The Effectiveness of Honey as a Substitute for Formalin in the Histological Fixation of Tissue

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Abstract

The preservative and antimicrobial powers of honey have been known for centuries. In addition, studies have shown that honey has acidic and dehydrative properties. The influence of these powers on tissue fixation was determined in this work to ascertain whether honey could prove to be a safe and acceptable substitute for formalin in the histological fixation of tissue. In this study, rat liver and kidney tissues were fixed at 37°C and at room temperature with and without agitation in concentrations of honey ranging from 10% to 100% diluted with distilled water. Tissues were fixed for 24 h, processed to paraffin wax, sectioned and stained by hematoxylin and eosin, and examined microscopically. Initial results obtained from this work concluded that tissues fixed in honey concentrations of 10% and 20% at room temperature with and without agitation gave comparable results with those obtained by formalin-fixed control tissues. Higher concentrations of honey were less successful and produced slower penetration rates, hardening of tissues, and difficulty in sectioning. Nonetheless, the results using low concentrations of honey for tissue fixation were encouraging and make further research using this and other brands of honey with a wider range of tissues, fixation times, and special stains of great interest. (*The J Histotechnol* 29:173, 2006)

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Key words: fixation, formalin substitute, honey, tissue fixative

Introduction

In cellular pathology, fixation preserves tissues by preventing autolysis and putrefaction. Although formalin is the most widely used tissue fixative, as the result of increasing concerns about its effect as a potential carcinogen, attempts have been made to find safer alternatives (1,2).

For centuries, honey has been shown to be a successful antibacterial agent, having the potential to preserve compounds without any harmful effects on users (3,4). As a result, honey has the capacity to serve as a natural food preservative, where it is able to enhance flavor, bind ingredients, and inhibit food-borne pathogens (5). In addition, the variety, content, and bioavailability of antioxidant compounds such as flavonoids and phenolic acid contained within honey render it as a potential source for the protection of tissues against cellular damage (6). The type of honey used in this work was collected from the Al-Qabel desert in the Sultanate of Oman. The two types of bee found in Oman are the *Apis millifera* and the *Apis florae*. In cultivated areas, they favor nests in citrus bushes or thorny trees such as the Simr (*Acacia tortilis*), which are most abundant in the region.

Materials and Methods

Fresh rat liver and kidney tissues were obtained by one of us (RAM) from the Department of Physiology at the Sultan Qaboos University, College of Medicine, Sultanate of Oman. The tissues were excised and dissected in to small segments (3–5 mm) using normal laboratory protocols and immediately placed into numbered universal containers. Dilutions of honey between 10% and 100% were made with distilled water, and the tissues were fixed for 24 h at room temperature (with and without agitation) and at 37°C. Control tissues were fixed in 10% neutral buffered formalin.

After fixation, the tissues were transferred to labeled cassettes and processed conventionally using alcohol for dehydration, toluene as clearing agent, and impregnated and embedded in molten paraffin wax at 60°C (Table 1).

Sections were cut at 4 microns on a rotary microtome and dried for 30 min at 60°C. Sections were stained using the hematoxylin and eosin method described in Table 2 and examined microscopically.

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Table 1. Tissue-Processing Schedule

Stage	*Reagent*	*Duration*
1	70% alcohol	2 h
2	80% alcohol	2 h
3	95% alcohol	2 h
4	Absolute alcohol	2 h
5	Absolute alcohol	2 h
6	Toluene	2.5 h
7	Toluene	2.5 h
8	Toluene	2.5 h
9	Paraffin wax	3 h
10	Paraffin wax	3 h

Table 2. Hematoxylin and Eosin Staining Schedule

Stage	*Reagent*	*Duration*
1	Xylene	2 × 5 min
2	Absolute alcohol	2 × 5 min
3	Tap water	2 min
4	Harris hematoxylin	10 min
5	Tap water	2 min
6	1% acid alcohol	3 s
7	Tap water	1 min
8	Lithium carbonate	5 s
9	Tap water	1 min
10	0.5% Eosin	1 min
11	Tap water	1 min
12	Absolute alcohol	3 × 1 min
13	Xylene	2 × 3 min

Results

The dilutions of honey were made in distilled water and their pH measured (Figure 1). The histomorphology of the tissue sections were rated according to cellular outline, cytoplasmic and nuclear detail, erythrocyte integrity, overall morphology, and staining. The liver and kidney control tissues fixed in 10% neutral buffered formalin showed excellent structural detail, with preservation of cellular outline and cytoplasm with nuclear and erythrocyte integrity (Figures 2 and 3).

