Advances in Research

21(7): 34-40, 2020; Article no.AIR.53361
ISSN: 2348-0394, NLM ID: 101666096

Efficacy of Various Concentrations of Honey Solution in the Fixation of Some Selected Tissues in Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author UA owner of the work and perform the design of the work. Authors AI and SYM source the materials for literatures review. Author OOO overall cross the work to ensure that everything is in order. Authors AU and UM carried out photomicrographs and interpretation of the results. Authors RIT, MOM and KHM managed the entire manuscript. Authors ATM, IM and AHM overall reviewer of the work and carried out dissection of the animal. Authors NO, MUK managed references. Authors HK, BAB and FAD take care of the animal used. Authors SMS and NAI managed the staining procedures and carried out screening of the slides. Authors AA and AIB carried out tissue processing and sectioning. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AIR/2020/v21i730217
Editor(s):
(1) Dr. Gamal Abdel-Hafez Mahmoud Bekhet, King Faisal University, Saudi Arabia.
Reviewers:
(1) Emmanuel Ifeanyi Obeagu, University Health Services, Michael Okpara University of Agriculture, Nigeria.
(2) Mona Said Elkutry, Ain Shams University, Egypt.
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Complete Peer review History: http://www.sdiarticle4.com/review-history/53361

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Original Research Article

Received 02 November 2019
Accepted 07 January 2020
Published 02 July 2020

ABSTRACT

Introduction: Honey is thought to preserve the cells by preventing autolysis and putrefaction. Thus, studies have shown that honey has a good preventive putrefactive property mainly because honey contains seven tetracycline derivatives, fatty acids, lipids, amylases and ascorbic acid and hydrogen peroxide. Therefore, honey is used as an agent for preventing autolysis and putrefaction.
Aim: This research aims to investigate the efficacy of some concentrations of the honey solution in fixation of some tissues (Such as kidney, liver, heart, lungs and muscle).
Methodology: A Wistar rat was sacrificed by vertical abdominal incision and the above-mentioned organs ware fixed in 10% formalin (positive control), 10% honey and 20% honey this grouped the research specimen into 3 groups. Histological examinations of these organs were carried out.
Results: Almost all the histological sections fixed in honey solution revealed normal nuclear as well as a cytoplasmic outline as compared with formalin-fixed organs, except for muscle which shown some level of swollen and fragmenting tissue.

Keywords: Honey solution; Fixation various concentrations and some selected tissues.

1. INTRODUCTION

Fixation is an initial and important step in tissue processing for microscopic examination. The primary aim of fixation is to preserve the tissues in a life-like state, prevent bacterial putrefaction, prevent autolysis, and increase the refractive index of the tissue [1]. Fixation is the process by which the tissues are preserved and protected from putrefaction and autolysis. The objective of fixation is to retain cellular components in their respective compartments and to present cells with a distinct and detailed microscopic appearance. Hippocrates discussed the biological effects of mercury and alcohol as a fixative as early as 400 BC. However, curiosity about the histological structure of the tissues began only with the invention of the microscope. Even then, the early microscope users were preoccupied with improving their scopes and cared little about the specimens [2].

The mechanisms by which fixatives act may be broadly categorized as dehydration, heat effects, cross-linking acid effects and combinations of these. An optimal fixative should allow high-quality histology and also preserve sufficient material for other technical analyses such as immunohistochemistry, western blot, *in situ* hybridization, and polymerase chain reaction. Fixation is the foundation for subsequent stages in the preparation of tissue processing for microscopic examination Al Saraj and Hubaity et al., 2003; Sabarinath et al. [1].

Honey is a sweet, sticky, yellowish-brown fluid made by bees from nectar collected from flowers. Honey is defined as the nectar and saccharine exudation of plants, which gathered, modified and stored as honey in honeycombs by honey bees (*Apis mellifera)* Al Saraj et al. 2010; Singh et al. [3].

