Continental Veterinary Journal
ISSN: 3079-0212 (ONLINE)
www.cvetj.com; editor@cvetj.com

RESEARCH ARTICLE
DOI: 10.71081/cvj/2024.029

Comparative Studies of Different Fixatives Used for Histopathology in Broiler Bird Tissues

Raheel Sharif[1]*, Muhammad Yasin Tipu[1], Ghulam Mustafa[1], Muhammad Rizwan[2], Kashod Aslam[3], Laraib Khan[4], Haziq Abdullah[6], Amina Yaseen[5], and Asjad Umair Shah[2]

[1]Department of Pathology, Faculty of Veterinary Sciences, University of Veterinary and Animal Sciences, Lahore, Pakistan; [2]Department of Medicine, Faculty of Veterinary Sciences, University of Veterinary and Animal Sciences, Lahore, Pakistan; [3]Center of Excellence of Molecular Biology, University of Punjab, Lahore; [4]Department of Medicine, Faculty of Veterinary Sciences, University of Veterinary and Animal Sciences, Jhang, Pakistan; [5]Department of Molecular and Biotechnology, University of Veterinary and Animal Sciences, Lahore, Pakistan; [6]Faculty of Veterinary Sciences, Bahauddin Zakariya University Multan Pakistan
*Correspondence: raheelsharif700@gmail.com

ARTICLE INFO

ARTICLE HISTORY: CVJ-24-920

Received: 20 September 2024
Revised: 02 November 2024
Accepted: 05 November 2024
Published online: 08 November 2024

Key words:
Histopathology
Broiler bird tissues

ABSTRACT

Formalin has been proven to be an effective fixative in ordinary tissue processing, but there are significant safety and health risks associated with its use. Formalin can be safely substituted with honey, jaggery, ethanol (70 %), and a mixture of ethanol, methanol and acetic acid (EMA=3:1:1). A total of 270 bits were collected from the liver, lungs, and intestines and fixed at different concentrations and durations. The aim of this study was to compare the tissue fixation properties of honey, jaggary, ethanol 70%, and EMA with those of formalin based on antibacterial properties, staining features of tissues, prolonged embedding of tissues in paraffin wax, and evaluation of nuclear staining, cytoplasm staining, cell morphology, clarity of staining, and uniformity of staining with H&E. This study found that honey, ethanol, EMA and formalin possess antibacterial properties and are effective fixatives for tissues. However, honey and jaggary showed poor fixation quality compared to EMA and formalin at a time duration of 24 h for liver and intestine tissues, while honey and jaggary gave comparable results to formalin, ethanol 70%, and a mixture of ethanol, methanol, and acetic acid (EMA) in the lungs. In addition, formalin and EMA caused tissue damage and shrinkage when embedding the tissue in paraffin wax for up to five hours, especially in the lungs. The study also found that the concentrations of honey and jaggary did not affect the staining quality of tissues, and a concentration of more than 50% could disturb the staining quality. These results suggest that natural fixatives such as honey and jaggary and chemical fixatives such as EMA (a mixture of ethanol, methanol, and acetic acid) could be considered as alternatives to formalin for tissue fixation in histopathological research.

To Cite This Article: Sharif R, Tipu MY, Mustafa G, Rizwan M, Aslam K, Khan L, Abdullah H, Yaseen A and Shah AU, 2024. Comparative Studies of Different Fixatives Used for Histopathology in Broiler Bird Tissues. Continental Vet J, 4(2): 214-221. http://dx.doi.org/10.71081/cvj/2024.029

INTRODUCTION

The goal of an effective histopathology procedure is to create microscopic preparations of tissues that are similar to their original form. Tissues were preserved using a variety of fixatives. It is challenging to create a fixative that is flawless in practice, and each fixative has its own benefits and drawbacks (Zuraw et al. 2022). The treatment of the tissue as soon as it was taken from the body was the cornerstone of all successful microscopic preparations. The tissue was immediately relocated as soon as possible and handled with the appropriate fixative solution. (Tan et al. 2020). Fixatives work in a variety of ways, including dehydration, heat effects, and acid effects, which cross-link molecules and mixtures of these by creating methylene bridges between amino acids and between amino acids and nucleotides (Jessy et al. 2019). Subsequently, thin (4-5um) tissue slices were cut once the tissue had been fixed and processed into paraffin blocks. One may view the tissue, its subcellular components, and the stroma that surrounds them were sliced into thin slices, labeled histochemically, and analyzed under a bright field microscope (Grizzle et al.

