10% processed honey (pH: 5.05); 10% NBF (pH 7.2-7.4). 24 hrs	H & E	Adequate staining: Unprocessed honey n=10 (tissue sections) Processed honey- n=11(tissue sections) NBF- n=12(tissue sections) Inadequate staining Unprocessed honey n=2(tissue sections) Processed honey- n=1(tissue section) NBF- n=0 (tissue section) Outcome: Processed honey is superior to unprocessed honey and comparable to NBF	Homogenization of connective tissue is seen with honey.



5	Patil S et al, ⁸ 2015, India	Observational	42	Goat buccal mucosa	30% jaggery, 20% Honey, 10% buffered formalin	6 months (the stains were evaluated at 48 hrs, 1 week, 3 months, 6 months)	H and E, PAS, and Masson-Trichrome staining	In HFT sections, the cytoplasmic and nuclear details were satisfactory but showed areas of uneven staining and difficulty during sectioning, leading to fold artefacts. Tissue morphology was preserved in jaggery and honey fixatives for 6 months, but cell and nuclear clarity decreased. Outcome: Natural alternatives can be used for screening camps and preserving museum specimens. They can also be used in forensics for retrieving stored tissue for histological examination.	Evident cellular and nuclear shrinkage was observed with jaggery and honey as compared to formalin during the final stages of the study.
6.	Narwal A et al ⁹ , 2023, India	Observational	5 sections from each tissue	fresh chicken and fish	20% vera, 10% Formalin, 20% Honey, 30% Jaggery, 20% Sugar solution	pH-4.6, Fixation time 24 hrs	H & E	10% Formalin yielded the best results in preserving nuclear and cytoplasmic details, followed by aloe vera and sugar solution. HFT and JFT both exhibited satisfactory results. Outcome: Aloe vera can be used as a potential substitute for 10% formalin	The tissue morphology was generally poor and showed abundant foldings in HFT and JFT.
7	Mandy Gunter and Philip Bryan ¹⁰ , 2009, UK	Observational	5	mastectomy specimens (patients) 5 mm in length	10% formalin, distilled water, 10% manuka honey, 10% commercial honey,	Low pH (exact values not mentioned)	IHC - Common leukocyte antigen (RP2/18, Clone RP2/22) Cytokeratin 5 (XM26), Cytokeratin	FFT with antigen retrieval gave better results than HFT.	It is likely to obtain false negative results in the absence of antigen retrieval.



					10%local honey		in AE1/AE3 (AE1, Clone AE3), Epithelial membrane antigen (Clone GP1.4)		
8	Rahma Al-Maaini and Philip Bryant ¹¹ , 2008, Oman	Observational	Tissues comprising the liver, kidney, brain, lung, heart, bowel, stomach, spleen, and tongue were excised and dissected into small segments (3–5 mm)	Fresh goat tissues	honey dilutions of 1%, 5%, 10%, and 20% in distilled water, 10% NBF-Positive control	pH-9 to 10, at 24hrs	Van-Geison, Gordon, and Sweets reticulin, Miller's elastic stain, Masson trichrome	10% Honey gave better results compared to others. Outcome: Honey can be used as a vehicle for long-term storage of tissue samples and resections, as well as in their preservation.	Development of molds.
9	B. Sabarina et al. ¹² 2014, India	Observational	30	pericoronitis and pericoronal abscess	n=13-FFT n= 17-HFT.	pH-3 to 4, Fixation time-24 to 48 hrs	H & E	Cellular and nuclear size, tissue architecture, and connective tissue staining were the same for FFT and HFT. Despite some background staining for HFT, nuclear details were similar. Outcome: Honey can be used as a nuclear fixative and an alternative to formalin.	Subjective nature of the criteria for assessing the quality by scores.
10	Majumdar et al ¹³ 2016, India	Observational	30	Fresh goat oral mucosa	10% NBF-positive control,	pH-5.5 for 30% jaggery, 4.5 for	IHC- Pancytok eratin, Desmin	superior staining quality of the epithelial component (Cytokeratin)with 30	Fermentation and crystallization occurring due to their high sugar content



					20% honey, and 30% jaggery solutions	20% honey, and 7.2 for 10% NBF, Time – 24 hrs		% JFT when compared with HFT and formalin. Outcome: Jaggery is a better fixative than honey	
11	Rahma Al Maini ¹⁴ 2006, Oman	Observational	4	Fresh rat liver and kidney tissues	10% Neutral Buffered formalin	Ph-4, 24 hrs	H&E	Tissues that were fixed in low concentrations of honey (10%) at room temperature showed results that were comparable to those of the formalin-fixed control tissues. However, if the tissues were fixed in all (100%) honey concentrations at 37°C, or in concentrations of honey of 30% or greater at room temperature, poor tissue preservation was observed. Outcome: Low concentrations of honey can be a safe alternative to formalin for tissue preservation.	Tissue shrinkage at a high concentration of honey
12	Patil S et al. ¹⁸ 2013, India	Observational	5	Goat meat (buccal mucosa)	20% honey, 20% sugar syrup & 30% jaggery syrup with formalin as a positive control	pH:4.5-5.5, Fixation time:24 hrs	H & E	In HFT, cytoplasmic and nuclear details were satisfactory, but there were areas of uneven staining due to fold artifacts during sectioning., Outcome: Jaggery gave the best results compared to other natural fixatives.	Honey's cost and limited availability make it impractical for large-scale use.

Abbreviations: H & E - Hematoxylin and Eosin; IHC- Immunohistochemistry;NBF- Neutral buffered formalin; HFT - Honey fixed tissue; JFT- jaggery fixed tissue;FFT- formalin fixed tissues, n-number



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