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RESEARCH ARTICLE

ECO-FRIENDLY NATURAL FIXATIVES – A SUBSTITUTE FOR FORMALIN?

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ABSTRACT

Fixation is the critical step in the preparation of tissues for routine histopathology in which Formalin is the universal fixative. The fundamental advantage stems from its continuous and almost universal use for over 100 years and all the accumulated scientific knowledge on it. Also, formalin is readily available, economical, fairly convenient to store, allows long-term storage, preserves lipids well, and has been accepted the perfect fixative, with no clear "all purpose" alternative found to date. However, formalin has well known disadvantages like high toxicity and classified as a human carcinogen. It binds severely to DNA, RNA and proteins, which makes them difficult or impossible to extract in a useful form for molecular tests. However we explored more economical, eco-friendly and readily available substances like sugar, honey and jaggery which have shown to have the potential to preserve compounds without any harmful effects. They are advantageous in that they are non- hazardous, compatible with routine processing, staining, do not require additional equipments and are easily available at any place. Hence the present study is undertaken with an aim to compare the tissue fixation abilities of honey, sugar syrup and jaggery syrup with that of formalin followed by H & E staining in order to determine the best fixative.

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INTRODUCTION

Formaldehyde was first discovered by Russian chemist Alexander M. Butlerov in 1859. Ferdinard Blum, (2014) has been credited as the first person to use formaldehyde as a tissue fixative. This fixative has been used for 150 years and represents an optimal compromise (Sabarinath, 2014). It is widely used for preservation of the morphology, antigenicity and molecular characteristics of most tissues, and is accepted by most pathologists after standardization of protocols (Gatta, 2012). However, it is toxic and was classified as carcinogenic to humans. It also binds to DNA, RNA and proteins, in such a way that it makes them difficult or impossible to extract in a useful form for molecular tests. There are issues, however, that may be improved by an alternative fixation method, such as natural fixatives. Literature revealed many economical, eco-friendly and readily available substances like honey, sugar and jaggery. These substances have the potential to preserve compounds without any harmful effects. They have the advantage of being non- hazardous, easily available and which do not require additional equipments (Patil, 2015).

Aim: The purpose of this study was to investigate the effects of different fixatives on the characteristics of tissues sent to the laboratory of pathology.

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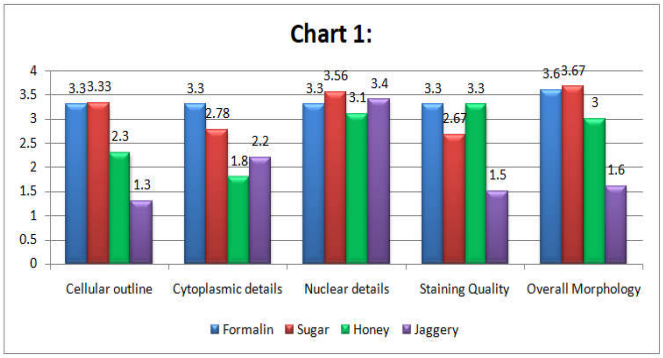
The tissues used in this study were sampled and fixed with four different fixatives like formalin, sugar syrup, honey syrup and jaggery syrup for 24 hours and to compare the tissue fixation abilities of these fixatives with that of formalin followed by H and E staining to determine the efficacy of these fixative.

MATERIALS AND METHODS

The study group included 40 samples obtained from patients who reported to the outpatient Department of Oral and Maxillofacial Surgery. The study included 4 groups, namely the formalin, honey, sugar and jaggery groups respectively which consisted of 10 tissue specimens each. Each bit was placed in four different containers containing 10% buffered formalin, 20% honey, 20% sugar and 30% jaggery syrup. 24 hours tissue fixation was attained at room temperature followed by conventional processing and staining with H & E. The tissue sections were assessed by two examiners for cytoplasmic, nuclear details and staining quality under light microscopy and the whole procedure was blinded. Each criteria was rated on a scale of 1- 4 and are elaborated in Table 1. The values were obtained compiled and analysed using ANOVA test.

RESULTS AND OBSERVATION

Mean values for tissue sections fixed with formalin, honey, jaggery and sugar were analyzed using ANOVA test. The values were high for tissues fixed in formalin, sugar and honey (Graph 1).