2009). Fixation is an initial and important step in histopathology to hold tissue in a realistic form for microscopic examination. The purpose of fixing was to extend the optical distinction of the tissue, while preserving it against bacterial putrefaction and autolysis (Bhattacharyya et al. 2018). In addition to bacterial putrefaction, the process of autolysis starts almost immediately once a person passes away. A delay in the fixation procedure may cause the diagnostic section of the tissue to deteriorate. Therefore, fixation of the tissue is extremely important immediately after its removal from the body. As a result, the tissue sample could be submerged in enough fixative solution to keep them preserved (Sezgin et al. 2023). Formalin, a widely used fixative, forms cross-links with proteins, ensuring tissue preservation, but poses health risks, including carcinogenicity. Formaldehyde in the form of 4% buffered formalin remains the most widely used fixative, partly due to its adaptability to a variety of tissues, convenience of use, affordability, and acceptability on a global scale. However, its preparation requires less time (Cortesia et al. 2014; Dakal et al. 2016). However, according to the IARC 2006, it also induced squamous cell carcinomas in the nasal respiratory epithelium of rats and mice, as well as nasopharyngeal cancer in humans (Nagaosss et al. 2023). Fixation with ethanol has been used to diagnose various diseases for years. It arrests tissue aging after death by dehydrating the body water. It has been demonstrated that ethanol preservation results in changes in size and shape, with the severity of these variations depending on the species (Rahman et al. 2022). Conventionally, ethanol (70%) is employed as a fixative for exfoliative cytology, although it has several drawbacks, such as being expensive, flammable, evaporating quickly, and requiring a purchase permit (Chandra et al. 2021). Acetic acid is regarded as a rapid, penetrative, and fixative agent in histopathology. It does so by causing nuclear proteins to clump together at no time. In addition, it stabilizes nucleic acids and aids in halting their loss. Acetic acid is a potent fixative that assists in the preservation of nucleic acids when used in combination with ethanol, but natural alternatives, such as honey and jaggery, offer safer fixation methods. Acidic bee honey also exhibits tissue-hardening, dehydration, and conserving qualities. In addition, it can penetrate the deepest tissue. These qualities imply that honey can be used as a tissue fixative (Aladi et al. 2023). According to Statius, the remains of Alexander the Great are preserved in the honey. All these details led us to consider honey's potential as a fixative in the contemporary environment that may be expanded (Salama et al. 2024). Honey's well-known antibacterial properties include its high sugar content, hydrogen peroxide content, and low ph. More recently, methylglyoxal and the antimicrobial peptide bee defensin-1 have been shown to be significant antibacterial components in honey. Honey's high sugar level, high hydrogen peroxide concentration, and low pH all contribute to its well-known antibacterial characteristics. More recently, it was discovered that honey contains important antibacterial compounds such as methylglyoxal and the antimicrobial peptide bee defensin-1(Kwakman et al. 2008). Jaggery is a traditional unrefined sugar that is produced by digesting sugarcane juice. This conventional sweetener is a combination of sugars and molasses. Jaggery and honey have similar makeup properties, and both have the potential to protect tissues ((Kwakman et al. 2008). The objective of my study is to compare the tissue fixation properties of Honey, Jaggery, Ethanol and a mixture of ethanol, methanol, and acetic acid (EMA=3:1:1) with formalin.

Objective

To compare the tissue fixation properties of Honey, Jaggery, Ethanol and a mixture of ethanol, methanol and acetic acid (EMA=3:1:1) with that of formalin.

MATERIALS AND METHODS

Study Design

A total of 270 bits were taken from the liver, lungs, and intestine (each organ consisted of 90 bits) and fixed at different concentrations and time durations. Formalin, ethanol, and EMA (a combination of ethanol, methanol, and acetic acid) were fixed at one concentration (27 bits of liver, lung, and intestine tissue required for these fixatives), whereas honey and jaggery were fixed at three different concentrations (81 bits of liver, lung, and intestine tissue for each fixative).

Isolation

Organisms

Clinical isolates of Staphylococcus aureus, Streptococcus pyogenes were used for the determination of antibacterial activity of different test solutions.

Procedure

A thick suspension of each organism, roughly matched to the 2 McFarland standards, was created in buffered saline. A 100 mL1 suspension of each organism was added to 5 mL1 of each test solution (honey, Jaggary, Ethanol 70%, EMA, Formalin and Normal saline). The initial colony forming units/mL1 of each organism was counted on 5% sheep blood agar. Growth was checked after 24 hours to confirm fixative's ability to fix the tissues and favored further tissue processing.

Histological analysis of tissues

The liver, lung, and intestinal tissues were isolated and fixed in 70% alcohol. Tissues were dehydrated using graded alcohol and xylene, followed by embedding in paraffin wax. Sections of 4 μm thickness were cut using a microtome and dried on glass slides at 40°C. Slides were stained sequentially with xylene, alcohol, hematoxylin (10 min), acid alcohol, eosin(6min), and alcohol. Finally, the slides were cleared with xylene and mounted using DPX. The samples were examined under a microscope for histological analysis (Grizzle et al. 2009). Various parameters, such as cell morphology, cytoplasm and nuclear staining, clarity, and uniformity of staining, were studied using the methods described by Abu- Bakar et al. (2020) with some modifications.

Laboratory analysis

Various parameters like cell morphology, cytoplasm and nuclear staining, clarity, and uniformity of staining were studied by the methods as described by (Abu- Bakar et al. 2020) with some required modifications.

Statistical analysis

All statistical analyses were performed using SPSS 13.0 for Windows. Fisher's exact test was used for comparing differences between the groups. Unless otherwise stated, a P value 0.05 was considered statistically significant (Alwahaibi et al.2022).

