General Medicine, ISSN: 1311-1817, VOLUME 27 ISSUES 4, Page: 2124-2131
Journal link: https://general-medicine.org
Abstract Link: https://general-medicine.org/abstract-2124-2131/
November 2025

REVOLUTIONIZING CONNECTIVE TISSUE DEMONSTRATIONS: EMBRACING NATURAL SOLUTIONS SUCH AS DATE SYRUP AND HONEY AS SUBSTITUTES FOR FORMALIN

Submission: 25 July 2025 | **Acceptance:** 20 September 2025 | **Publication**: 26 October 2025

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Abstract:

Objective: Formalin, sometimes referred to as formaldehyde, is a fixative that is frequently employed in anatomical research to preserve biological materials. However, since it causes cancer, it poses a health risk. Natural alternatives, such as date syrup and honey, which have historically been employed as antibacterial and preservative substances, were taken into consideration to solve this issue. These compounds have been investigated for their potential as formalin substitutes because of their acidic and dehydrating qualities. The purpose of the study was to determine if date syrup and honey may serve as natural fixing agents instead of formalin in lab settings.

Methods: The researchers generated varied amounts of date syrup and honey depending on their pH, glucose, and fructose levels. After anesthetizing albino rats using chloroform vapor, they removed their liver, kidney, heart, and muscle cells. A 10% solution of formalin was employed as a control after the tissues had been cleaned and preserved in mixtures of date or honey syrup that varied in intensity. The cells were examined over a period of ten days, and following staining, a panel evaluated and rated the integrity of the tissues.

Results: As their effectiveness did not differ substantially (P-value >0.05) from 10% formalin, the study's results suggest that higher amounts of 65 percent to 85 percent date syrup and honey are appropriate for long-time gross preservation of organs and tissues. The properties of good tissue staining are achieved at lower concentrations of 45%.

Conclusions: To reduce and eventually eliminate the usage of formalin, modest amounts of date syrup and honey (65 percent and 45 percent, respectively) may be used as substitutes.

Keywords: formalin, syrup, histopathology

Introduction:

All histopathological and cytological studies place a high priority on maintaining the anatomic material in its original condition. This process involves a sequence of chemical events that stop autolysis, putrefaction, and the deterioration of cells and tissues. The essential and overriding goal of tissue fixing agents in pathological diagnosis is to maintain cells and tissue constituents [1]. Fixation causes a molecular change in the tissue that makes sectioning and staining simpler while keeping structure. Tissue fixation has not made any significant improvements during the last century. The most common fixing agent in anatomic pathology laboratories for routine pathological and immunohistochemical diagnosis is formaldehyde (10 percent buffered formalin). It is widely utilized, simple to apply, and suitable for a range of tissues. Additionally, it is affordable, well-known on a global scale, and requires minimal preparation time. [2] There is a health danger when formaldehyde is inhaled repeatedly or for a long period into the upper respiratory system, nose, or eyes. There have been efforts to create environmentally friendly, safer alternatives by replacing formaldehyde with less dangerous compounds. Non-formalin fixing agents don't include any aldehyde elements, hence there's no risk of toxicity concern. Organic substitutes currently have more potential due to their advantageous qualities. The research found that date syrup and honey's antibacterial properties are dehydrating and acidic. Research has emphasized honey's anti-autolysis and tissue-strengthening effects as well as date syrup's auto-preserving qualities in addition to its ability to treat injuries and fight microbes [3]. These solutions comprised honey solutions with a 10% ethanol base and honey solutions with a 10% water base. In the present investigation, we employed date syrup and honey as fixing components to preserve tissue specimens. Employing stains and various date syrup and honey concentrations, we investigated their cellular characteristics. To overcome formalin's toxic effects, this research used naturally occurring substances like date syrup and honey as substitutes [4].

Methods:

Study Design: The investigation took place from March to April 2023 at the Mayo Hospital in Lahore, Pakistan. Laboratory rats were acquired from Biotechnology and Biochemistry Department and housed in a sturdy metallic cage before being sacrificed to extract connective tissues (liver, muscle, kidney, and heart).

