Honey as a Natural Alternative for Formalin Fixative - A Systematic Review

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ABSTRACT

Fixation is the first step in histopathological tissue processing which is independently performed with 10 % formaldehyde or formalin for over many decades. The fixation of tissues is performed to retain cellular components in their respective compartments and to withstand tissue processing, avoid decomposition, putrefaction and autolysis, an ideal fixative is required to impart mechanical rigidity. Due to the increasing concern about the potential carcinogenicity of formalin, opting for more secure choices is vital. One such safe natural alternative for fixation is honey. Honey has been proven to have medicinal properties that qualify it to be used as a fixative. The aim of this study is to do a systematic review on the efficacy of honey as a tissue fixative in histopathological laboratories. The articles for this review were searched from PubMed, Google search and manual search from the year 2009 - 2019 using the keywords Honey, natural substitute, natural alternative, neutral buffered formalin, 10 % formalin, formaldehyde, tissue fixative and tissue fixation. The final of 9 articles were included in the review which compared the efficacy of honey as a natural alternative tissue fixative with the gold standard formalin. Once the articles to be reviewed were finalised, data was collected from each article, tabulated and was verified and interpreted. Honey as a fixative yielded satisfactory results with respect to cellular details and the results of maintaining the structural morphology of tissues were good. Yet, the staining properties of honey fixation did not yield an exact outcome. It, despite everything, stays to be in difficulty as differentiating results continue. Yet honey has likewise demonstrated comparable outcomes to that of formalin in histopathological tissue processing. There are also few noticeable limitations for using honey as fixative which is not observed in formalin. The formalin will continue to dominate as the best fixative in tissue processing when comparing all the parameters till newer alternatives are available for fixation. Still honey has the potential to be used as an alternative to formalin in histopathological laboratories. With an added benefit of honey being eco-friendly, easily available, cost effective, nontoxic and non-inflammable, it can also be used as an effective alternative.

KEY WORDS
Honey, Natural, Alternative, Tissue Fixative.

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BACKGROUND

Fixation in an imperative step before histopathological tissue processing for light microscopic examination in laboratories. The specimen is prepared first by 'Fixing' it using a chemical, formaldehyde and this prevents further deterioration and decay process (Autolysis) of the tissue specimen.[1] Formaldehyde was discovered by a Russian Chemist Alexander M. Butlerov in 1859.[1,2] Formaldehyde still remains as gold standard fixative in preservation of tissue specimens because of its ease of availability and its cost effectiveness. But the International Agency for Research on Cancer (IARC) classified formaldehyde as 'carcinogenic to humans'.[3] The U.S. Occupational Safety and Health Administration (OSHA, 2004) stated that the permissible exposure limit is 0.75 ppm as an 8 hour time weighted average.[4] Exposure of formalin more than this estimated value causes health ill effects such as irritation of eyes, nose, throat and allergic skin reaction.[2] Also the primary criteria for long term specimen preservation with morphology & anatomy preserved in its best possible condition is still a challenge. Hence, the need for a safe natural alternative leads to the innovative idea of usage ''Honey'' as fixative.

Honey is the natural sweet substance, produced by honeybees from the nectar of plants. Honey is a mixture of sugars and trace amounts of other compounds like chrysin, pinobanksin, vitamin C, catalase and pinocembrin.[2] Honey has been proved to have dehydrating and preserving properties similar to gold standard formaldehyde which makes it ideal to be used as an eco-friendly fixative in pathological laboratory.[2] There are also few properties exerted by honey other than fixation. It has a strong medicinal value by its antioxidant, antimicrobial, anti-inflammatory and antimutagenic effects.[5] Many evidences suggest that honey turned out to be more effective in treating wounds. This was stated by Samarghandian et al. in his study; honey and health: a review of recent clinical research.[5] With all these positive properties, many studies have attempted to explore the natural substance honey as a substitute for fixation of tissues with different concentrations.

The systematic reviews have an important role in modern health care. They are used to appraise evidence, information policy, construct guidelines and assess cost effectiveness of interventions.[6] Our primary aim was to evaluate the efficacy of honey as a tissue fixative in histopathological laboratory and reporting the systematic review. This study can establish the original impact of eco-friendly, natural and safer alternatives for gold standard fixative, formaldehyde.