RESULTS

Fixative properties of six different solutions like Honey, Jaggary, Formalin, EMA, Ethanol 70% and Normal saline was checked. normal saline exhibited the development of staphylococcus and streptococcus on blood agar (Fig. 1), demonstrating that it lacks the fixation capabilities. Additionally, it was found that there was no growth of staphylococcus and streptococcus on blood agar in Honey, Jaggary, Formalin, EMA and Ethanol 70% solutions (Fig. 2) which proved their antibacterial capability and fulfilled the first property of fixation of tissue. Staining features of liver fixed in H 10, H 20 and H 30 at different time durations were show in figures4,5,6,8,9respetively. Hematoxylin and eosin-stained liver, lungs and intestine sections of concentrations of honey fixed groups at different time durations were shown in figures 3,7,10,11,15,16,17respetively. Comparison of different fixative groups on chicken lungs with hematoxylin and eosin staining method using Fisher's exact test were shown in table 2,3 and 4.

Fig. 1: *Streptococcus pyogenesis* and *Staphylococcus aureus* growth on blood agar (Normal Saline)

Fig .2: No growth observed on blood agar (Formalin, Ethanol, EMA, Honey.

There were noticeable differences in sectioning and formation of ribbons in all groups. As honey fixed tissues and jaggary fixed tissue could not be sectioned smoothly and no formation of ribbons occurred when the tissues embedded in paraffin for a duration of three hours. The sections of tissues could be properly achieved if time of tissue embedded in paraffin increased up to five hours. Therefore, all the tissues were fixed in different groups in paraffin for five hours to check their effects on nuclear staining, cytoplasm staining, and cell morphology, clarity of staining and uniformity of staining.

Liver, Lung and intestine sections of EMA fixed groups at different time durations (I-IX). Green arrow heads indicate clarity of structure seen in lungs in IV, V, VI of Fig. 11. While there is no clarity seen in ethanol fixed liver and intestine in all durations of Fig. 11.

DISCUSSION

Researchers need to investigate alternate options for tissue fixation in histopathological research to address the safety issues and public health risks associated with formalin exposure. In this regard, it is important to find a safe substitute for formalin, a known human carcinogen. To this end, our study evaluated the comparison of natural fixatives and chemical fixatives with that of neutral buffered formalin to check their tissue fixative effectiveness. To the best of our knowledge, this is the first study to compare the fixative capability of four fixatives: honey, jaggary, and Ethanol70%, EMA (a mixture of ethanol, methanol, and acetic acid), and formalin.

The present study assessed four important aspects of fixation: 1) Determination of antibacterial properties of different fixatives. 2) Effect of concentration and duration of fixation on staining features of tissues. 3) Effect of prolonged embedding of tissues in paraffin wax. 4) Evaluation of nuclear staining, cytoplasmic staining, cell morphology, clarity of staining, and uniformity of staining with H&E. The first assessment in our study was to evaluate the antibacterial properties of all five fixatives, which is one of the key components of tissue fixation. The findings of our study are in line with those of previous studies, suggesting that honey, ethanol, EMA, and formalin possess antibacterial properties that fulfill the first challenge of tissue fixation (Mastracci et al. 2019). The comparative studies of all fixatives at different time durations in different tissues (liver and intestine) revealed that honey and jaggary showed poor fixation quality compared with EMA (a mixture of ethanol, methanol, and acetic acid) and formalin for 24h. The findings of our study were comparable with those of a previous study (Bhat et al. 2021) that EMA and formalin were stronger fixatives and had the capability to fix the tissue more rapidly. While honey and jaggary might be weaker fixative and diffused into the tissue slowly. That's why favorable results were obtained to formalin and EMA. While honey and jaggary gave comparable results to formalin, ethanol 70% and EMA in case of lungs. It meant that lungs were more delicate in structure in comparison with liver and intestine. That's why honey and jaggary more quickly diffused to the lungs when we fixed the tissues for a duration of 24h. In our study, it confirmed that honey and jaggary gave comparable results to formalin and EMA even surpassing the ethanol 70% in fixing liver and lungs at a time duration of 48 and 72h.

