Original Article

The Effectiveness of May Grunwald Giemsa Staining on Tissues Fixed With Date Molasses

Hatice Yaren Kuloğlu*

Department of Histoloy and Embriology, Faculty of Veterinary Medicine, Aksaray University, Aksaray, Turkey.

Use your device to scan and read the article online

How to Cite This Article Kuloğlu, H. Y. (2024). The Effectiveness of May Grunwald Giemsa Staining on Tissues Fixed With Date Molasses. *Iranian Journal of Veterinary Medicine, 18*(4), 525-534. http://dx.doi.org/10.32598/ijvm.18.4.1005514

http://dx.doi.org/10.32598/ijvm.18.4.1005514

ABSTRACT

Background: Formaldehyde is a reactive chemical that bonds randomly with various cellular elements. This substance, which is also found in the natural structure of the organism, is used in many areas, from industry to household materials, from the production of coatings in dentistry to the fixation of cadavers in laboratories. Formaldehyde is commonly used in laboratories to fix tissues.

Objectives: In this study, the staining properties of tissues fixed with date molasses, a natural sugar, using the May-Grunwald-Giemsa (MGG) staining method were compared with the staining properties of tissues fixed with buffered formol-saline.

Methods: Liver, spleen, kidney, skin, testicle, small intestine, large intestine, brain, cerebellum, and lung tissue samples of 4 rats of both genders were used as materials in the study. Each tissue underwent routine histological tissue tracing and was stained with MGG.

Results: When all tissues were evaluated anatomically, the general morphology of the tissues showed that the tissue integrity was preserved. There was no color change in the tissues. In addition, tissue hardness was at the required level, supporting previous studies. Histologically, regarding chromatin distribution, nucleus separation and cytoplasm staining, tissues fixed with date molasses and tissues fixed with buffered formal saline showed similar properties, except for the tissues subjected to intense maceration (skin and small intestines). Pale staining was observed in some tissues fixed with buffered formal saline (liver, skin, heart), but the nucleus and cytoplasm structures were better distinguished at higher magnifications. Considering the chromatin distribution, nuclear separation and cytoplasm staining in other tissues (spleen, kidney, testicle, large intestine, brain, cerebellum and lung), tissues fixed with date molasses showed similar properties to tissues fixed with buffered formal-saline.

Conclusion: We recommend that researchers interested in the subject prolong the fixation period (6 months) and compare these results with those of tissues fixed for 24 hours.

Keywords: Buffered formal saline, Date molasses, Fixation, Histological, May-Grunwald-Giemsa

Article info:
Received: 15 Mar 2024
Accepted: 18 May 2024
Publish: 01 Oct 2024

*** Corresponding Author:**
Hatice Yaren Kuloğlu, *Assistant Professor.*
Address: *Department of Histoloy and Embriology, Faculty of Veterinary Medicine, Aksaray University, Aksaray, Turkey.*
Phone: *+90 (382) 2882888*
E-mail: *haticeyyaren@gmail.com*

Introduction

Formaldehyde (CH_2O), an important member of the aldehyde family, is obtained as a liquid from methanol oxidation. Formaldehyde is a highly reactive, colorless, pungent, irritating, low molecular weight toxic gas due to its strong electrophilic properties. It can rapidly turn into gas at room temperature, burn and dissolve very well in water (Smith, 1992; Shaham et al., 1996). Formaldehyde is not stored in the body but is metabolized to formic acid in the liver and erythrocytes via the dehydrogenase enzyme. This chemical agent is excreted through urine and feces or by respiration as oxidized to carbon dioxide (Smith, 1992; Usanmaz et al., 2002). Formaldehyde is highly irritant to mucous membranes (Smith, 1992) and tends to combine strongly with proteins, nucleic acids, and unsaturated fatty acids in a non-enzymatic way. This combination creates denaturation in proteins, causing cytotoxicity, inflammatory reaction, necrosis, allergy, and mutagenic effects. It shows fixation function and antimicrobial activity in tissues that have lost vitality (Bolt, 1987; Heck & Casanova, 1999; Usanmaz et al., 2002).