Graph 1. Mean values of different fixatives

Table 1: Histomorphological criteria

Histomorphologic Criteria	Rating
• Cellular outline • Cytoplasmic detail • Nuclear detail • Staining quality • Overall morphology	Each histomorphologic criteria was rated on a scale of 1- 4 • Poor • Satisfactory • Good • Excellent

The tissue fixed in formalin gave the ideal results which acted as the positive control for the study (Fig 1). On the other hand, interestingly tissue fixed with sugar syrup (Fig 2) showed a good overall morphology, nuclear and cellular outline than formalin fixatives except cytoplasmic details and staining quality. Similarly, tissue fixed with honey (Fig 3) showed good nuclear and staining quality but poor overall morphology, cytoplasmic and cellular outline. In our study, the tissue sections with jaggery fixation (Fig 4) showed significant cellular swelling and tissue autolysis. Hence all three natural substances were able to preserve the tissue over a period of 24 hours with sugar and honey giving the best results when compared to jaggery syrup. To sum up the overall results, the tissue fixation ability was in the following order: Formalin > Sugar > Honey> Jaggery Photomicrograph of the tissues fixed in: Fig 3: Honey, Fig 4: Jaggery (H & E, 40X)

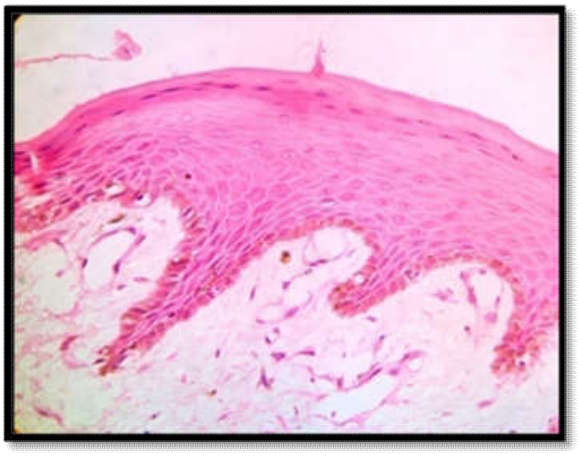


Figure 1: Formalin

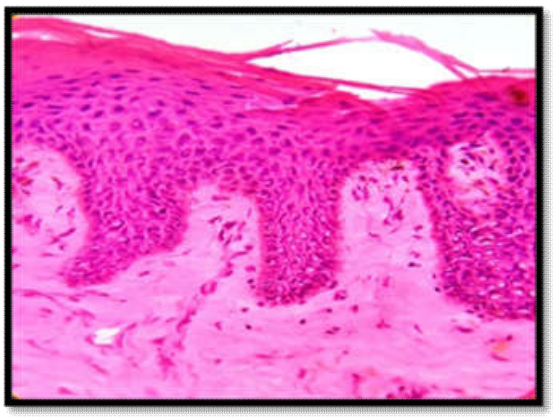


Figure 2: Sugar

Photomicrograph of the tissues fixed in: Fig 1: Formalin, Fig 2: sugar (H & E, 40X)

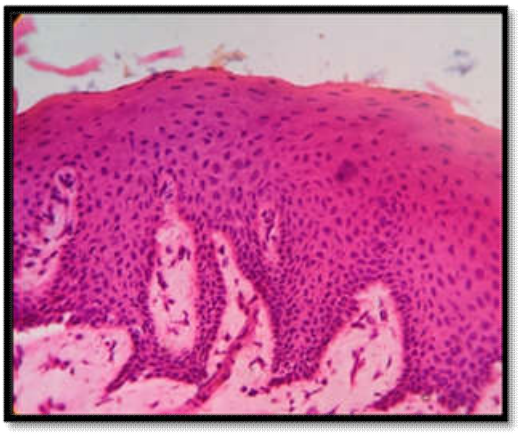


Figure 3: Honey

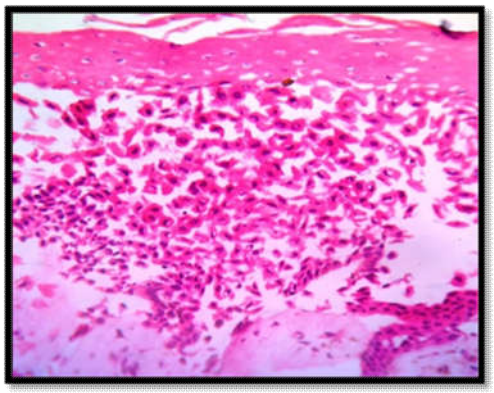


Figure 4: Jaggery

DISCUSSION

The aim of fixation is the preservation of cells and tissue constituents in a condition identical to that existing during life and to do this in a way that will allow the preparation of thin, stained sections. In practice, the purpose of fixation is:

- To prevent or arrest autolysis and bacterial decomposition and putrefaction,
- To coagulate the tissue as to prevent loss of easily diffusible substances,
- To fortify the tissue against the deleterious effects of the various stages in the preparation of sections, and To leave the tissues in a condition which facilitates differential staining with dyes and other reagents (Drury, 1980).