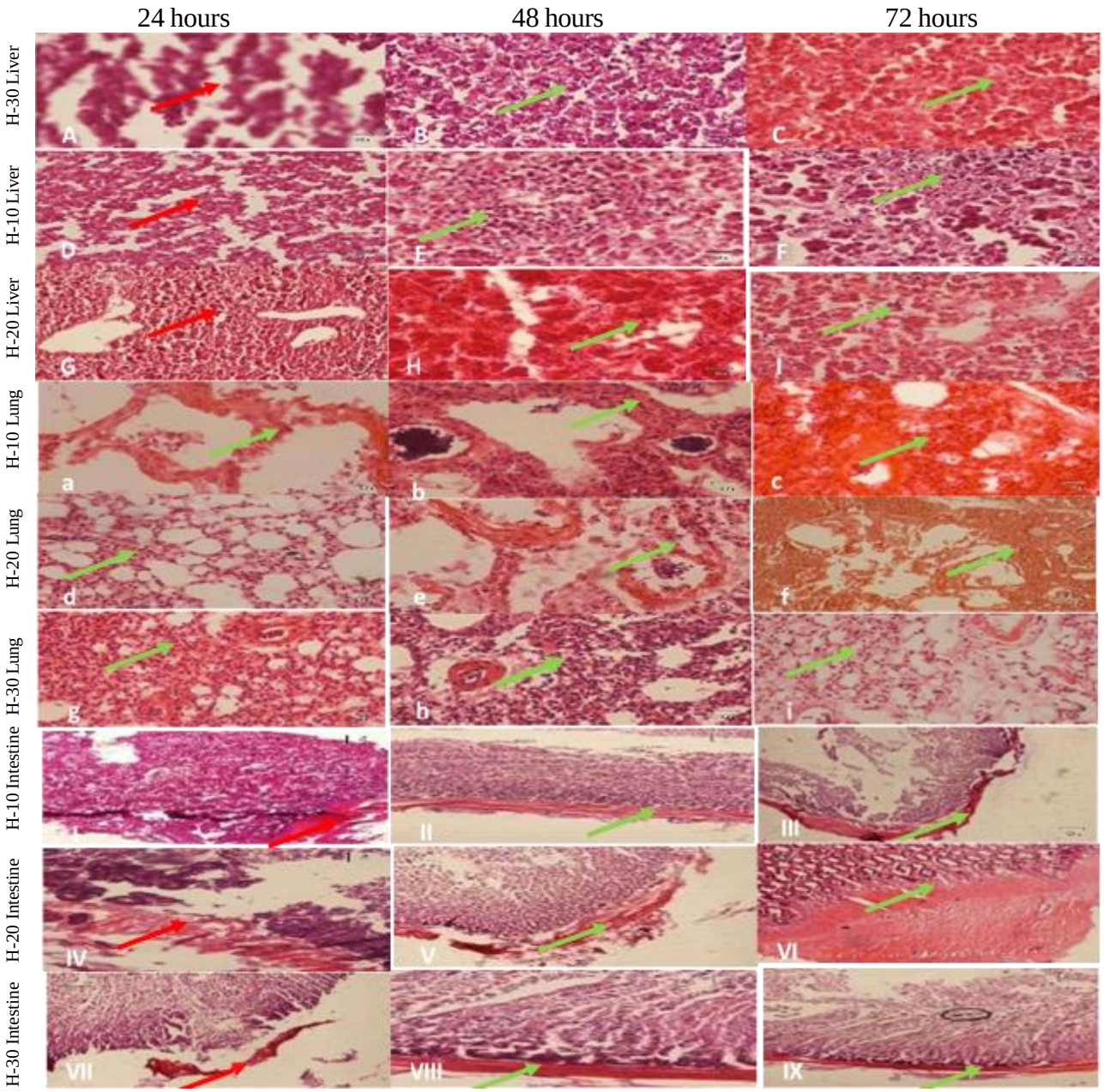

Fig.3: Hematoxylin and eosin-stained liver, lungs and intestine sections of concentrations of honey fixed groups at different time durations. Green arrow head indicates the nucleus well preserved in 48 and 72 hours in liver in B, C, E, F and H, I of Fig.3. While red arrow head indicates no clarity seen in 24 hours in liver in A, D and G of Fig. 3. Arrow head indicates the nucleus well preserved in lungs especially in 24 and 48 hours in a,b,d,e and g,h shown in Fig. 3. While in intestine, green arrow head indicates the muscularis mucosa intact in 48 and 72 hours in II, III, V, VI and VIII, IX seenin above Fig.3. While the red arrow head shows the distortion of muscularis mucosa occurs in 24 hours in I, IV and VII of Fig. 3.

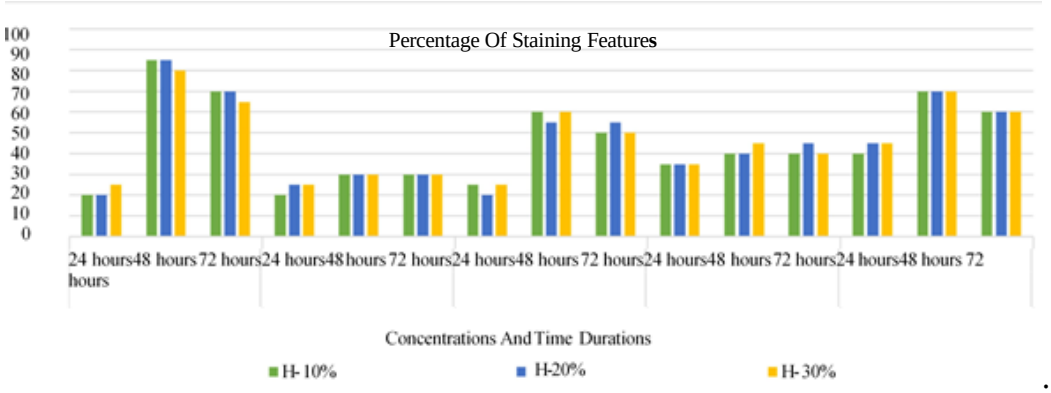

Fig. 4: Staining features of liver fixed in H 10, H 20 and H 30 at different time durations.

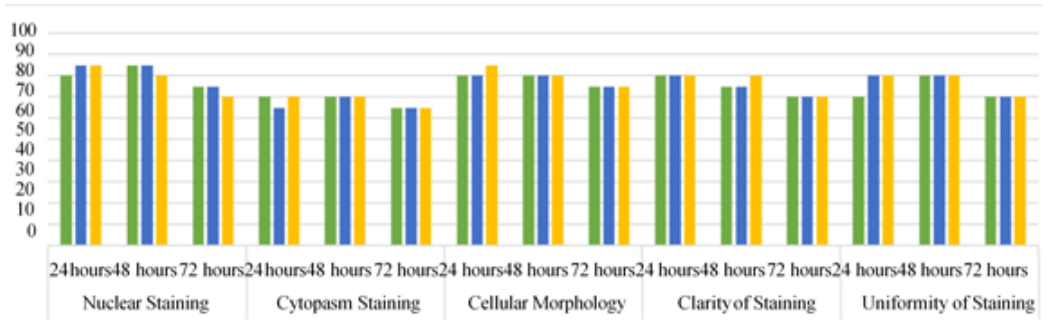

Fig. 5: Staining features of lung fixed in H 10, H 20 and H 30 at different time durations.