At room temperature, the morphology of the liver fixed with low concentrations of honey at room temperature were well preserved with the morphology of the hepatocyes, lobules, sinusoids, bile duct, and portal triad well defined (Figure 4). The cytoplasm stained strongly eosinophilic because of the high content of organelles. When high concentrations of honey were used, the architecture and morphology of the liver showed poor preservation of cellular detail (Figure 5).

The morphology of the rat kidney fixed at room temperature with honey up to a concentration of 20% also showed good preservation. The microscopic structure of the glomeruli and the nuclei of the proximal convoluted tubules were well preserved (Figure 6). However, kidney tissue fixed with high concentrations of honey showed only partial nuclear preservation with loss of cellular outlines and glomerular shrinkage (Figure 7). Agitation at room temperature facilitated the exchange of reagent and resulted in increased penetration rates and improved fixation and staining.

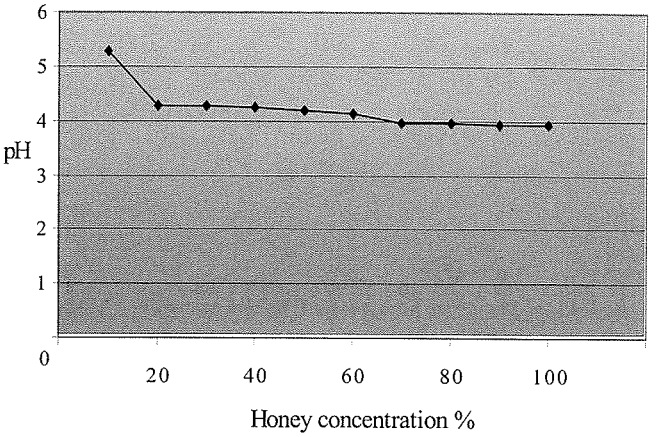

Figure 1. The relationship between pH and the concentration of honey.

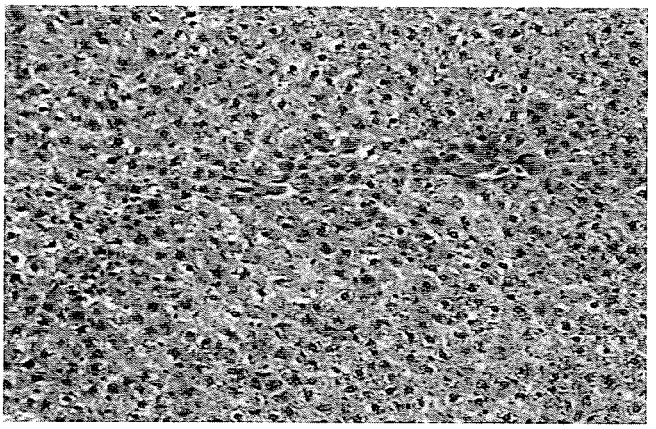

Figure 2. Rat liver fixed in 10% neutral buffered formalin at room temperature and stained with hematoxylin and eosin (magnification ×100).