Histology and pathology laboratories are where tissue samples are stained and examined under a microscope for sample evaluation. Exposure to formaldehyde used in these processes threatens the health of technicians, histologists, pathologists, anatomists and scientists working in the laboratory. Therefore, finding healthy and natural alternatives to formaldehyde is of utmost importance. Numerous studies have proven that honey can replace formaldehyde in routine histochemical and immunohistochemical staining procedures (Rahma & Bryant, 2006). However, honey is not widely available worldwide, and its high cost makes it unsuitable for practical use. Due to the high cost of formaldehyde and honey, it is important to search for substances that can overcome these shortcomings. Various studies conducted on honey, sugar and jaggery reveal that these natural fixatives fulfill almost all the requirements of an ideal fixative (Patil et al., 2013; Patil et al., 2015).

This study compared the staining properties of tissues fixed with date molasses, a natural sugar, using theMay-Grunwald-Giemsa (MGG) staining method with those fixed with buffered formol-saline. Using natural fixatives, we aim to derive a safe and pleasant working environment for healthcare professionals.

Materials and Methods

Experimental procedure

In this study, experimental animals were obtained from the Selçuk University Experimental Medicine Application and Research Center. Laboratory studies were carried out at Aksaray University Central Research Laboratory. Liver, spleen, kidney, skin, testicle, small intestine, large intestine, brain, cerebellum, and lung tissue samples of 4 rats of both genders were used as materials. Tissue samples were divided into two parts and fixed in 30% date molasses (group a) and 10% buffered formal saline (group B) for 24 hours at room temperature.

Preparation of fixative solutions

Group A was prepared by diluting date molasses obtained from the market with distilled water to a final % date sugar concentration of 30%. Since there is no study on date molasses in the literature, this ratio was determined experimentally. The sugar composition of date molasses obtained from the market was analyzed in the Konya Provincial Food Control Laboratory.

Group B consists of 10% buffered formaldehyde, made of 10% buffered formal saline solution with a pH of 7 prepared with 0.1 M phosphate buffer. After 24 hours of fixation, tissue samples were washed, dehydrated, and polished using known histologic techniques and then blocked in paraffin. MGG staining method was applied to 5 µm thick sections taken from the blocks. After the staining process, the preparations were covered with entellan and examined under a light microscope.

Results

Date molasses solution (30%)

Liver

Tissue and cell integrity were generally preserved. Hepatocytes were clearly distinguished. Maceration was observed in some parts of the bile ducts. The staining quality was good and clear (Figure 1a).

Spleen

Pale staining was observed. Red and white pulp areas were well differentiated (Figure 2a).

Kidney

Renal corpuscles and nephrons were well differentiated. The cell nucleus was well stained, but chromatin detail was not visible. The vessel walls were well stained (Figure 3a).

Skin

Pale staining was observed. Details were not distinguishable in hair follicles and epithelial tissue (Figure 4a).

Testicle

Tubules were stained quite well. However, Leydig cells could not be distinguished. The spermatozoa in the lumen were stained well (Figure 5a).

Small intestine

The lamina epithelial layer in the villus intestinalis was macerated. Tissue integrity was disrupted. However, cell nuclei were well differentiated (Figure 6a).

Large intestine

Tissue integrity was preserved and staining quality was quite good (Figure 7a).

Heart

Cardiac muscle and transverse bandings were well distinguished. The tissue was generally well stained. Cell nuclei in capillaries and endothelial cells were well differentiated (Figure 8a).

Brain

Pale staining was observed. However, the cell nucleus was well differentiated. The layers were clearly distinguished from each other (Figure 9a).

Cerebellum

Nerve fibers were observed. Purkinje cells were well differentiated. The layers were clearly distinguished from each other. Str. Granulosum layer was well stained (Figure 10a).

Lung

A very pale staining was observed. However, cell nuclei were well distinguished at high magnifications (Figure 11a).

Formol solution (10%)

Liver

Pale staining was observed. Chromatin distribution was evident in hepatocytes at high magnifications (Figure 1b).

Spleen

The tissue was stained quite well. Red and white pulp areas were well differentiated (Figure 2b).

Kidney

Renal corpuscles and cells forming nephron tubules were well stained. Chromatin distribution was evident (Figure 3b).

