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Efficacy of Honey and Local Anesthesia soft tissue stabilizing and preservative processing

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Abstract

Fixatives play an important role in tissue processing for pathological diagnosis. Using formalin as a fixative in tissue specimens is the gold standard. There exists an area of interest in search of a new alternative for formalin due to it being expensive, carcinogenic, and not easily available. Honey has various properties, like dehydrant, anti-bacterial and anti-oxidant. Local anesthetics (LA) which are available in every clinic, have minimal hazards. This study was performed to identify the effectiveness of honey and local anaesthetic solution as an alternative fixative for formalin in tissue processing.

Keywords: fixative, formalin, honey, local anesthesia, tissue fixatives

Introduction

Fixation is the most important step in 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 proper diagnosis. Formalin is the gold standard fixative, which was in use since 19th century after its preparation and standardization done by Ferdinand Blum. Formalin is considered the gold standard fixative in routine hematoxylin and eosin (H&E) procedures because it is economical, easily available; feasible and provides rapid fixation with an ease of processing. Despite all these benefits, the health and safety threats associated with formalin usage are a concern. Formalin exposure, even for a short span of time, is extremely irritating to the eyes, nose and throat and can lead to breathlessness and coughing. Long-term exposure causes serious allergic responses in the skin, eyes and respiratory tract. According to the 11th report on carcinogens by Environment Health and Safety Information (EHSI), formalin was classified as 'reasonably anticipated to be a human carcinogen.' It is also associated with nasal and

lung cancer and has a probable connection to leukemia and brain cancer. Furthermore, the International Agency for Research on Cancer (IARC) has declared formaldehyde to be a Group- II carcinogen and that it is associated with causing nasopharyngeal cancer. Occupational Safety and Health Administration (OSHA), also asserts that formalin is unsafe and encourages its replacement with less perilous substances. To overcome these hazardous effects and also to use and utilize biocompatible, cost effective, easily available, natural alternatives that retains the properties of fixatives are in search. The present study was designed with the aim to analyze the efficacy of honey and local anesthesia in fulfilling the above-mentioned quality and criteria.

The other solution preferred was Local Anesthetic solution, which is available in every dental clinic. A dental clinician often comes across with patients exhibiting oral lesions in his/her routine practice. Hence, the ability of general practitioners to diagnose a suspicious lesion necessitates a thorough knowledge regarding the application of an appropriate diagnostic aid. Definite diagnosis cannot be solely formulated only on the basis of clinical examination; hence, biopsy procedure for histopathological study becomes indispensable for confirmation of lesion. Local Anesthesia interacts with cell membrane and based on this concept; it was here attempted to use it as fixative.

With this background, the present study intended to find natural, non- toxic, easily available, economical substitute for formalin to minimize its hazardous effects and to make clinics as well histopathology lab- a biofriendly environment.

Materials And Methodology

The study was designed as a cross- sectional study and animal tissue (Goat) tongue was used for the study. The sample size was calculated using G-power software. The tissues were fixed in three fixatives medium 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 Anesthetic solution (n=20)

Sixty sections each of 1cm in size were sectioned from goat tongue. Twenty sections were kept in each of all 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 thickness. All tissue sections were stained with routine Hematoxylin & Eosin (H & E) staining and examined under microscope. All the slides were examined under following criteria's and scored individually (Table-1).

Table1: Evaluation Criteria

Features	**Scores & Criteria**	
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

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 color 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 fore mentioned criteria by individually two examiners and the values were tabulated and analyzed using Chi-square test. The following were the results analyzed and graphed based on the scoring given by two examiners: (Table- 2,3) & Graph- 1-7.

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 anesthetic fixed (n=20) (%)	x^2 p value
Cytoplasmic staining	100	90	80	.000
Nuclear staining	100	80	80	.012

Cell morphology	100	80	95	.001
Nuclear morphology	100	80	70	.000
Clarity of staining	100	70	80	.000
Uniformity of staining	100	75	75	.000

The Figures (1-3) and graphs (1-7) represents the results of three fixatives evaluation criteria, i.e the scoring and evaluation done by two examiners.

And also represents the comparison of the result of three fixatives.