The speed of fixation depends on the rate of diffusion of fixative into the tissue and the rate of chemical reactions with various components. In practice, it is assumed that these processes require at least 1 hour/mm of tissue thickness, but routinely the tissues are fixed for 24 to 48 hours (Srinivasan, 2002). Ideally, the specimen should be placed in a larger volume of the fixative that is ten times the volume of the specimen and the fixative must surround the specimen on all sides (Satyakumar, 2013). Fixation is a complex series of chemical events, and differs for the different groups of chemical substances found in tissues (Bancroft, 2002). Neutral buffered formalin has been the standard fixative in histopathology for many decades; however, new technologies and increasing time constraints have made this common fixative less widely applicable (Dapson, 1993). The most common fixative that is being used throughout is formalin. Formalin is a solution containing methylene glycol and a little formaldehyde. The mechanism of fixation takes place by the formation of intermolecular bridges. Normal formalin fixation takes place in three steps: First, the methylene glycol quickly penetrates the tissue; second, some methylene glycol is slowly converted to formaldehyde by dehydration; third, formaldehyde binds very slowly to the proteins in the tissue by cross linking. Various factors that affect fixation are the size and thickness of the tissue, presence of mucous, blood and fat, the amount of agitation, and the temperature maintained. Apart from formalin fixation, different methods have been tried to demonstrate proper fixation (Satyakumar, 2013).

Other alternative for fixatives are:

- Aldehydes (formaldehyde, glutaraldehyde, glyoxal),
- Oxidizing agents (osmium tetroxide, potassium permanganate, potassium dichromate),
- Other cross – linking agents (carbodimides), Physical (heat, microwaves)

Miscellaneous -- mercuric chloride, picric acid, non - aldehyde - containing fixatives, dye stuffs (Bancroft, 2002). All tissues must be adequately supported before they can be sectioned for microscopical examination. Permanent tissues are more commonly taken through a series of reagents and finally infiltrated and embedded in a stable medium which when hard, provides the necessary support for microtomy. This treatment is termed tissue processing (Drury, 1980). However, formalin has well known disadvantages. The International Agency for Research on Cancer has evaluated that there is sufficient evidence for the carcinogenicity of formaldehyde both in humans and in experimental animals. Formaldehyde is therefore considered to be carcinogenic to humans. It is highly toxic and classified as a human carcinogen therefore, represents a risk to anyone handling the solution (Wakefield, 2008; Moelans, 2011). Each fixative has advantages and disadvantages and does not fulfil all the aims of tissue preservation which includes molecular loss in fixed tissues, swelling or shrinkage of tissues during the processing, variation in histochemical and immunohistochemical staining. Many tissue components are soluble in aqueous acidic or other liquid environments. To minimise the loss of various molecular and macromolecular components including proteins, peptides, mRNA, DNA, lipids, cytoplasmic membranes, rough endoplasmic reticulum, nuclear membranes, mitochondria etc, there is a need to develop universal ideal fixative. A good fixative should be able to destroy the infectious agents within, thereby minimizing the enzymatic degradation and prevent breakdown of tissue (autolytic cystic changes) during long term storage (Pal, 1999). An ideal fixative should fix the tissue as quickly as possible because delay in fixation results in loss of mitotic index by 30-50%. The fall in mitotic index may result in errors in the grading carcinomas. Lastly it should have long shelf life, compatible for wide variety of tissues, easily disposable and support long term tissue storage (Cross, 1990). Therefore, we explored more economical, eco-friendly & readily available substances like honey, sugar & jaggery syrup. In this study, to standardize the dilution of sugar, honey and jaggery by using different conc. (10%, 20%, 30% and so on). Hence we considered 20% for our study and pH of all the three substances ranged between 4.5 to 5.5 which were in favour of fixation. In our study, the tissue sections with jaggery fixation showed significant cellular swelling and tissue autolysis which are not in accordance with the results obtained by Patil et al who noted a good overall morphology, nuclear, cytoplasmic and staining quality. In our study, among natural fixatives, sugar syrup and honey syrup showed better results when compared to the jaggery syrup.

Conclusion

The search for an alternative to formalin fixation, which offers better technical performance and greater protection for health workers, is unavoidably needed. Although, extensive research has to be done on eco-friendly fixatives and should be implemented in routine histopathology. Natural substitutes like sugar, honey and jaggery are a boon when health hazards of formalin are considered. To sum up the overall results, formalin, sugar and honey showed more equivalent results when compared to jaggery. Thus we conclude that the eco-friendly natural fixatives have the novel qualities and can be used as a safe alternative to formalin in histopathology.

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