Skin

Epithelial tissue hair follicles and other layers of the skin were evident and well stained (Figure 4b).

Testicle

The cells in the tubules and chromatin distribution were quite evident. Tissue integrity was preserved and the cells were stained well (Figure 5b).

Small intestine

Although disintegration was observed in some villi, it was generally observed that the tissue was well stained (Figure 6b).

Large intestine

Tissue integrity and cellular lines were evident (Figure 7b).

Heart

Pale staining was observed. Transverse bandings were not well differentiated (Figure 8b).

Brain

Pale staining was observed. However, the layers were distinct, and nerve cells and nuclei were well differentiated (Figure 9b).

Cerebellum

Normal staining properties were observed. The layers were well differentiated. Neuron nuclei were well differentiated (Figure 10b).

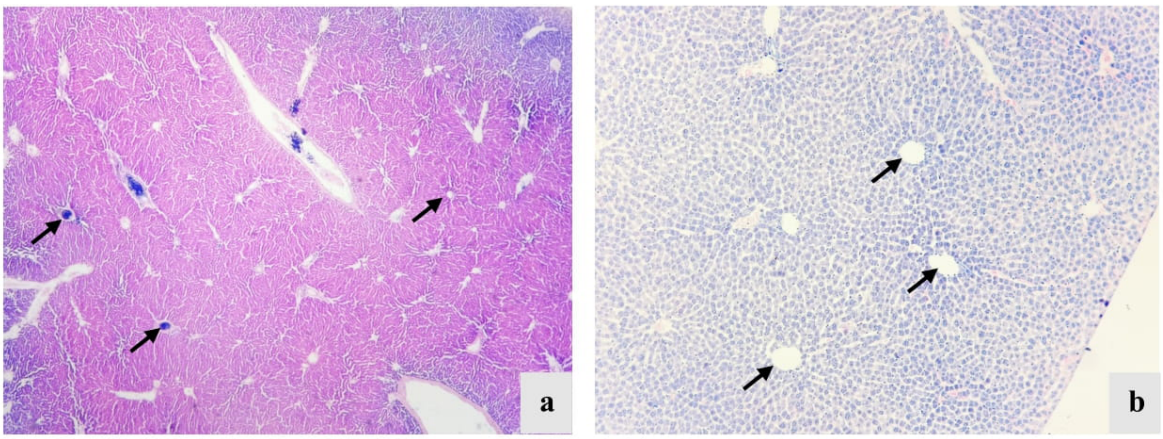

Figure 1. Liver, MGG staining method

a) The tissue fixed with date molasses solution (30%), b) The tissue fixed with formol solution (10%)

Notes: Vena. centralis: Arrows.

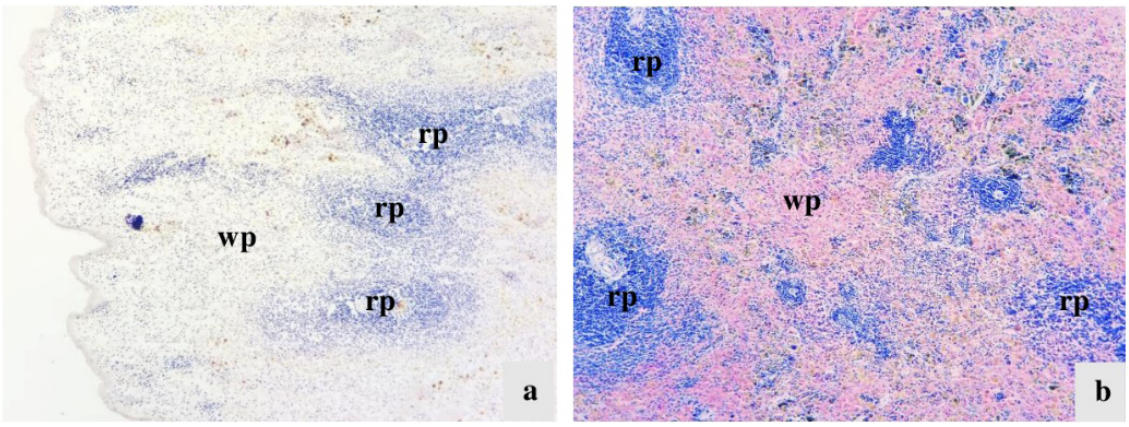

Figure 2. Spleen, MGG staining method

a) The tissue fixed with date molasses solution (30%), b) The tissue fixed with formol solution (10%)

Notes: Red pulp: Rp areas; White pulp: Wp areas.