METHODS

Search Strategy for Identification of Studies

The search strategy was in accordance with the Cochrane guidelines for systematic reviews. The articles included in this study were extracted from PubMed and back references of the articles till the year 2019. The internet search was also done to obtain relevant articles of our interest. The studies which assessed and compared the efficacy of honey as fixative were included in this study. The titles of articles and abstracts were reviewed. The text of the selected articles was retrieved and further analysed.

Search Methodology

The search methodology applied in PubMed was using the following keywords: (((honey) and ((((natural alternative) OR natural substitute) or alternative) or substitute)) and (((((formalin) or formaldehyde) or 10 % formalin) or neutral buffered formalin) or formol)) and (((fixative) or tissue fixative) or tissue fixation). Filters: published in the last 10 years. In addition, internet search was also done using the keywords "honey" and "safer alternatives" and "fixative" and "formalin". Articles which had used honey as natural fixative with control groups were considered for the review.

Inclusion Criteria

- Original research articles done with natural bees honey as fixative as alternative to formaldehyde were included.
- Articles published in English language were included in the review.
- Articles published in the last 10 years (2009 - 2019) were included.

Exclusion Criteria

- Studies with no control group and review articles were excluded.
- Studies published in other languages were excluded.
- Studies that used natural fixatives other than honey were excluded.
- Studies conducted with different applications of honey other than tissue fixation were excluded from the review.

Methods of Review

The initial search yielded 158 results. Additional filters were added for restraining the search to last 10 years (2009 - 2019), yielding 128 results. 124 articles were excluded based on the exclusion criteria, title and abstract screening reviews. 4 articles were approved for full text review from PubMed search and an additional of 5 articles was included from Google search, manual search & cross references. After the final full text review, 9 articles were included in this systematic review. Data was extracted from the full text articles and reviewed and extracted content. The Figure 1 presents the search flowchart.

Data Extraction

Once the articles to be reviewed were finalised, data was collected from each article, tabulated and was verified and interpreted.

Outcomes

The outcomes in this review examined and analysed the efficacy of the honey as a natural alternative for formalin fixative.

DISCUSSION

The data was extracted and synthesized from n = 9 articles. Table 1 provides a summary of the included studies. The results of the n = 9 articles showed almost similar results. In the study conducted by Amirtaksha battacharya et al.[7] analysed based on epithelial preservation, epithelial staining, connective tissue preservation, connective tissue staining for 24 hrs, 48 hrs, 72 hrs. With 100 % honey & 10 % Neutral Buffered Formalin (NBF). It was observed that formalin gave better and comparable results than honey in fixation. Statistically significant differences were obtained between honey and formalin fixative in nuclear details and cytoplasmic staining (p value < 0.01).

The study conducted by Vidushi lalwani et al.[2] analysed nuclear staining, cytoplasmic staining, tissue morphology, clarity of staining and uniformity of staining with 10 % unprocessed honey, 10 % processed honey & 10 % NBF. The nuclear staining of processed, unprocessed honey and NBF showed 100 % staining efficiency. It was observed that 92 % adequate staining in processed and unprocessed honey as compared to NBF in terms of cytoplasmic staining, 75 % adequacy in tissue morphology in processed than unprocessed honey as compared to NBF, which showed 92 %. There was no statistical significant difference between tissues fixed in processed honey and unprocessed honey compared to formalin for adequacy of diagnosis. But the assessment of artefacts showed statistical significance between 2 groups of honey and formalin. (P value = 0.004)

Shankargouda patil et al.[8,9] analysed the nuclear details, cytoplasmic details and staining qualities with 20 % honey, 10 % NBF & distilled water. The results of this study showed that cytoplasmic and nuclear details were satisfactory but showed areas of uneven staining of tissues preserved with honey. Honey was able to preserve the tissue over a period of 24 hrs. But formalin fixation after 48 hrs. Significantly showed better results than honey. The same author has done a longitudinal study over 6 months and found that the cellular and nuclear clarity gradually decreased with evident shrinkage compared to formalin. No statistical significance between tissue fixed with honey and formalin was noticed (p value = 0.563) at the end of 6 months.