Honey is as old as the written history - dating back to 2100 B.C. -Sumerian and Babylonian cuneiform writings. Honey was man's first and most reliable source of sweetener. History knows examples of things preserved in honey for decades and even centuries. Use of honey in funerary practices in many different cultures is well documented. Burmese priests have a custom of preserving their chief abbots in coffins full of honey. Alexander the Great, as Statius records – his remains shall be preserved in honey [4]. Herod I, King of Judea (40-4 B.C.) - executed his beautiful wife Marianne's body and preserved it in honey for 7 years. Arabs still preserve meat in honey and mummification in honey by Egyptians is very well known. All these facts made us wonder whether we could broaden the horizon of use of honey as a fixative in this modern world of "GO GREEN" [3]. Honey is produced from many floral sources and its content varies with its origin. Honey contains lysosomes (hydrolytic enzymes active at acid pH against several bacterial species), several minerals, trace elements such as potassium, sodium, chlorine, etc.vitamins such as B_1 (Thiamine), Riboflavin, Niacin, B_6 (Pyridoxine),

Pantothenic acid and B_{12} and C (Ascorbic acid) [5]. The antimicrobial activity of honey is due to its inhibitory effect on the growth of around 60 species of bacteria, which includes aerobes, anaerobes and gram-positive and gram-negative organisms, but its efficiency as a fixative is concealed [3].

Honey is thought to preserve the cells by preventing autolysis and putrefaction. Thus, studies have shown that honey has a good preventive putrefactive property mainly because honey contains seven tetracycline derivatives, fatty acids, lipids, amylases and ascorbic acid and hydrogen peroxide [6]. Therefore, honey is used as an agent for preventing autolysis and putrefaction [3].

2. MATERIALS AND METHODS

2.1 Study Location

The research was carried out in the Laboratory of the Department of Histopathology, School of Medical Laboratory Science, Usmanu Danfodiyo University Sokoto to investigate the efficacy of some concentrations of the honey solution in fixation of some tissues.

2.2 Study Design

Five organs (Kidney, lung, heart, liver and muscle) were used and fixed both in 10% honey, 20% honey and 10% formalin respectively. They were subjected to subsequent processing and staining procedure as shown in Table 1.

2.3 Honey Procurement

Forest honey (honey from forests collected by the traditional method) was purchased from Kaya of Giwa local government area, Kaduna State, Nigeria. The honey was tested for purity and genuineness by dropping on the floor to observed flatness and spreading, and by flaming to the observed formation of smoke or burning [7].

2.4 Experimental Animals

The experimental Animals used for this research were 2 Wistar rats, which were purchased from Animal House Ahmadu Bello University Zaria and kept in well-ventilated metal cage at the animal house of Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto, and they were fed with animal feed (sun seed) *ad libitum* before the day of animal sacrifice.

2.5 Animal Sacrifice

The Wistar rats were anaesthetized by using chloroform vapour, and they were put into sleep, the longitudinal abdominal incision was made. The organs were carefully harvested and washed with normal saline, then weighed and fixed in 10% formal saline and various concentration of pure honey (10% and 20%) for 72 hours.

2.6 Laboratory Analysis

The tissues fixed both in 10% pure honey, 20% pure honey and 10% formol saline were washed in normal saline, then were grossed and subjected to conventional tissue processing. The kidney lung, heart, liver and muscle were stained using Hematoxylin (H) and Eosin (E) by staining technique and PAS staining technique by Bancroft and Layton [8].

3. RESULTS

The sections from tissues fixed in 10% honey, 20% honey and 10% formal saline were compared for the following; Cytoplasmic staining Nuclear staining overall tissue architecture, and Clarity of staining. Out of all the slides analyzed, only clarity of staining was absent in the majority

Table 1. Summary of the study design

Organs	Pure Honey (Test)		Formalin (Control)	Duration of Fixation / Day	Staining Techniques*
Kidney	10%	20%	10%	3	H and E PAS
Lung	10%	20%	10%	3	H and E
Heart	10%	20%	10%	3	H and E
Liver	10%	20%	10%	3	H and E G & S
Muscle	10%	20%	10%	3	H and E

**meaning of the staining techniques- Hematoxylin, E - Eosin*

of formalin-fixed tissues. Rests of the parameters were fulfilled in all (100%) of the formalin-fixed tissues. For the sections fixed with honey, almost all of it did not show clarity of staining. Both the skeletal muscle fixed in 10% honey and 20% honey showed unpreserved tissue architecture (presented with some level of swollen and fragmenting). Both cytoplasmic and nuclear staining remained well in all the honey-fixed tissues.

Periodic Acid Schiff's (PAS) positive materials were demonstrated clearly by Periodic Acid Schiff's staining of both 10% and 20% honey-fixed kidney. Also, reticular fibres were demonstrated by staining both 10% and 20% honey-fixed liver with Gordon and Sweet [9] method.