With 50 tissue samples in each group, the tissues were divided into three categories. Various amounts of honey (45%, 65%, and 85%) were used to fix the tissues in Group A, date syrup (45%, 65%, and 85%) was used to repair the tissues in Group B, and 10% neutral buffered formalin was used to fix the tissues in Group C. There are 150 tissue samples altogether since each tissue sample was divided into five equal pieces. (Table 1-2)

Table 1: Timetable for processing tissues

Stage	**Reagent**		**Duration (Mins)**
		%	
one	Alcohol	70%	45
two		80%	45
three		90%	45
four		100%	60
five		100%	60
six		100%	60

seven	xylene		90
eight			90
nine	Paraffin wax impregnation and infiltration		45
ten			45
eleven			60
twelve			90

Using a pH meter and a refractometer, respectively, the pH and glucose levels of the date syrup and honey were ascertained. The juice produced from pitted and unpitted dates was boiled and evaporated to create the date syrup. Fresh honey was collected from the forest, and after passing the light match test, its quality was confirmed by inspecting its flatness, spreading, and burning.

Table 2: Schedule for hematoxylin and eosin staining

Stage	**Regent**		**Duration**
		%	
one	Sections are deparaffinized and rehydrated.		30 sec
two	xylene		4×3 minutes
three	ethanol	100	2×3 minutes
four		95	1×3 minutes
five		80	1×3 minutes
six		70	1×3 minutes
seven	Desalinated water		1×3 minutes
eight	Hematoxylin Harris		1×3 minutes
nine	water from tap		1×5 minutes
ten	Alcohol acid	1	60×30 sec
eleven	water from tap		1×5 minutes
twelve	alcohol	70	1×3 minutes

The tissues were fixed in various concentrations of date, honey syrup, or formalin for a period of 24 hours, after which they underwent a series of steps including alcohol-induced dehydration, decolorization with xylene, and deparaffinization. Hematoxylin and eosin were used to stain the deparaffinized sections of the tissues that had been fixed in date syrup honey and, whereas formalin-fixed tissues had to first be de-alcoholized using a normal xylene processing procedure.

Comparing the caliber of the specimens of tissue dyed with eosin and hematoxylin allowed researchers to assess the effectiveness and potency of the various amounts of date syrup honey in fixing connective tissues.

Statistical Analysis: GraphPad Prism version 5 was used to analyze the data after it was input into Microsoft Excel 2021. An examination of homogeneity was conducted to see how equivalently different the parameters are. When a significant difference (p-value < 0.05) was found, one-way and two-way ANOVA (Analysis of Variances) analyses were conducted at the 95% level of confidence along with Bonferroni posthoc assessment.

Results:

The research assessed the efficiency of formalin, date syrup, and honey as fixatives for tissue samples taken during biopsies. Six factors were taken into consideration while grading the samples: morphology, clarity, staining, and nuclear and cytoplasmic features. Utilizing an electronic microscope, the samples were examined by two independent examiners, and Graph Pad Prism version 5 was used to measure inter-observer variability.

The findings indicated no substantial variance (p-value >0.05) between the two fixing agents when the physical and chemical features of the date syrup and honey employed for fixation were examined. While date syrup had a glucose content of 74.64% and a fructose content of 74.63%, honey had a fructose and glucose content of 83.99% and 75.18%, respectively.

Figure 1 shows an evaluation of the effects of various quantities of honey, date syrup, and 10 percent formalin with a buffer on gross fixation. After one week, cells preserved in 45 percent date syrup and honey demonstrated moderate fixing with a total score of 9 (50%) that differed substantially (p-value < 0.05) from the results obtained with 10% formalin. With total scores of 18 (100%) that were comparable (p-value > 0.05) to their formalin-fixed equivalents, tissues treated in 65% and 85% date syrup and honey provided excellent preservation for up to 2 weeks.