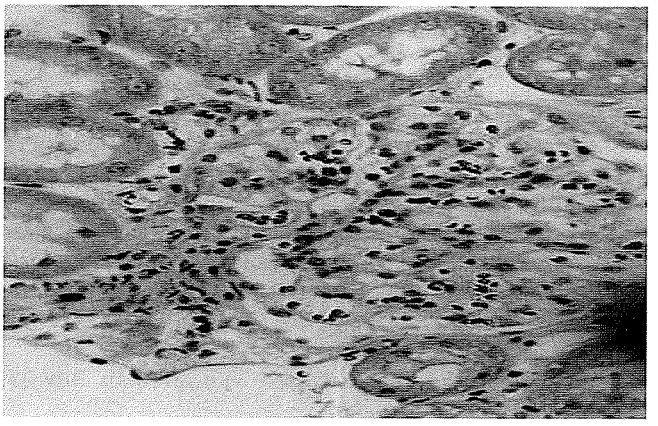

Figure 3. Rat kidney fixed in 10% neutral buffered formalin at room temperature and stained with hematoxylin and eosin (magnification ×400).

At 37°C, the architecture and morphology of the liver and kidney tissues fixed at all concentrations showed significant changes when compared with the control sections. In the liver, the cellular outline, nuclear, and cytoplasmic detail of the hepatocytes was poorly preserved (Figure 8 and 9). In the kidney, the microscopic features of the glomeruli and renal tubules showed poor nuclear detail (Figure 10 and 11).

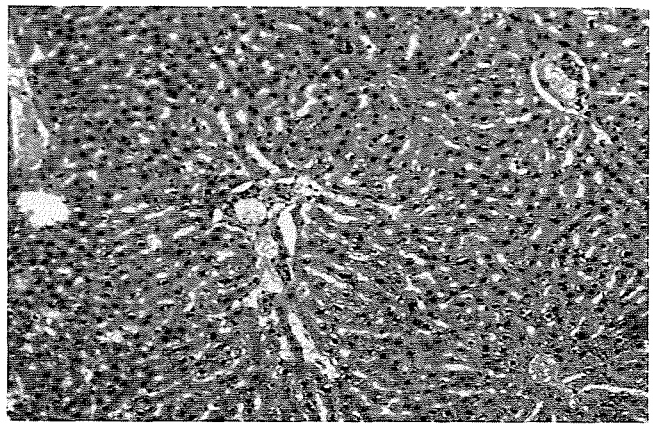

Figure 4. Rat liver fixed in 10% honey at room temperature and stained with hematoxylin and eosin (magnification ×100).

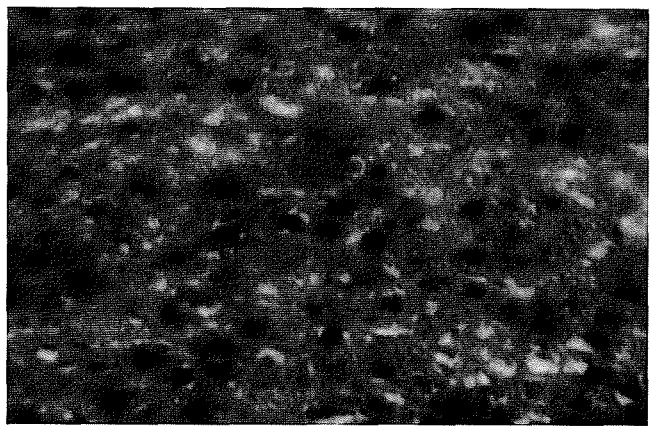

Figure 5. Rat liver fixed in 100% honey at room temperature and stained with hematoxylin and eosin (magnification ×400).

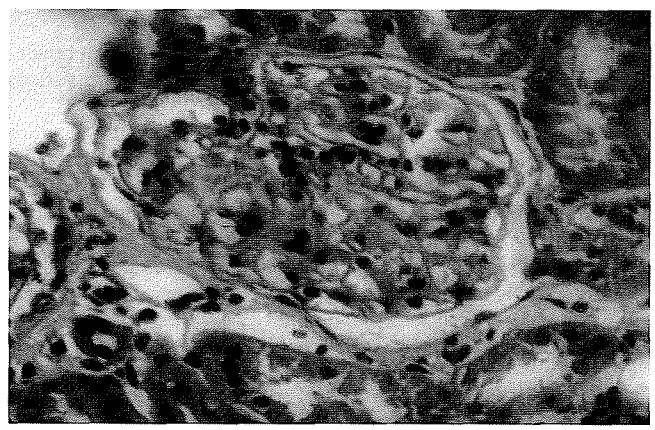

Figure 6. Rat kidney fixed in 10% honey at room temperature and stained with hematoxylin and eosin (magnification ×400).