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

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

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

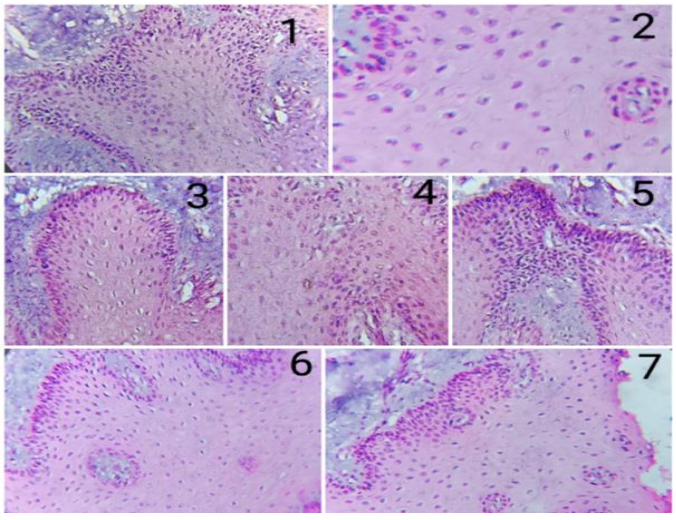


Figure 1: H & E-stained tissue section with formalin fixation 40x (1- Cytoplasmic staining; 2- Nuclear staining; 3- Cell morphology; 4- Nuclear morphology; 5- Clarity of staining; 6- Uniformity of staining; 7- Tissue architecture)

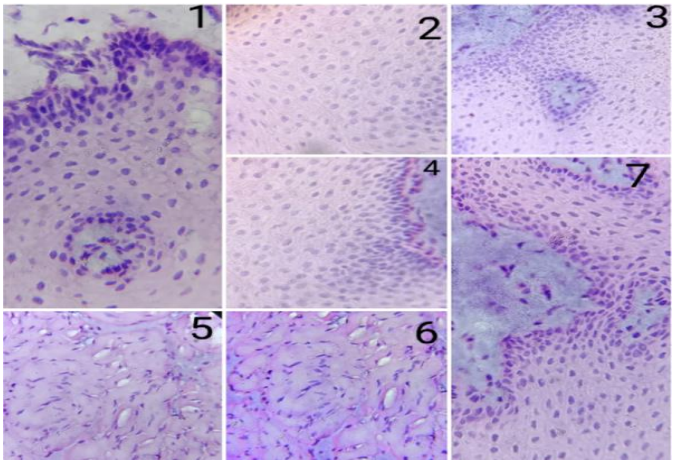
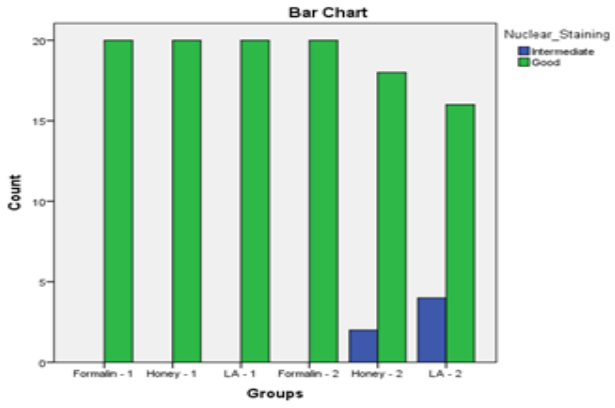
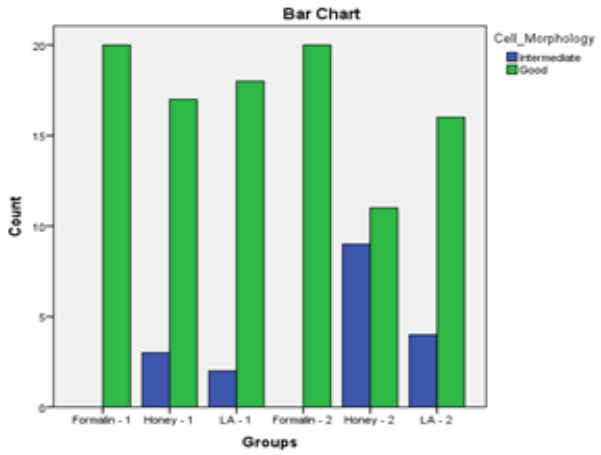


Figure 2: H & E-stained tissue section with honey fixation 40x (1- Cytoplasmic staining; 2- Nuclear staining;

3- Cell morphology; 4- Nuclear morphology; 5- Clarity of staining; 6- Uniformity of staining; 7- Tissue architecture)



Graph 1: Nuclear Staining (Formalin- 100%; Honey- 90%; LA- 80%)



Graph 2: Cytoplasmic Staining (Formalin- 100%; Honey- 80%; LA- 80%)

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

Discussion

Fixation in an essential step which is done before histopathological tissue processing for all types of microscopic examination. The specimen is first fixed by using a chemical, formaldehyde which prevents deterioration and decay process (Autolysis) of the tissue specimen. Russian Chemist Alexander M. Butlerov, in 1859, discovered formaldehyde. Formaldehyde still remains as exemplar fixative in preserving tissue specimens because of its easy availability and its cost effectiveness. International Agency for Research on Cancer (IARC) denotes formaldehyde as 'carcinogenic to humans. The U.S. Occupational Safety and Health Administration in 2004 stated that the permissible exposure limit is 0.75 ppm as an 8-hour time weighted average. Exposure of formalin more than this estimated value causes health ill effects such as irritation of eyes, nose, throat and allergic skin reaction.