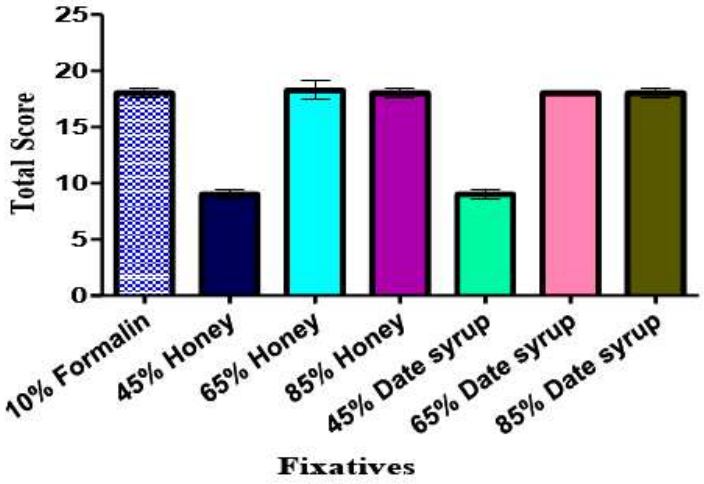

Figure 1: Fixing agent overall fixation score

Table 3: Physical and chemical characteristics of date syrup and honey

Parameter	Date	Honey	p
Fructose	74.63	83.99	0.264
Glucose	74.64	75.18	
Refractive index	1.473	1.474	
pH	4.1	6.09	
Temperature	26 Degree	26 Degree	
Color	Dark brown	Deep amber	

Figure 2 shows an evaluation of the staining capabilities of tissues preserved in various date syrup and honey concentrations. The 65% and 85% ratios of both date syrup and honey produced excellent

nuclear and cytoplasmic staining that was intense, clear, and preserved tissue morphology. With a reasonable degree of tissue shape preservation, the 45% concentration produced moderately strong, clear, and cytoplasmic nuclear staining. When 65% and 85% of date syrup and honey fixing agents were compared to their formalin-fixed equivalents, the variations in staining qualities were not significantly different (p-value=0.210). The comparison of 10% formalin and 45% date syrup for clarity and morphology revealed statistically significant variations (p=0.0026).

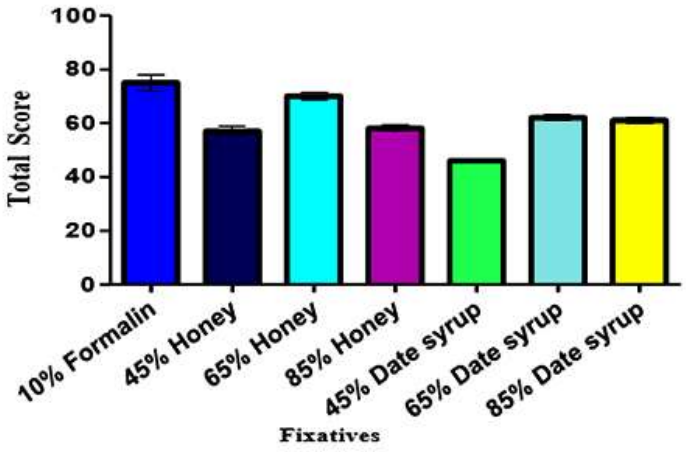

Figure 2: Fixatives' stains-causing abilities

Discussions:

Since there aren't any more secure or effective substitutes in our natural surroundings, formalin must be used as a fixing agent and embalming solution [5]. The main advantage comes from its practically universal usage for over a century and all the scientific knowledge that has been gathered about it [6]. Furthermore, formalin preserves lipids, is affordable, simple to store, and allows for preservation over time. It has come to be regarded as the fixing agent that gets nearest to being perfect as no evident "all-purpose" alternative has yet to be developed. [7,8] Contrarily, formalin has two well-known downsides. Formalin is quite poisonous, to begin with. Nasopharyngeal cancer has been associated with formaldehyde, which is categorized as a human carcinogen. A previous study showed strong evidence to suggest a cytotoxic and genotoxic mode of action for the carcinogenesis of breathed formaldehyde in the pulmonary nasal mucosa [9].

