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Original Research Paper

BEE Honey As A Locum for Routine Formalin Fixative

Dental Science

KEYWORDS : fixation, formalin, honey.

*** Dr. Ritesh Srii** — BDS, MDS, Lecturer, Department of Oral Pathology and Microbiology, Kathmandu University School of Medical Sciences, Dhulikhel, Kavrepalanchowk, Nepal. * Corresponding author

Dr. Vinay Marla — BDS, MDS, Lecturer, Department of Oral Pathology and Microbiology, Kathmandu University School of Medical Sciences, Dhulikhel, Kavrepalanchowk, Nepal.

ABSTRACT

Introduction: *Formaldehyde (4% buffered formalin) is the most commonly used fixative in biology. However, use of formalin causes health hazards due to its toxicity which have been reported by Occupational Safety and Health Administration (OSHA). To achieve a formalin- free laboratory for pathological specimen preservation we aimed to use a natural substitute, bee honey.*

Materials and methods: *Our study constituted of 30 histopathology tissue specimens each, which were fixed in honey and formalin. The tissues were then processed routinely, sectioned at 5µm; and stained using Hematoxylin and Eosin (H&E) stain. The slides were viewed by two independent examiners under light microscope to evaluate the staining intensity and the preservation of the structure and cellular components. The entire procedure was blind folded.*

Results: *The tissues fixed in bee honey gave good comparable results with that of the formalin fixed tissues microscopically; displaying good preservation of both nuclear and cellular structures, and staining qualities for the routine haematoxylin and eosin stain.*

Conclusion: *It can be concluded that in the near future, carcinogenic fixatives like formalin could be eliminated and replaced by natural fixatives like honey which gives satisfactory results.*

INTRODUCTION

An essential part of all histological and cytological techniques is preservation of cells and tissues in their natural state. In order to ensure this, tissues or cells are subjected to a process called fixation which involves a complex series of chemical events by which biological tissues are preserved from decaying, thereby preventing autolysis or putrefaction and this process differs for different groups of chemical substances found in the tissues (Bancroft JD, 2001; Nowacek JM, 2003; Bosetti C, 2007; Wood MF, 2011).

The field of tissue fixation and processing has not shown any significant changes in the past 100 years. Formaldehyde, employed as 4% buffered formalin, has remained the most commonly used fixative universally, largely because of its ease of use and applicability across a range of tissues, economic viability, international acceptance and also because its preparation requires less time. On the other hand, formalin also has disadvantages like toxicity which can affect the health professionals. This has gained the attention of two agencies like U.S. Environmental Protection Agency (EPA) and the International Agency for Research on Cancer (IARC) who have classified formalin as a probable human carcinogen with potential toxicity (Cristina Zanini, 2012). In recent years, attempts have been made to find safer alternatives. This is motivated by the Occupational Safety and Health Administration (OSHA) regulation standards, declaring formaldehyde as hazardous and advocating it's substitution with less dangerous substances (Mariani-Costantini R, 2011).

For centuries, honey has been thought to have antibacterial properties with the additional potential to preserve compounds without causing any harmful effects on its users. Mellified man, or human mummy confection, was a legendary medicinal substance created by steeping a human cadaver in honey. In ancient Rome, honey was used to preserve meat for several days. Antibacterial properties of honey are the result of the low water activity causing osmosis, hydrogen peroxide effect, and high acidity. This combination of high acidity, hygroscopic and antibacterial effect makes honey a plausible way to turn human cadaver into a mummy. (Bogdanov S, 2009; Mariani-Costantini R, 2011; Kwakman PHS and Zaat SAJ, 2012).

After considering these properties, we aimed to use honey as a fixative agent for the preservation of tissue specimens; and to study their cellular and structural characteristics by using routine stain and to compare its effectiveness with the currently, universally accepted formalin fixation.

MATERIALS AND METHODS

This study was conducted with a sample size of 30 tissue specimens in each group. Ethical approval was obtained from the institutional review board prior to conducting the study. Tissue specimen were biopsied from gingiva and pericoronal region following which they were cut into two halves; each half placed in two separate groups, group A (working solution) - where the tissue specimens were fixed with diluted form of honey and group B – where tissue specimens were fixed using 10% formalin.

Agmark graded pure Coorg bee honey was used in this study which is commercially available. Fresh working solution was prepared just before the biopsy procedure by using honey and water in the ratio of 1:9. Tissue specimens were kept in the respective solutions for a period of 24 hours at room temperature after which it was taken through routine tissue processing. The processed tissues were embedded in paraffin wax; and 5µm thick sections were prepared and stained using H & E. The tissues were examined by two oral pathologists under light microscope and the whole procedure was blind folded. The histomorphological criteria examined are enlisted in Table 1. Inter-Observer variability was determined using Kappa statistics.

Table- 1: The histomorphological criteria and evaluation are detailed below

Histomorphologic criteria	Rate on a scale of 1-4
For H& E stain Cellular outline Nuclear detail Staining quality Overall morphology Specificity of the stain Staining intensity	 Poor Satisfactory Good Excellent

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Composition of working solution:
Concentrated form of Coorg honey- 10ml

Distilled water - 90ml

Preparation of working solution:
10 ml of concentrated form of honey was mixed with 90ml of hot water. This prepared solution was then allowed to cool and pH was maintained at 4.5-5.0; and the tissues were immediately immersed in it.

OBSERVATION AND RESULTS
The biopsied samples were observed by two independent examiners under light microscope and the procedure was blinded. The scores given by the two observers were compared for inter-observer variability using Kappa statistics. A Kappa value of 0.833 suggested a high agreement between the observers. So the scores given by the first observer were considered.