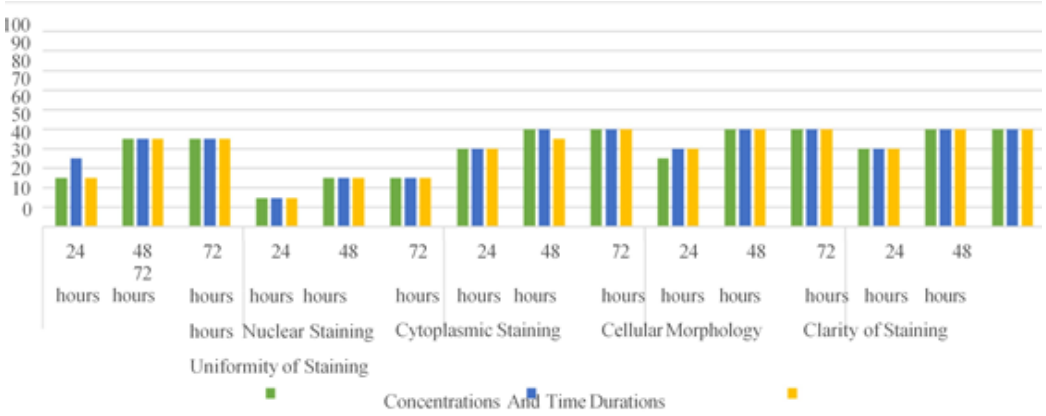

Fig. 6: Staining features of intestine fixed in H 10, H 20 and H 30 at different time durations.

The findings of our study agree with those of a previous study by Shi et al. (2024). In comparison with formalin and EMA, the honey fixed intestine, jaggary fixed intestine and ethanol 70% fixed intestine did not give satisfactory results. However, no significant difference was found among honey, jaggary and ethanol 70% in the quality of fixation of the intestine. Furthermore, there was significant tissue damage and shrinkage of tissue observed for EMA- fixed tissue at a time duration of 48 and 72h compared to 24h. There was no significant tissue damage or shrinkage in formalin-fixed tissues. The results of our study are in concordance with a previous study by (Belsare et al. 2012) who found that a fixation time of 6 to 12 h for EMA produced the best quality sections with minimal tissue damage and antigen retrieval.

In our study, we found that no reasonable difference existed in the staining quality of tissues between the different concentrations of honey and jaggary, which supports the results of a previous study (Abubakar et al. 2020). The only difference between different concentrations of honey and jaggary is that the tissues can be preserved for a longer duration by increasing their concentration. However, a concentration of more than 50% may disturb staining quality.

Formalin and EMA cause the tissue damage and shrinkage of tissues when we embed the tissue especially lungs in paraffin wax for up to five hours due to its delicate and intricate structure. As a result, the air gaps in the lung might close up during over embedding of tissue. These findings are similar to those of Ekiz et al. (2019) who found that prolonged exposure of formalin-fixed tissue in paraffin wax can damage the tissue, resulting in poor tissue morphology and complicating the interpretation of the results.

While in honey fixed tissue and jaggary fixed tissue, there is no shrinking of tissue or abnormal cell shape present. This implies that fixatives that have the ability to rapidly fix the tissue are more corrosive to tissue as we extend the duration of embedding time in paraffin wax. The honey fixed and jaggary-fixed livers showed clear cell boundaries, hepatocytes, and sinusoids, which were comparable to formalin and EMA when the paraffin embedding time was increased to 5h.

While ethanol fixed liver shows the worst cell morphology. The findings of the current study show prominent pneumocytes and alveoli in honey-fixed and jaggary-fixed lungs, which agrees with the results of a previous study (Schmiedeberg et al. 2009), whereas over-embedding of tissue in paraffin wax destroys the prominence of alveoli in formalin and EMA. Additionally, ethanol 70% shows the intermediate results (Karimi-Maleh et al. 2021).

The use of natural fixatives like honey and jaggary for tissue fixation has a number of benefits, including the fact that they are easily accessible, nonhazardous, suitable for regular processing, staining, and equipment-free. In addition, jaggary is far more affordable and widely accessible than honey (Schmiedeberg et al. 2009). It costs around one-sixth as much as honey. Few shortcomings of using natural fixatives include tissue section folding, high eosin staining, and rupture in section continuity. Patients with suspicious lesions are encouraged to have a biopsy right away.

A few advantages of using a chemical fixative such as EMA (a mixture of ethanol, methanol, and acetic acid) are that EMA can fix tissues more quickly than NBF, which is consistent with the results of Frost et al. (2015). The disadvantage of using EMA is that it causes tissue shrinkage and distortion of cell morphology if the tissue is preserved for a long period of hours.