Honey is the natural sweetener, produced by honeybees from the nectar of plants. Honey is a complex mixture of sugars and trace amounts of other compounds like chrysin, pinobanksin, vitamin C, catalase and Pino cembrin. Various studies in the past proved, Honey have dehydrating and preserving properties similar to formaldehyde which makes it ideal to be used as an eco-friendly natural and cost-effective fixative in patho logical laboratories. There are also few properties exerted by honey other than fixation. It has a strong medicinal value by its antioxidant, antimicrobial, anti-in flammatory and antimutagenic effects. Many evidences suggest that honey turned out to be more effective in treating wounds.

They also stated that all studies have indicated consistently that formalin fixed tissues show preferred outcome over honey in all the aspects. Honey has likewise demonstrated comparable outcomes to that of formalin in histopathological tissue processing. They recommended that a natural substitute like honey which is economical, nontoxic and non-allergenic can be considered for an efficient use in laboratories.

Bhattacharyya A et al (2018) conducted a study by fixing tissues of goat tongue in formalin as positive control, honey, jaggery, sugar syrup and distilled water as negative control in forty tissues, five tissue sections in each group. The results showed statistically significant differences between jaggery with other natural fixatives for both nuclear details and cytoplasmic staining. Their concluded that jaggery can be used as effective fixative as formalin.

Kasetty et al (2018), utilized forty soft tissue specimens obtained from 2 goat tongue were used. All the tissues were directly immersed in local anesthesia, Distilled Water, Normal Saline solution and formalin for 12 and 24 h each. They also found that there was significant difference in the efficacy of all these three fixatives.

They also recommended the usage of local anesthetic solution as an emergency fixative.

Lamubol et al (2018) used oral tissues which were obtained during impaction or tooth extraction procedures were included in the study. The tissues were sectioned and fixed in 4 different fixatives: 30% jaggery, 70% ethanol, 2% mepivacaine with 1:100000 epinephrine, and formalin for 24 and 72 hours. They concluded that fixative efficacy scores of 70% ethanol and 30% jaggery at 24 and 72 hours were not statistically different from those of formalin. Both 70% ethanol and 30% jaggery provided acceptable fixative efficacy at 24 hours. But only 30% jaggery maintained fixative efficacy at 72 hours.

Conclusion

Fixation is the most important step 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 form of Neutral buffered formalin (10%) in most of the histopathological laboratories, imposes danger as it has numerous side effects on respiratory system mainly and also affects central nervous system, skin. Various studies are being conducted by researchers to found an economical and effective alternative. We also attempted a study here using local anesthesia and honey as an alternative to formalin and recommend that it can used as indispensable/ essential fixatives at times or in case when formalin is not available or can be used a transport medium until transfer.

References

1. Jalava P, Kuopio T, Juntti-Patinen L, Kotkansalo T, Kronqvist P, Collan Y. Ki67 immunohistochemistry: a valuable marker in prognostication but with a risk of misclassification: proliferation subgroups formed based on Ki67 immunoreactivity and standardized mitotic index. Histopathology. 2006;48:674–682
2. Jalava P, Kuopio T, Juntti-Patinen L, Kotkansalo T, Kronqvist P, Collan Y. Ki67 immunohistochemistry: a valuable marker in prognostication but with a risk of misclassification: proliferation subgroups formed based on Ki67 immunoreactivity and standardized mitotic index. Histopathology. 2006;48:674–682
3. Oliver RJ, Sloan P, Pemberton MN. Oral biopsies: Methods and applications. Br Dent J 2004;196:329-33.
4. Al-Saraj A. Use of saturated sodium chloride solution as a tissue fixative. Iraqi J Vet Sci 2010;24:53-8.
5. Ficarra G, McClintock B, Hansen LS. Artefacts created during oral biopsy procedures. J Craniomaxillofac Surg 1987;15:34-7
6. M R, A RP, G S, Jk SB, Ndvn S. Transit fixatives: an innovative study. J Clin Diagn Res. 2015 Mar ;9 (3): ZM01-3. doi: 10.7860/JCDR/2015/11083.5615. Epub 2015 Mar 1. PMID: 25954725; PMCID: PMC4413175.
7. Lam-Ubol A, Kitrueangphatchara K, Putthanuparp T, Araya Khun R, Kwan thong R, Choonhawarakorn K. Nonformal in Fixative Agents: A Comparative Study of Fixative Efficacy and Histomorphology. Int J Surg Pathol. 2018 Dec;26(8):701-706. doi: 10.1177/ 106689 6918 779450. Epub 2018 Jun 6. PMID: 29873279.