The tissues fixed in bee honey gave reasonably good results with nuclear and cellular structures maintained, for both H & E. Both the general stain uptake and the maintenance of general tissue architecture including nuclear and cytoplasmic size, were comparable to formalin fixed tissues, except that the collagen fibres in honey fixed specimen had a more hyalinised appearance (Figure 1). The scores given for the various parameters in the two groups showed a normal distribution (Table: 2). The mean values of the scores given for each parameter were compared using the independent 't' test; where there was no significant differences (p>0.05) for all the variables (Table 3).

Figure 1: Photomicrograph of H & E stained tissue sections fixed in honey at magnification of 10x(A) and 40x(B) showing good cellular morphology with proper nuclear and cytoplasmic details.

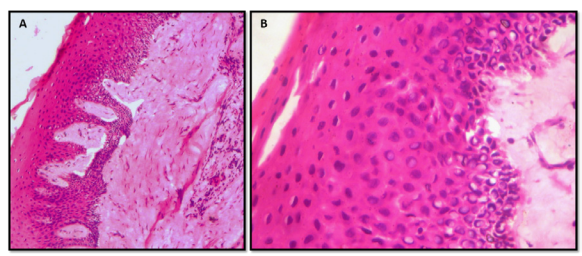

Table 2: Kolmogorov-Smirnov test for determining normality of data.

GROUP		CELLULAR OUTLINE	NUCLEAR DETAIL	STAINING QUALITY	OVER-ALL MORPHOLOGY	EASE OF SECTIONING	SPECIFICITY OF STAIN	STAINING INTENSITY
HONEY	MEAN	3.70	3.13	3.80	3.73	3.23	3.83	3.07
	Std dev	0.466	0.346	0.407	0.450	0.430	0.379	0.254
FORMALIN	MEAN	3.83	3.17	3.93	3.90	3.23	3.87	3.10
	Std dev	0.379	0.379	0.254	0.305	0.430	0.346	0.305
p- value		0.952	1.000	0.952	0.799	1.000	1.000	1.000

Table 3: Independent t test for comparing the scores between honey and formalin for H & E stain.

Parameter	Mean difference	p- value
CELLULAR OUTLINE	-0.133	0.229
NUCLEAR DETAIL	-0.033	0.723
STAINING QUALITY	-0.133	0.134
OVERALL MORPHOLOGY	-0.167	0.098
EASE OF SECTIONING	0.000	1.000
SPECIFICITY OF STAIN	-0.033	0.723
STAINING INTENSITY	-0.033	0.647

DISCUSSION
Honey has been used as a medicine for thousands of years and its curative properties have been well documented. Honey has been shown to inhibit the growth of a wide range of bacteria, fungi, protozoa and viruses. The antibacterial and anti-oxidative effect of honey depends on its osmotic effect, acidity, and because of components like hydrogen peroxide and phenol inhibine formed by enzymatic reaction and phytochemical factors (Kwakman PHS and Zaat SAJ, 2012).

The tissues fixed in low concentrations of honey at room temperature gave comparable results to formalin fixed ones. Properties of honey such as high osmolarity, low pH and the presence of components such as hydrogen peroxide and phenol inhibine, all contribute to its anti-oxidative and antibacterial effects (White JW, 1963; Molan PC, 1992; Molan PC, 1998; Rahma A and Bryant P, 2006).

Apart from its antibacterial properties, honey has been found to prevent decay in tissues immersed in it for up to 30 days without showing any signs of autolysis. The tissue

hardening property makes its action similar to other fixatives which act by cross linking the proteins.

Honey when diluted generates hydrogen peroxide owing to inactivation of the enzyme, glucose oxidase, which oxidizes glucose to gluconic acid and hydrogen peroxide. Gluconic acid is non-corrosive, mildly acidic, less irritating, non-odorous, non-toxic, easily biodegradable, non-volatile organic acid (Ramachandran S, 2006). The possible mechanism of action 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 catalyzed by gluconic oxidase enzyme is thought to have wide applications in food and pharmaceutical industry as preservative and in the process of pickling of food; as gluconic acid prevents the putrefaction of the food and has antiseptic properties also (Ramachandran S, 2006).

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 (Ramachandran S, 2006; Mandy G and Philip B 2009).This mechanism is somewhat similar to the HOPE (Hepes-glutamic acid buffer-mediated organic solvent protection effect) technique which consists of incubation of fresh tissues in a protective solution comprising of a mixture of amino acids at pH 5.8 to 6.4. This solution is thought to penetrate the tissues by diffusion (Srinivasan M, 2002).

In our study, we got satisfactory results with tissue fixed in honey, in comparison with the formalin fixed tissue. It has been said that honey that is not well filtered may contain various artefacts in it, including viable spores such as clostridia which may cause false positive reactions. But we used Agmark certified pure honey, which did not result in any such artefacts.

The use of formaldehyde in developing countries, where aspiration devices are rarely used and safe disposal of toxic wastes may be non-existent or problematic, combined with the known health hazards should be terminated. This makes it an ideal time for finding and adopting a new suitable substitute such as honey, with action similar to that of formalin and which has the ability to give comparable results, thus aiding in the elimination of formalin in future scientific fields.

CONCLUSION

A natural substitute like honey which is economical, nontoxic and non-allergenic can be a boon, when health hazards of formalin are considered. Honey can be a suitable replacement as a fixative for the surgical pathology diagnostic work. In our study, preservation of tissue integrity by fixing the tissue in honey gave comparable results with good cellular morphology and characteristics, with the fixing time being similar to that of formalin. So considering this property of honey, it can be concluded that use of bee honey at low concentrations can be a positive step towards abolishing or significantly decreasing the use of formaldehyde in the near future.

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