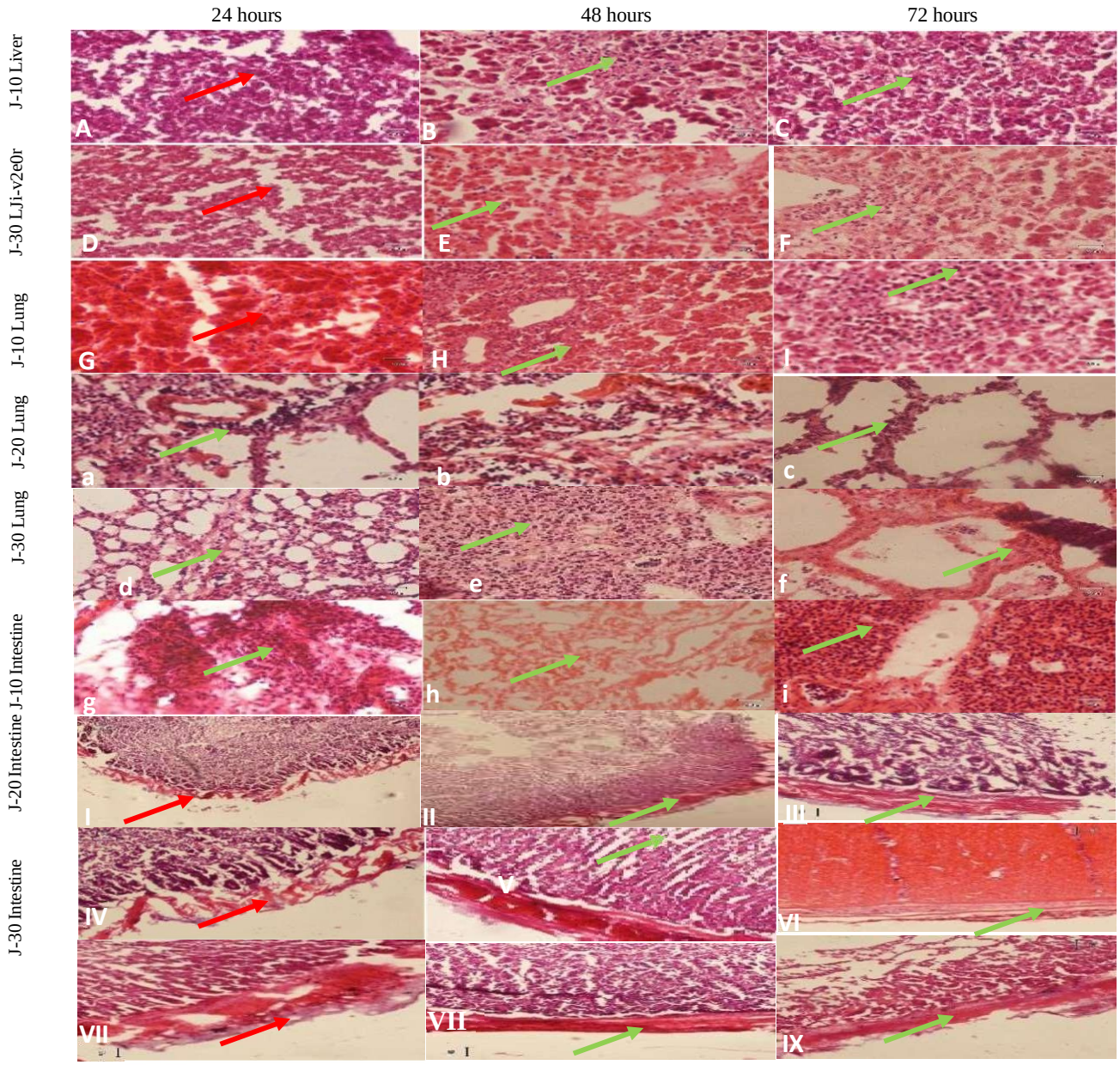

Fig.7: Hematoxylin and eosin-stained liver, lungs and intestine sections of concentrations of honey fixed groups at different time durations. Green arrow head indicates the nucleus well preserved in 48 and 72 hours in liver in B, C, E, F and H, I of Fig.7. while red arrow head indicates less clarity seen in 24 hours in liver in A, D and G of Fig.3. But clarity is much evident as compared to Honey. Arrow head indicates the nucleus well preserved in lungs especially in 24 and 48 hours in a,b,d,e and g,h shown in Fig.7. While in intestine, green arrow head indicates the muscularis mucosa intact in 48 and 72 hours in II, III, V, VI and VIII, IX seen in above Fig.7. and the red arrow indicates the distortion of muscularis mucosa occurs in 24 hours in I, IV and VII. But the clarity is more as compared to Honey fixed tissues.

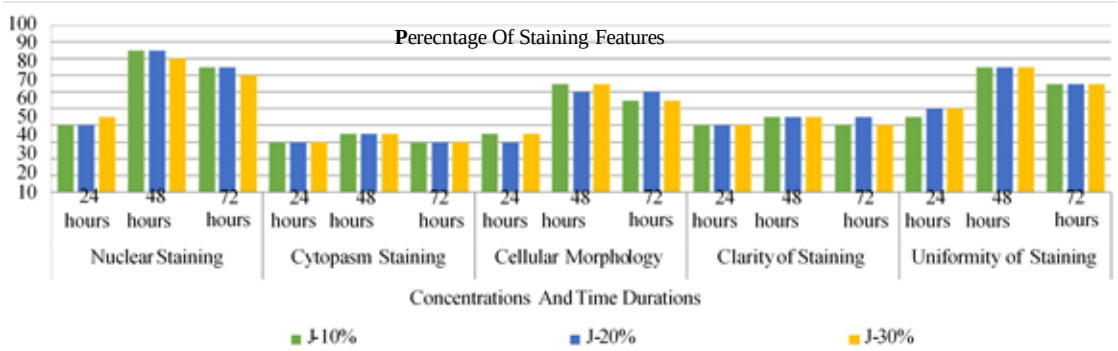

Fig. 8: Staining features of liver fixed in J 10, J 20 and J 30 at different time durations.