The study conducted by Sri R et al.[10,11] analysed based on H&E (Haematoxylin and Eosin) staining and PAS (Periodic Acid-Schiff) & Mason trichrome & IHC (Immuno-Histo-Chemistry) with 10 % honey & 4 % NBF. It was observed that good reasonable results were obtained in tissues fixed with honey with nuclear and cellular structures maintained. Also they conducted the study using high concentrations of buffered formalin – 10 %.

The tissues fixed in bee honey gave good comparable results with that of formalin fixed tissues in maintaining the nuclear and cellular structures. No statistically significant difference was seen, suggesting that honey was equivalent to NBF fixative in all parameters (p > 0.05).

M.I Udonkang et al.[12] analysed the nuclear & cytoplasmic staining and preservation of tissue morphology with 20 %, 50 %, 70 %, 90 %, 100 % of honey & 10 % buffered formalin for 48 hrs. The results showed 100 %, 90 %, and 70 % concentrations of honey gave good intensity and clear nuclear & cytoplasmic staining with moderate preservation of tissue morphology. Statistical results showed tissues fixed in 20 % and 50 % honey showed putrefaction changes than 70 %, 90 % & 100 % honey after 72 hours and were statistically significant (p value 0.04). Minor differences in nuclear and cytoplasmic staining (p value = 0.391), intensity and clarity of histopathological details (p value = 0.252) among the honey fixed group were not statistically significant.

