



EVALUATION OF TISSUE FIXATION USING ORGANIC SUBSTITUTES – A COMPARATIVE STUDY

Oral Pathology

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ABSTRACT

Fixation of tissue is an initial and important step in tissue processing in microscopical examination. It is a critical step in the preparation of tissues. Fixation is considered to preserve the tissues in a life-like state, prevent bacterial putrefaction, prevent autolysis and to increase the refractive index of the tissue. The present study used a natural fixative instead of chemicals in order to prevent the deleterious effects of the chemical fixatives. The aim of the study is to compare the tissue fixation ability of Apple cider vinegar and Honey Lemon Ginger Tea extract with that of formalin using H and E stain and to determine the best fixation among the two. Tissue sections were assessed for cytoplasmic, nuclear details and staining quality under light microscope. Results were analysed by Kruskal Wallis ANOVA. Preservation of tissue by Apple cider vinegar gave good results than honey ginger lemon tea after comparing with formalin.

KEYWORDS

Formalin, Fixation, H and E, Apple cider vinegar, Honey lemon ginger tea

INTRODUCTION

In microscopical examination, fixation of tissue is an initial and important step in tissue processing. In histopathology, it is a critical step in the preparation of tissues. Fixation is considered to preserve the tissues in a life-like state, prevent bacterial putrefaction, prevent autolysis and to increase the refractive index of the tissue. The most commonly used fixatives are aldehydes. They provide to become stable the structural details of cells and tissues prior to examination by light or electron microscopy (1).

The recent fixative of choice is formalin. Initially, the mechanism of formalin fixation was based on the formation of cross-links. Formalin has been recognized as a fixative for routine surgical pathology, and most clinicians are attentive of it by its pungent odour (2).

For tissue stabilisation and a number of histological staining, organic and inorganic reagents have been used successfully. One of the most commonly used fixatives in histology is still formalin, i.e. formaldehyde dissolved in aqueous solutions. A biopsy is most often the only way to diagnose oral lesions and diseases (3).

Formaldehyde is a highly toxic systemic poison that is absorbed well by inhalation. The vapour is a severe respiratory tract and skin irritant and may cause dizziness or suffocation. Contact with formaldehyde solution may cause severe burns to the eyes and skin (4).

Apple cider vinegar (ACV) is recently one of the seriously talked about topic amidst the home remedy seekers. Hippocrates formulated a mixture of honey lemon ginger tea and apple cider vinegar for treatment of various diseases. Apple cider vinegar is a subgroup in the vinegar family. Vinegar is generally produced from fruit juices such as grape, apple, plum, coconut, and tomato; rice and potato. While apple cider is made from overwhelming apples and then bacteria and yeast are added to the liquid to start the alcoholic fermentation process. The sugars are converted to alcohol and in the following second fermentation phase, the alcohol is converted into vinegar by acetic acid-forming bacteria (Acetobacter) (5).

Ginger, lemon and honey tea is a revitalizing beverage often used to help relieve cold and flu symptoms. All these ingredients have their own natural medicinal qualities and when they are combined, they create a calming and relaxing tea that can ease symptoms and boost your immune system. Ginger, lemon and honey tea can provide other health benefits, too (6).

Thus, due to apple cider vinegar's and honey ginger lemon tea easy

application and low cost and toxicity, this study aimed to assess the tissue fixation using organic substituent's. To simulate its effect as an alternative therapy for formalin fixation.

MATERIALS AND METHODS :

Thirty tissue samples were obtained from normal gingiva i.e. from gingivectomy (non-idiopathic) patients, cut into three pieces and placed in 10% formalin, Apple cider vinegar, Honey ginger lemon tea. 24 hours tissue fixation was attained at room temperature followed by conventional processing and staining. Tissue sections were assessed for Cellular outline, nuclear details, staining quality and overall morphology under light microscopy. Each criteria was rated on a scale of 1 - 4 (Excellent, Good, Average and Poor) and whole procedure was blinded. Results were analysed by Kruskal Wallis ANOVA test. The histomorphological criteria examined are elaborated below.

HISTOMORPHOLOGICAL CRITERIA

Histomorphologic Criteria is based on following details.

1. Cellular outline
2. Nuclear details
3. Staining quality
4. Overall morphology

Each histomorphologic criteria was rated on scale of 1-4

1. Excellent
2. Good
3. Average
4. Poor

RESULTS

A combined comparison was carried out between the mean value of Formalin and its natural substitutes (Apple cider vinegar, Honey ginger lemon tea and Formalin) by Kruskal Wallis ANOVA non parameter test. The highest mean score of 2.62 was obtained for tissue section fixed with 10% formalin which acted as a positive control. Followed by Apple cider vinegar showed good Cellular outline, Nuclear detail, Staining quality, Overall morphology.

With Apple cider vinegar fixation, the tissue section showed a fair cytoplasmic details, with mean score of 2. The lowest mean score of 1.15 was obtained for tissue section fixed with honey ginger lemon tea. It showed significant cellular swelling and poor staining with H & E (autolysis). Thus out of 2 natural substitutes, Apple cider vinegar was superior.

DISCUSSION

Formalin has long been universal standard fixative in histopathology

& clinical routine worldwide. The fundamental advantage 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 suitable to store, allows long-term storage, preserves lipids well, and has been accepted as the closest thing there is to the perfect fixative, with no clear "all-purpose" alternative found to date. On the other hand, formalin has two well-known disadvantages. Firstly, formalin is highly toxic. The International Agency for Research on Cancer (IARC) classifies formaldehyde as a human carcinogen that can cause nasopharyngeal cancer (7).

Lu *et al* found strong evidence that can support a genotoxic and cytotoxic mode of action for the carcinogenesis of inhaled formaldehyde in respiratory nasal epithelium (8).

In the present study, we use Bragg's Apple Cider Vinegar because it is made from certified organic raw apples and is unfiltered, unheated, unpasteurized with 5% acidity. It contains the floating "mother" which occurs naturally as strand-like enzymes of connected protein molecules. It has a antibacterial, antifungal, antiviral properties and cytotoxicity aspect.

For centuries, honey has been shown to be a successful antibacterial agent having the potential to preserve compounds without any harmful effects on users. 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. Later, several studies proved that honey can be a safe alternative to formalin in conventional histochemical and immunohistochemical staining methods (9)

Since honey is not universally available and it is impractical to use honey in large scale due to its high cost, sugar and jaggery have started being chosen as a substitute for formalin.

S. Patil to compare preservation properties of formalin with that of natural products like honey, sugar syrup and jaggery syrup, and found that out of the three natural products tissue fixation ability was good in jaggery syrup followed by honey and sugar syrup (3). So in our study we choose a concentration of apple cider vinegar and Honey ginger lemon tea for tissue fixation comparable to formalin and found that both Apple cider vinegar gave good results according to the histomorphological criteria i.e. very close to formalin to fix the tissue.

CONCLUSION :

Natural substitutes like Apple cider vinegar and Honey lemon ginger tea which are easily available with no known toxicity are a boon when health hazards of formalin are considered. Among the two natural fixatives investigated, Apple cider vinegar has really all novel qualities to be an excellent substitute for formalin in tissue fixation.

In our study we concluded that, when the preservation of tissue by Apple cider vinegar and Honey lemon ginger tea was compared to that of formalin, Apple cider vinegar showed better preservative result over a period of 24 hours than Honey lemon ginger tea.

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