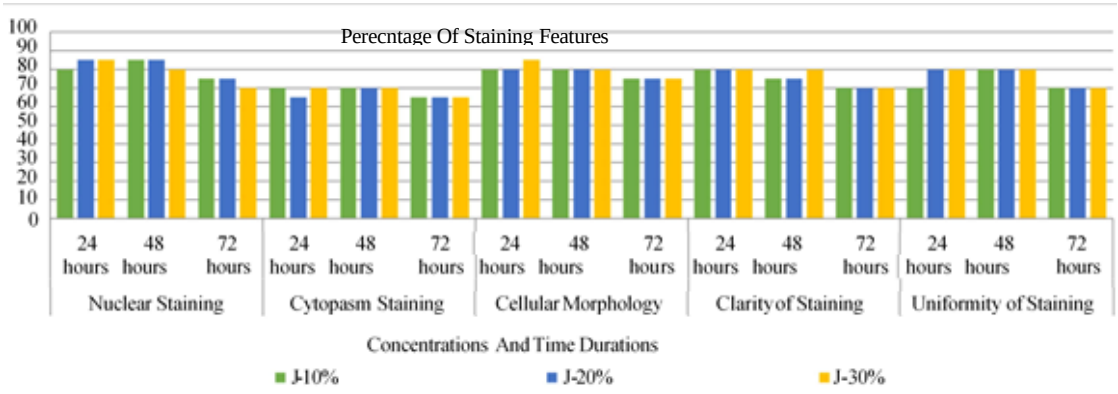

Fig. 9: Staining features of lung fixed in J 10, J 20 and J 30 at different time durations.

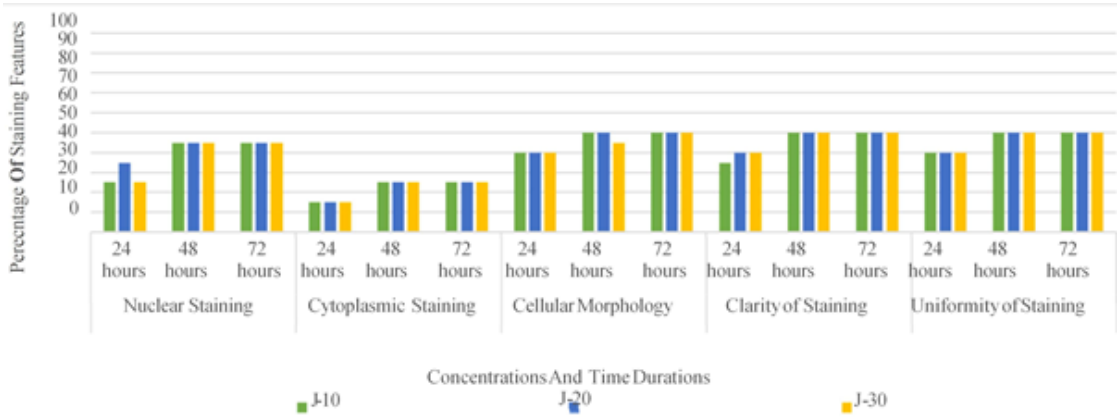

Fig. 10: Staining features of intestine fixed in J 10, J 20 and J 30 at different time durations.

Conclusions

Histopathology, historically reliant on formalin since the 19th century, now confronts its carcinogenic risks. Natural alternatives like honey and jaggary, alongside chemical substitute EMA, offer comparable fixation quality without health hazards. Jaggary emerges as a safe substitute for formalin in tissue preservation. In contrast, 70% alcohol fixation shows reduced efficacy and may cause cellular shrinkage and deformation, impacting specimen quality significantly.

Funding

No funds were provided by any national or international funding agencies.

Ethical Statement

No Ethical permissions were required for this article.

Availability of Data and Material

The data can be obtained from the corresponding author on a reasonable request.

Acknowledgment

We acknowledge the technical support of the Department of Pathology, Institute of Microbiology, and the Department of Clinical Medicine, University of Veterinary and Animal Sciences Lahore, Pakistan for publication of this article.

Authors Contribution

RS conceived and designed the experiment and performed the study. MYT supervised and coordinated the experiment. GM carried out the lab analyses. RS also performed the statistical analyses of experimental data. MR KA and HA prepared the manuscript for publication.

REFERENCES

Zuraw A, Aeffner F. 2022. Whole-slide imaging, tissue image analysis, and artificial intelligence in veterinary pathology: An updated introduction and review. Veterinary Pathology 59(1):6-25.

Tan WC, Nerurkar SN, Cai HY, Ng HHM, Wu D, Wee YTF, Lim TKH. 2020. Overview of multiplex immunohistochemistry/immunofluorescence techniques in the era of cancer immunotherapy. Cancer Communications 40(4):135-153.