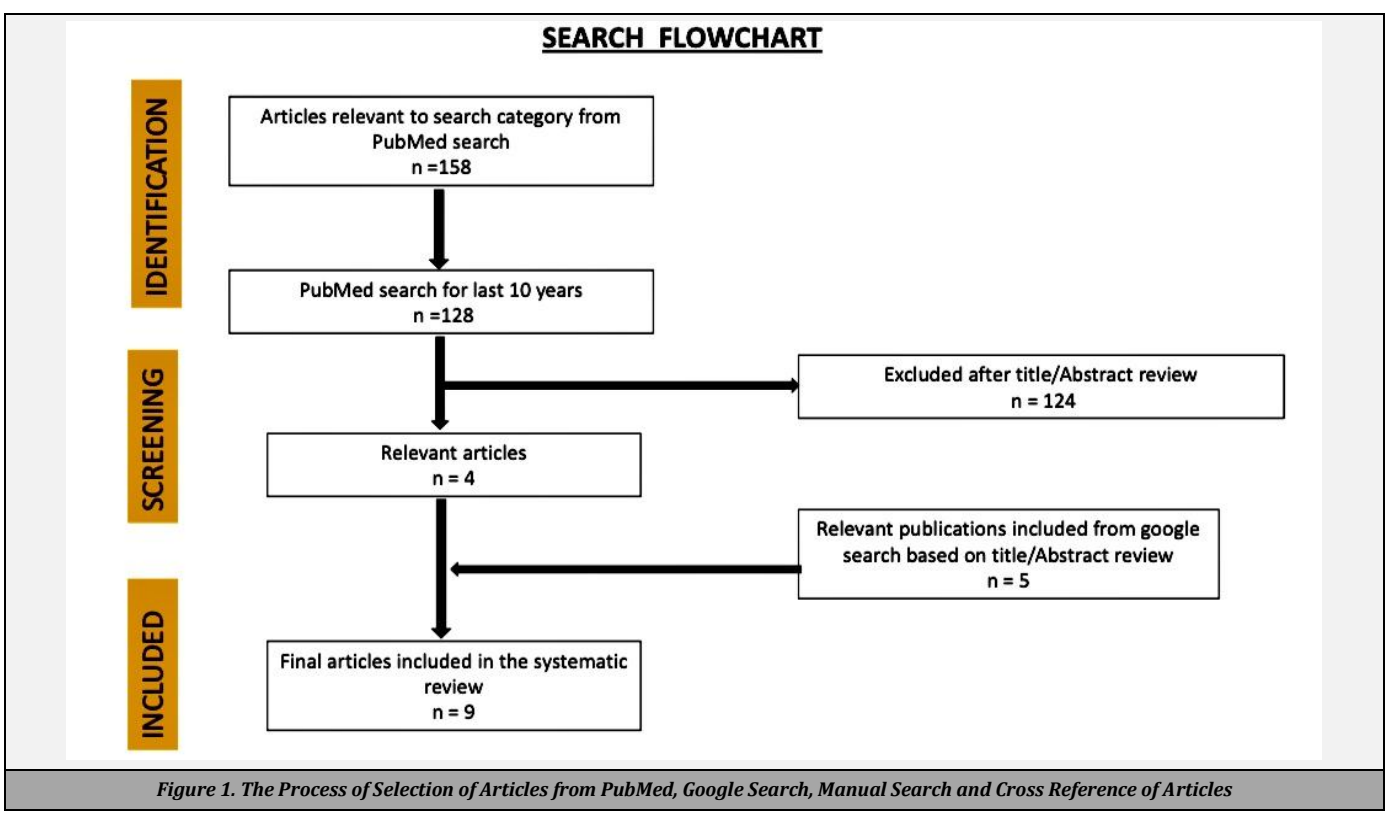

Figure 1. The Process of Selection of Articles from PubMed, Google Search, Manual Search and Cross Reference of Articles