4. DISCUSSION

The findings of this study, both the tissues fixed in 10% honey and 20% honey showed good staining property and clarity as similar to those fixed in 10% formalin (positive control). Except for skeletal muscle (which showed unpreserved tissue morphology), all the issues fixed in both 10% honey and 20% honey showed normal preserved overall tissue architecture, which is similar to those fixed in 10% formalin. These findings were in line with work reported Eroschenko, [10]; Sabarinath et al., [1]; Rajanikanth et al., [11], Dhengar et al. [12], this could be due to the same animal model used in both types of research.

Also in this study sections showed similar connective tissue fibre (such as reticular fibre in liver tissue) in both the honey fixed tissue and formalin-fixed tissue. Finding correspond to the work of Al-Maaini and Bryant [13] and Ozkan et al. (2012); Bracamonte et al*.,* [14], which showed better staining of connective tissue with honey fixation. This research gives an insight into how natural substances such as honey can be used in fixation of tissues.

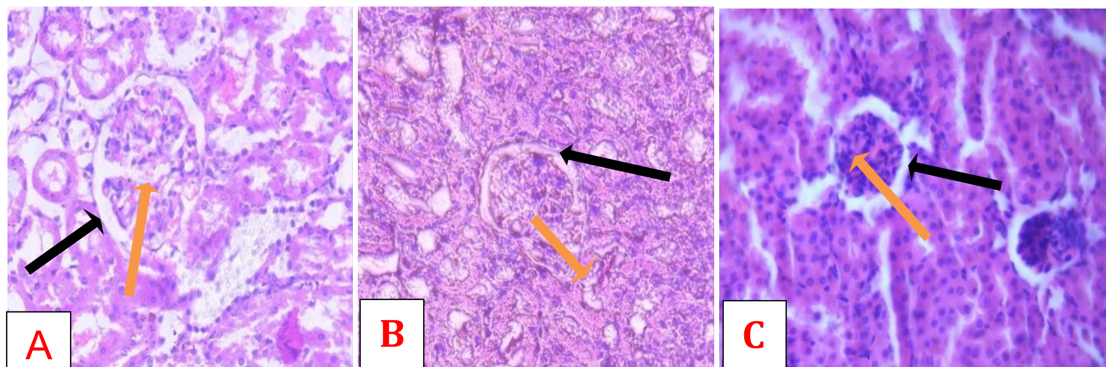

Fig. 1. Photomicrographs of Kidney tissue (A) formalin-fixed (control), (B) 10% honey-fixed and (C) 20% honey-fixed kidney tissue sections stained with H and E method, showing tucked glomeruli (black arrow) and capsular space (black arrow) (X 400)

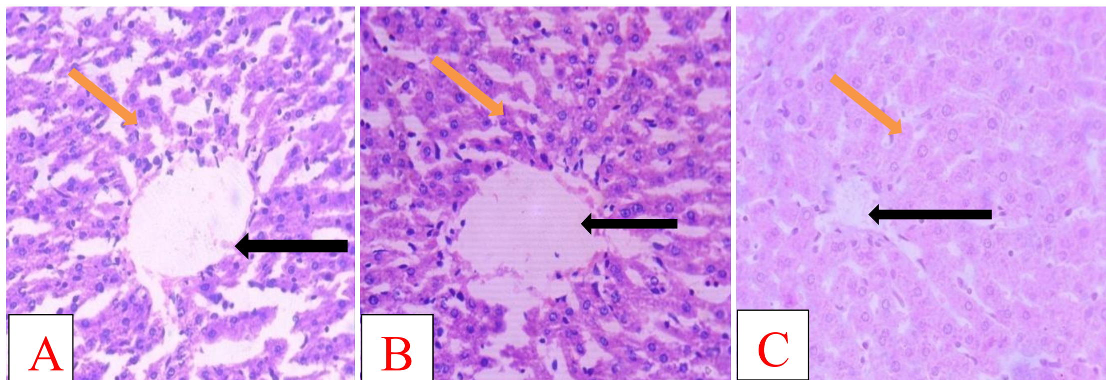

Fig. 2. Photomicrographs of Liver tissue (A) formalin-fixed (control), (B) 10% honey-fixed and (C) 20% honey-fixed liver tissue section stained with H and E method, showing central canal (black arrow) and hepatocyte (yellow arrow) (X 400)