Shi Y, Zhou M, Chang C, Jiang P, Wei K, Zhao J, He D. 2024. Advancing precision rheumatology: applications of machine learning for rheumatoid arthritis management. Frontiers in Immunology 15:1409555.

Grizzle WE. 2009. Special symposium: fixation and tissue processing models. Biotechnic & Histochemistry 84(5):185-193.

Jessy J. 2019. Modulation of tumor antigen-reactive anti-Gal antibodies by lipoprotein (a) concentration and its effect on tumor susceptibility. Doctoral dissertation, SCTIMST.

Bhattacharyya A, Gupta B, Singh A, Sah K, Gupta V. 2018. Probing natural substitute for formalin: Comparing honey, sugar, and jaggery syrup as fixatives. National Journal of Maxillofacial Surgery 9(1):14-21.

Sezgin Y. 2023. The importance of technical quality for diagnosis in pathology laboratory. Current Studies on 109(5), 165-173.

Shi Y, Zhou M, Chang C, Jiang P, Wei K, Zhao J and He D, 2024. Advancing precision rheumatology: applications of machine learning for rheumatoid

arthritis management. Frontiers in Immunology 15:1409555.

Cortesia C, Vilcheze C, Bernut A, Contreras W, Gomez K, de Waard J. 2014. Acetic acid, the active component of vinegar, is an effective tuberculocidal disinfectant. Scientific Journal of Microbiological Sciences 5(2): e00013–14.

Nagao T, Kerr AR. 2023. Noninvasive diagnostic. Oral submucous fibrosis: A guide to diagnosis and management. Cham: Springer International Publishing13:197.

Rahman M, Islam R, Hasan S, Zzaman W, Rana MR, Ahmed S, Sunny AR. 2022. A comprehensive review on bio-preservation of bread: An approach to adopt wholesome strategies. Foods 11(3):319.

Chandra S, Sah K, Amaranath BJ and Ahmad S, 2021. Utilization of processed honey and jaggery as an oral cyto-fixative. Indian Journal of Dental Sciences 14(1):6-10.

Aljadi AM. 2003. Isolation and identification of phenolic acids in Malaysian honey with antibacterial properties. Turkish Journal of Medical Sciences 33(4):229-236.

Salama Y, Chennaoui M. 2024. Biochemical properties and therapeutic activities of honey: A review. Journal of Material and Environmental Sciences 15:301-324.

Kwakman PH, Van den Akker JP, Güçlü A, Aslami H, Binnekade JM, de Boer L, Zaat SA. 2008. Medical-grade honey kills antibiotic-resistant bacteria in vitro and eradicates skin colonization. Clinical Infectious Diseases 46(11):1677-1682.

Abubakar K, Mailafiya MM, Chiroma SM, Danmaigoro A, Zyoud TY, Abdul Rahim E, Abu Bakar Zakaria MZ. 2020. Ameliorative effect of curcumin on lead-induced hematological and hepatorenal toxicity in a rat model. Journal of Biochemical and Molecular Toxicology 34(6): e22483.

Mastracci L, Gambella A, Bragoni A, Pigozzi S, Pastorino L, Vanni I, Tosi I, Campora M, Fiocca R, Grillo F. 2019. Coping with formalin banning in pathology: under vacuum long-term tissue storage with no added formalin. Histochemistry and Cell Biology 151(6):501-511.

Bhat AH, Hussein S. 2021. Fixation and different types of fixatives: Their role and functions: A review. International Journal of Clinical and Diagnostic Pathology 4:113-119.

Ekiz R, Demiraslan Y. 2021. Investigation of cadaver preservative properties of solution obtained with a mixture of honey and ethyl alcohol in different proportions: A pilot study on sheep heart. Journal of Histotechnology 70(2):229-243.

Alwahaibi N, Al Dhahli B, Al Issaei H, Al Wahaibi L, Al Sinawi S. 2023. Effectiveness of neutral honey as a tissue fixative in histopathology. F1000Research 11:1014.

Schmiedeberg L, Skene P, Deaton A, Bird A. 2009. A temporal threshold for formaldehyde-crosslinking and fixation. Journal of Histochemistry and Cytochemistry 4(2): e4636.

Dakal TC, Kumar A, Majumdar RS, Yadav V. 2016. Mechanistic basis of antimicrobial actions of silver nanoparticles. Frontiers in Microbiology 7:1831.

Belsare A, Mushrif M. 2012. Histopathological image analysis using image processing techniques: An overview. Signal & Image Processing 3(4):23.

Karimi-Maleh H, Orooji Y, Karimi F, Alizadeh M, Baghayeri M, Rouhi J, Tajik S, Beitollahi H, Agarwal S, Gupta VK. 2021. A critical review on the use of potentiometric-based biosensors for biomarkers detection. Biosensors and Bioelectronics 184:113252.

Armstrong J, Sargent C, Singh D. 2009. Glucocorticoid sensitivity of lipopolysaccharide-stimulated chronic obstructive pulmonary disease alveolar macrophages. Clinical and Experimental Immunology 158:74-83.

Frost AR, Eltoum IE, Siegal GP, Emmert-Buck MR, Tangrea MA. 2015. Laser microdissection. Current Protocols in Molecular Biology 112(1):25A-1.