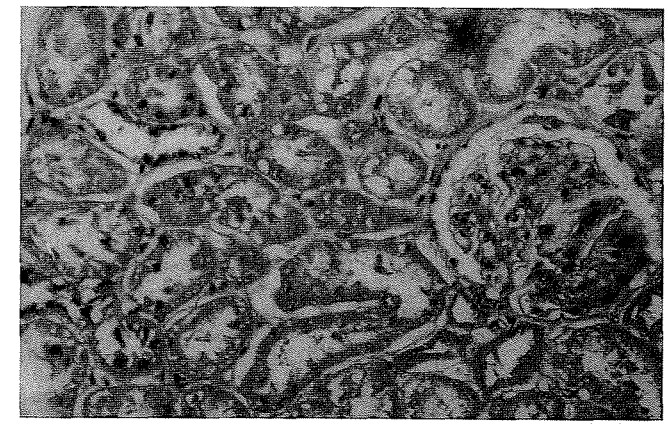

Figure 7. Rat kidney fixed in 100% honey at room temperature and stained with hematoxylin and eosin (magnification ×200).

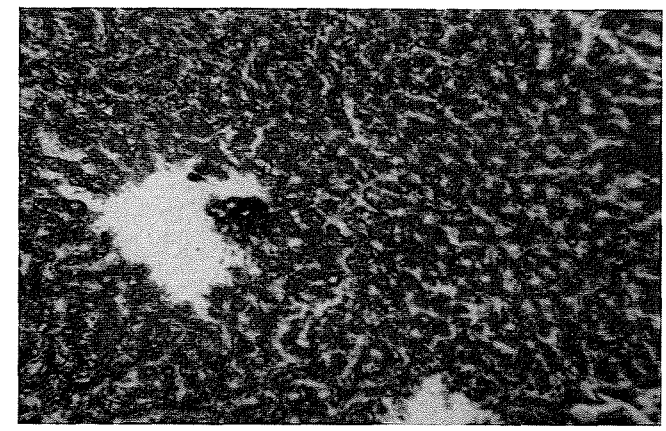

Figure 8. Rat liver fixed in 10% honey at 37°C and stained with hematoxylin and eosin (magnification ×100).

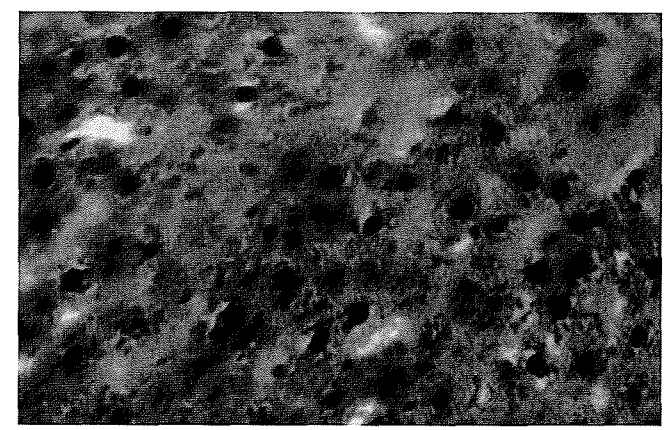

Figure 9. Rat liver fixed in 100% honey at 37°C and stained with hematoxylin and eosin (magnification ×400).

Discussion and Conclusion

Formalin is regarded as the gold standard for the fixation of tissue in cellular pathology. However, it remains a potential carcinogen for the laboratory worker and, for this reason, attempts have been made to formulate tissue fixatives that do not contain formaldehyde (1,7). Honey contains several compounds that help to protect tissues against damage, and its value as a preservative has been known for centuries (3,4,6,8,9).