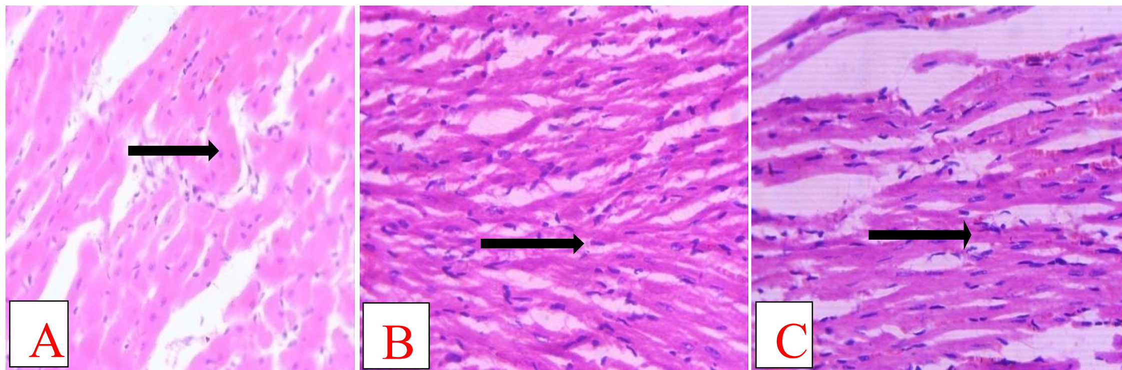

Fig. 3. Photomicrographs of Heart tissue (A) formalin-fixed (control), (B) 10% honey-fixed and 20% honey-fixed heart tissue section stained with H and E method, showing normal smooth muscle fibres (black arrow) (X 400)

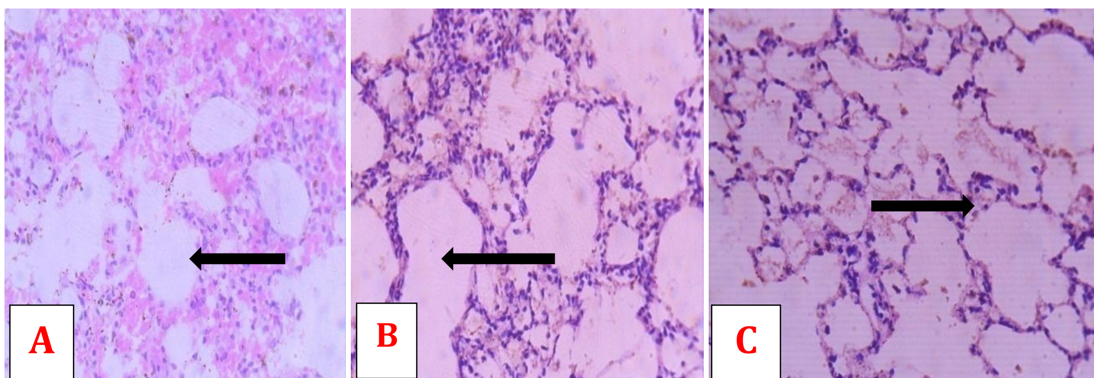

Fig. 4. Photomicrographs of Lung tissue (A) formalin-fixed (control), (B) 10% honey-fixed and (C) 20% honey-fixed lung tissue section stained with H and E method, showing alveolar air spaces (black arrow) (X 400)