Fu	Probing natural substitute for formalin: comparing honey, sugar and jaggery syrup as fixatives	Honey as an alternative fixative for oral tissue: an evaluation of processed and unprocessed honey	Revelation in the field of tissue preservation – A preliminary study on natural formalin substitute	Natural sweeteners as fixatives in histopathology: A longitudinal study	Bee honey as a safer alternative for routine formalin fixative	Honey as fixative & safer substitute for formalin in histology.	Bee honey as a locum for routine formalin fixative	Fixative properties of honey in comparison with formalin	Honey as a substitute for formalin?
Author	Amritaksha Bhattacharya, bhavana Gupta (…), Vivek Gupta Lucknow, India	Vidushi Lalwani, R Surekha, (…), Shamala Ravikumar Karnataka, India	Shankargoud A Patil, BR Premlatha, (…) BS Ganavi Bangalore, India	Shankargoud A Patil, Roopa S. Rao, (…), Barnali Majumdar Bangalore, India	Srii R, et al., Nepal, India	M.I. Udonkang, Kommomo A ubi, Imeobong J Inyang Calabar, Nigeria	Srii R, et al., Nepal, India	B Sabarinath, B Sivapathasun Dharam, M Sathyakumar Kanchipuram, India	N Oskan, E salva(…), B Tuzunser Istanbul, Turkey
Year	2018	2015	2013	2015	2017	2018	2016	2014	2011
Sample Size	n = 40	n = 36	n = 5	Not mentioned	n = 10	Not mentioned	n = 30	n =30	Not mentioned
Concentration of Solution	100 % honey 10 % NBF	10 % unprocessed honey 10 % processed honey 10 % NBF	20 % honey 10 % NBF Distilled water	20 % honey 10 % NBF over 6 months at interval	10 % honey 10 % NBF	20 %, 50 %,70 %, 90 %, 100 % honey 10 % buffered formalin for 48 hrs	10 % honey 4 % buffered formalin	Not mentioned 10 % formalin	10 % honey 10 % NBF Alcoholic formalin
Parameters Analysed	•Epithelial preservation •Epithelial staining •CT preservation •CT staining for 24 hrs., 48 hrs., 72 hrs.	.•Nuclear staining •Cytoplasmic staining •Tissue morphology •Clarity of staining •Uniformity of staining	•Nuclear details •Cytoplasmic details •Staining quality	For H & E staining •Cellular outline •Cytoplasmic detail •Nuclear detail •Staining quality •Overall morphology For PAS & Masson trichrome •Specificity of the stain •Staining intensity	For H & E staining •Cellular outline •Cytoplasmic detail •Nuclear detail •Staining quality •Overall morphology For PAS & Masson trichrome & IHC •Specificity of the stain •Staining intensity	•Nuclear & cytoplasmic staining •Preservation of tissue morphology	For H & E staining •Cellular outline •Cytoplasmic detail •Nuclear detail •Staining quality •Overall morphology •Specificity of the stain • Staining intensity	• Cytoplasmic details • Nuclear details •Cytoplasmic staining •Nuclear staining For 24 – 48 hrs.	For histomorphology •Cellular outline •Cytoplasmic detail •Nuclear detail •Erythrocyte integrity •Overall morphology •Staining intensity
Statistical Analysis	One-way Anova & post hoc analysis	Kruskal Wallis test & post hoc analysis	Kruskal wallis anova & kappa statistics	Kruskal Wallis Anova & Mann Whitney U test	Kappa statistics & independent 't' test & chi square test	Student t test	Kappa statistics & independent t 't' test	Student 't' test	Kruskal Wallis test & Mann Whitney U test
Results	Statistically significant results between honey and formalin for nuclear details and cytoplasmic staining.	•92 % adequate staining in processed than unprocessed honey •75 % adequacy in tissue morphology in processed than unprocessed honey as compared to NBF, which showed 92 % There was no statistically significant difference between tissues fixed in processed honey and unprocessed honey compared to formalin for adequacy of diagnosis. But the assessment of artifacts showed statistical significance between 2 groups of honey and formalin. (p value = 0.004)	K value = 0.815 The cytoplasmic details and nuclear details were satisfactory but showed areas of uneven staining. Honey was able to preserve the tissue over a period of 24 hrs. 0 % NBF gave ideal results which acted as positive control for the study.	K value = 0.563. The cellular and nuclear clarity gradually decreased with evident cellular and nuclear shrinkage compared to formalin. Nevertheless, at the end of 6 months honey were good for H&E staining	Satisfactory results were obtained with No significant differences noted in tissues fixed with honey in comparison with formalin fixed tissue.	100 %, 90 %, 70 % concentrations gave good intense and clear nuclear & cytoplasmic staining with moderate preservation of tissue morphology	K value = 0.833. The mean values of the scores given for each parameter were compared and there were no significant differences between tissue fixed with honey and formalin. The tissue fixed in bee honey gave good comparable results with that of formalin fixed tissues. Good preservation of the structure and cellular components.	The results showed statistically significant differences between honey and formalin for both nuclear details and cytoplasmic staining	The results showed significant difference in tissues fixed between alcoholic formalin & honey but significant results seen between NBF & honey. Honey fixed tissues showed weak nuclear and cytoplasmic details but better cellular morphology
Conclusion	Formalin> honey Eco friendly natural fixative honey can be used in place of formalin which showed comparable results.	Formalin > Processed honey > unprocessed honey Processed honey has better fixative properties compared to unprocessed honey.	Formalin > honey But eco-friendly natural fixatives have all novel qualities to replace formalin.	Formalin > honey Formalin fixation for 48 hrs. resulted in significantly better results when compared to honey. The quality of fixation could not be assured as the tissues fixed by honey gradually deteriorated over a period of time.	Formalin > honey Considering the novel qualities of bee honey it could be concluded that at low concentrations it could help to decrease and abolish the use of formalin.	Formalin > honey 70 – 100 % honey are suitable for long term gross preservation while 20-50 % concentrations give excellent staining characteristics.	The tissues fixed in honey showed satisfactory results compared to formalin	Formalin > honey Honey can be used as a nuclear fixative and an alternate to formalin.	10 % formalin > honey > Alcoholic formalin Honey fixation was almost similar to NBF & Alcoholic formalin.
Limitations	•Liable to moulds over time •Breach in continuity of tissue section •Intense eosin staining •Folding of sections	•Homogenization of connective tissue	•Liable to moulds over time hence usage of thymol crystals	•Growth of moulds over a period of time •Special attention is required during sectioning of honey as they tend to breach due to fragility.	Honey fixed tissues exhibited a more hyalinised appearance of the collagen fibres in H & E staining and special stains.	Not mentioned	Not mentioned	Homogenization of connective tissue.	Not mentioned

Table 1. The Table Presenting the Summary of the Articles Included in the Systematic Review

In the study conducted by Sabarinath et al.[13] cytoplasmic & nuclear details & staining for 24 - 48 hrs. were analysed with honey & 10 % NBF. It was observed that both honey and formalin showed statistically significant differences in nuclear details and cytoplasmic staining characteristics. The p value for all the parameters analysed showed statistical significance ($p < 0.05$).

In the study conducted by Oskan et al.[14] analysed histomorphology characteristics based on cellular outline, cytoplasmic detail, nuclear detail, erythrocyte integrity, overall morphology & staining intensity with 10 % honey, 10 % NBF & alcoholic formalin. The results of this study were observed to be honey fixed tissues showed weak nuclear and cytoplasmic details but better cellular morphology, but the preservation of morphology was similar to NBF. There were no significant differences ($p > 0.05$) among honey and formalin in terms of cytoplasmic details but in contrast significant differences were seen in tissue morphology ($p < 0.05$).