The presence of amino and organic acids for instance, gives natural honey a pH of approximately 4.0 (Figure 1). Even at low concentrations, honey has a pH of less than 5.5,

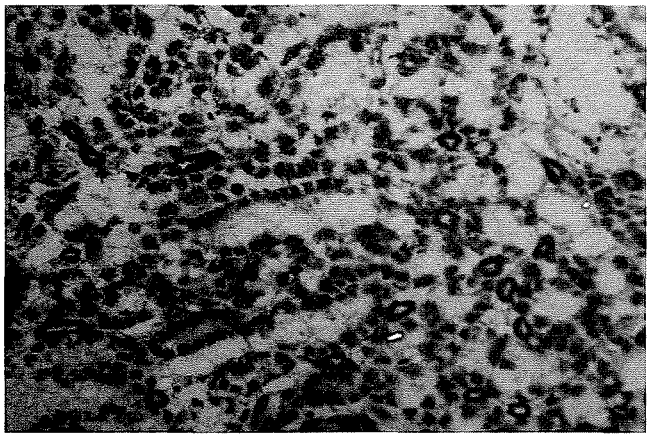

Figure 10. Rat kidney tissue fixed in 10% honey at 37°C and stained with hematoxylin and eosin (magnification ×200).

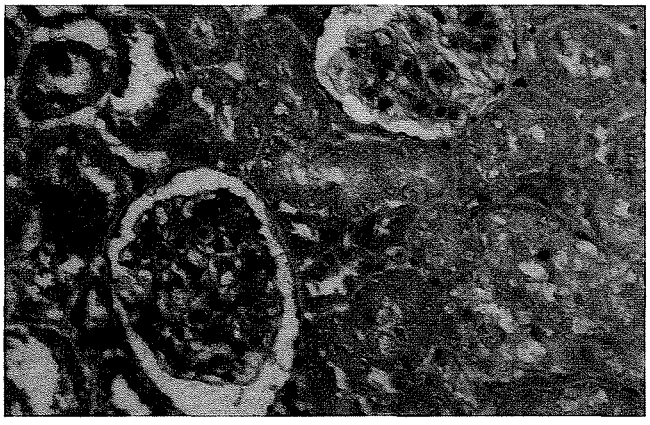

Figure 11. Rat kidney tissue fixed in 100% honey at 37°C and stained with hematoxylin and eosin (magnification ×200).

which is important in slowing down and inhibiting bacterial growth. A low pH also prevents the action of the enzyme glucose oxidase because, in concentrated honey, the acidity produced lowers the pH to a point that prevents the enzyme from functioning (10). When honey is diluted, the pH increases, and the activity of the enzyme dramatically increases with the release of antiseptic at a level that is antibacterial but not damaging to tissue. These preservative and antibacterial powers are the product of a combination of factors that also include antioxidant activity, production of hydrogen peroxide, and osmolarity (6,11). However, the high concentration gradient (osmolarity) of sugar found in concentrated honey causes tissues dehydration and shrinkage (see Figures 7 and 11). Tissue shrinkage occurs when the concentration outside the tissue is much higher than inside, leading to a rapid exchange of fluid by diffusion (12). Only when honey is diluted does it become less hypertonic with only minimal or no tissue changes apparent.

The results in this work supports these data since it has shown that tissues fixed in low concentrations of honey at room temperature gave results comparable with those of formalin-fixed control tissues (Figures 2 and 3). However, fixation in all honey concentrations at 37°C (Figures 8–11) and concentrations of honey of 30% or greater at room temperature (Figures 5 and 7) continually gave poor tissue preservation with some tissue shrinkage and, as such, are not recommended for routine histological fixation. Initial results using low concentrations of honey as a safe alternative to formalin are encouraging. Further parallel studies with other brands and types of honey are proposed to determine whether the properties that influence tissue fixation are universal or limited to certain honey types. Combined with a wider range of tissues, special stains and immunocytochemistry, these studies should be of great interest.

Acknowledgments

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