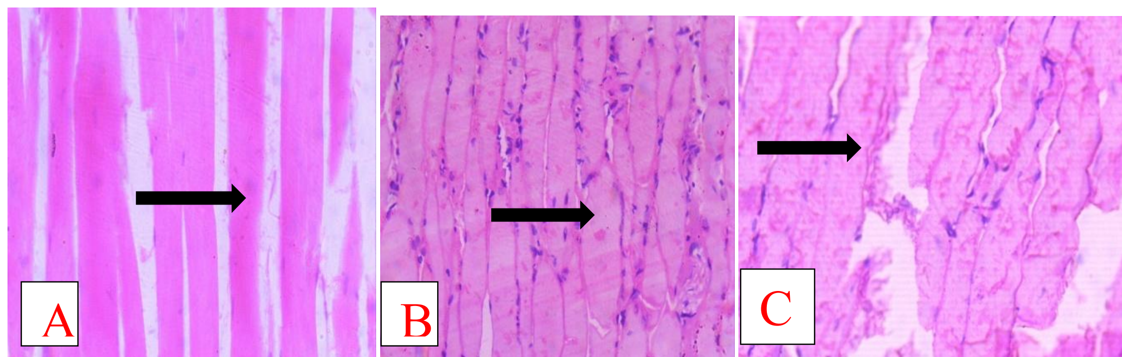

Fig. 5. Photomicrographs of skeletal muscle tissue (A) formalin-fixed (control), (B) 10% honey-fixed and (C) 20% honey-fixed skeletal muscle tissue section stained with H and E method, showing striated muscle bundle (black arrow) (X 400)

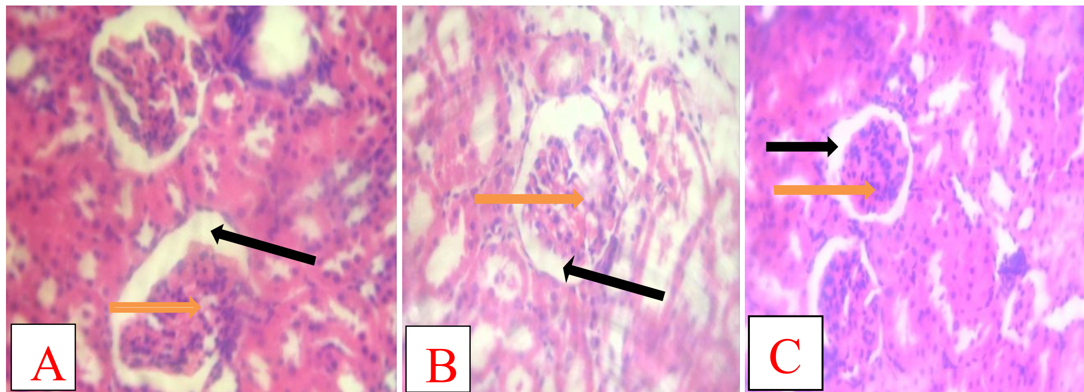

Fig. 6. Photomicrographs of Kidney tissue (A) formalin-fixed (control), (B) 20% honey-fixed kidney tissue sections stained with Periodic acid Schiff's method, showing tucked glomeruli (yellow arrow) and capsular space (black arrow). (X 400)

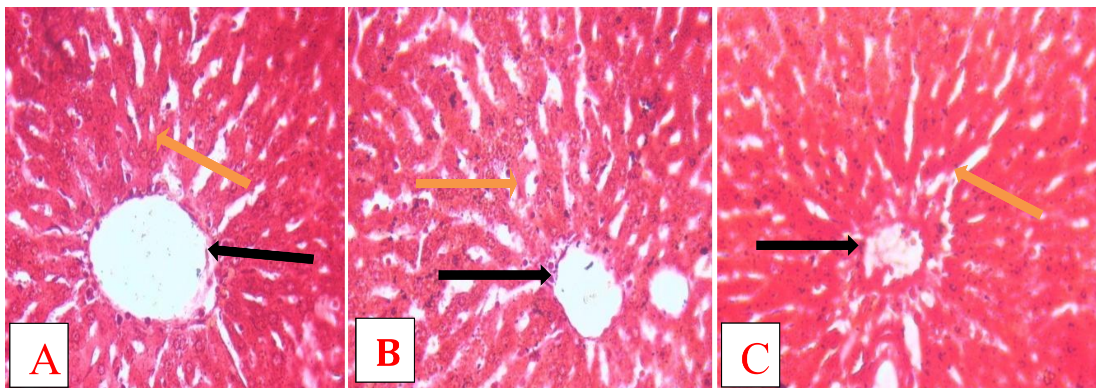

Fig. 7. Photomicrographs of Liver tissue (A) formalin-fixed (control), (B) 10% honey-fixed and (C) 20% honey-fixed liver tissue section stained with Gordon and Sweet's [11] method, showing reticular fiber (black arrows) and background substances (yellow arrow) (X 400)

5. CONCLUSION

The component in honey that is efficiently serving as a fixative is a matter of further research. In the absence of formalin or as a substitute to it, honey can be used as a successful alternative. Honey can thus be a cheaper, pleasant smelling, easily and forever available, bio-friendly and non-toxic. However, honey as a fixative has its limitations for use.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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