Honey can be used as a natural alternative for formalin because of its ease of availability. And also, honey has shown to possess many positive properties compared to other fixatives. The articles included in the study were reviewed for tissues fixed with different concentrations of honey compared with gold standard formalin based on various functional parameters. The microscopic characteristics are established by cytological and histological examination that provides diagnosis of certainty.[15] Even though the main motives for choosing these substitutes is due to their ease of availability, eco-friendly nature, non-toxicity, cost-effectiveness and minimum armamentarium required, the preservation of tissue morphology is critical to provide an accurate diagnosis without any compromise in details.[16]

Studies showed that concentrations of honey used for fixation showed variations in results. Few studies done by Sri R et al, Vidushi Lalwani et al and Naziye Oskan et al.[10,2, 14] showed that 10 % honey provided comparable results to that of formalin with slightly minor histomorphological features but that does not interfere with diagnosis. Shankargouda Patil et al. identified 20 % honey gave good staining efficacy and preservation of tissue.[9] The author Udonkang et al. also stated 20 % - 50 % honey gave excellent tissue staining characteristics which was similar to that of NBF, also the authors mentioned its statistically significant.[12] 70 % - 100 % honey was found to be suitable for long term gross preservation. Thus, variations in concentrations of honey used as fixative provides much positive results in different aspects of preservation of tissues as compared to NBF.

The cellular details include nuclear and cytoplasmic details. There is a noteworthy contrast in results between safe alternative honey and NBF. The usage of honey as fixative has produced satisfactory results for the same when compared to NBF but none of the studies proved that honey was better compared to NBF in terms of nuclear & cytoplasmic staining. Also, the cellular and nuclear clarity seem to be gradually decreased along with evident cellular shrinkage in tissues preserved in honey for an extensive period of time. But none of the studies had indicated any loss of cellular details after fixation of tissue with honey, which qualifies honey as a viable fixative and preservative for a shorter duration by the author Udonkang.[12] In contrast, Shankargouda Patil et al.[9] stated that long term preservation decreases the nuclear and cellular clarity when compared to formalin.

The structural morphology of the tissues is better preserved with honey fixation. The author Shankargouda Patil et al.[9] noticed no evident shrinkage or swelling of the tissues over a period of 6 months when compared to NBF. There seems to be no imbibition of honey into the tissues. This could be because of its thick viscosity causing no swelling and since it's a non-chemical fluid there is no chemical reaction between the tissue and the fluid to cause any shrinkage. The mechanism of honey in the process of fixation is thought to be due to the conversion of carbohydrates to gluconic acid. The gluconic acid produced by the dehydrogenation reaction catalysed by gluconic oxidase.[17] The other hypothesis which 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 the tissue fixation.[18] So honey can be opted as a fixative for preservation of structural components of tissue similar to that of NBF but not suitable for longer period storage.

The staining of tissues can be used to highlight structural components as well as to enhance the tissue contrast and tissue differentiates for better visualization under light microscopy.[19] The staining qualities include the nuclear and cytoplasmic staining intensity and clarity. The H&E staining after tissue fixation was found to be intense & clear in few studies and in contrast few other studies have shown uneven staining. Hence, the staining qualities for the tissues fixed with honey still remains to be a dilemma.

All studies have indicated consistently that formalin fixed tissues show preferred outcome over honey in every aspect. Yet honey has likewise demonstrated comparable outcomes to that of formalin in histopathological tissue processing. Thus, a natural substitute like honey which is economical, nontoxic and non-allergenic can be considered for an efficient use in laboratories.

Yet there are few limitations on using a natural honey as fixative. Liable to molds over time which causes breach in continuity of tissue section and makes the tissue fragile. There is also folding and homogenization of tissue sections. Honey fixed tissues exhibited a more hyalinised appearance of the collagen fibres in H & E staining and special stain.

CONCLUSIONS

Formaldehyde is routinely used in developing countries. Aspiration devices are rarely used. Safe disposal of toxic wastes may be non-existent or problematic. Therefore, finding and receiving another appropriate substitute such as honey with a performance which is almost similar to that of formalin helps in the elimination of formalin while fixing tissues for histopathological study.

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