Different organ systems are affected by the numerous health dangers that come with formalin. It may irritate the mucous membranes and skin, resulting in contact dermatitis caused by allergies and alterations to the nasal mucosa's inflammatory response. Additionally, the pulmonary tract may be impacted, resulting in symptoms like asthma, sneezing, and coughing. Additionally, gastrointestinal issues such as vomiting, nausea, diarrhea, and lack of appetite may result from exposure to formalin. It may produce lacrimation and eye irritation. Formalin may have an impact on the cardiovascular system, producing a rise in heart rate and respiration, as well as the brain and spinal cord, resulting in migraines, dizziness, and sleep disturbances. The hematopoietic system, which may result in low blood cell counts, and the system of kidneys, which may result in kidney failure, can both be affected by formalin exposure. Additionally, formalin may hurt the female reproductive organs, resulting in irregular menstruation and spontaneous abortion.[10] However, fixation keeps tissue proteins in place such that the tissues may be kept after being eliminated from the patient's body and still seem realistic [11].

A fixative must effectively maintain the tissues' constituent parts to allow correct staining and microscopy. Because honey has therapeutic and antimicrobial properties, humans have used it as medicine for over a thousand years [12]. Honey has been discovered to possess antibacterial and antioxidative properties that can hinder the growth of different types of bacteria, fungi, protozoa, and viruses. These properties can be attributed to its low water content, inhibin formed by enzymatic reaction, phytochemical components, and hydrogen peroxide content. Honey's antibacterial activity comes from the production of hydrogen peroxide by an enzyme in honey, while its antioxidative properties are due to the presence of phytochemicals such as flavonoids [13].

Inverted sugars make up the majority of honey's concentrated aqueous inverted sugar solution [14]. In terms of dry weight, honey's carbohydrate content ranges from 95 percent to 97 percent [15]. The two sugars that make up the majority of honey's physical and nutritional properties are glucose and fructose [16,17]. While other elements, such as weather and processing, may also alter the composition and properties of honey, the color, density, scent, flavor, and other chemical and physical qualities are inherited from the plants from which it is produced [18]. Long thought to possess antibacterial properties and the capacity to retain substances without harming its customers, honey possesses these properties. A human body was soaked in honey to create the mythical medicinal substance known as "mellified man," often referred to as "human mummy confection." In ancient Rome, meat was preserved with the use of honey. Honey has antibacterial properties due to its elevated level of acidity, low water activity, and hydrogen peroxide action. Because of its antibacterial effects, hygroscopicity, and high acidity, honey is an efficient method to transform a human corpse into a mummy [19]. Honey's action is analogous to that of other fixing agents that function through protein cross-linking in that it renders tissue stiff [20]. Date syrup, on the other hand, includes tannins, which are used therapeutically as an astringent and deterrent in gastrointestinal issues [21,22].

Our initiative is the first of its type since there is currently no literature on the use of date syrup as a formalin replacement. Honey includes a variety of artifacts, some of which may be living spores like clostridia that might cause erroneous positive results. The honey utilized in the current investigation did not produce any such artifacts. In industrialized countries, the use of formaldehyde is almost entirely forbidden due to its potential toxicity.

Conclusions:

The use of data and honey syrup as natural alternatives to formalin for tissue fixation has shown encouraging results. Even more than anticipated, date syrup has shown to be comparable to honey in quality. These organic materials offer several benefits, including the fact that they are safe, easy to process and stain, and don't need any special tools. To stop the development of mold, thymol crystals may be used as an antibacterial. Even if immiscibility, stickiness, and discoloration were some of the problems, they may be fixed with frequent stirring and thorough washing. Lower concentrations (45%) of honey are appropriate for tissue staining whereas higher concentrations (65-85%) are suitable for long-term preservation. Finally, the future requirement for formalin may be reduced or perhaps eliminated with the use of natural fixatives. It is important to continue investigating and using natural fixatives.

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