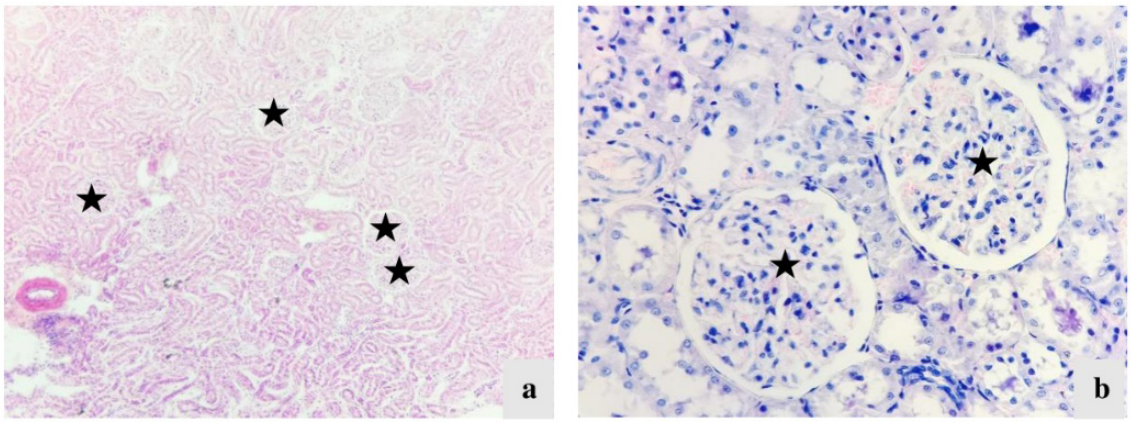

Figure 3. Kidney, MGG staining method

a) The tissue fixed with date molasses solution (30%), b) The tissue fixed with formol solution (10%)

Notes: Renal corpuscle: Stars.

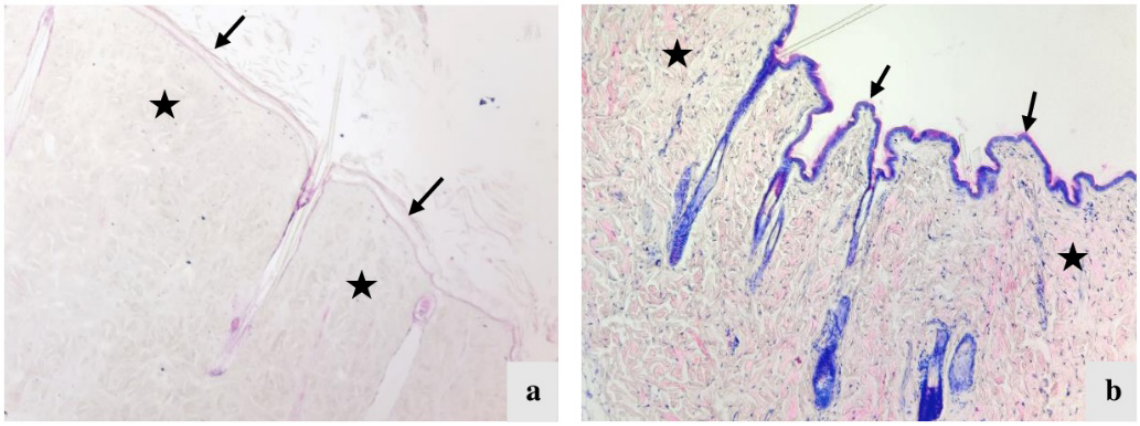

Figure 4. Skin, MGG staining method

a) The tissue fixed with date molasses solution (30%), b) The tissue fixed with formol solution (10%)

Notes: Epidermis: Arrows; Hypodermis: Stars).

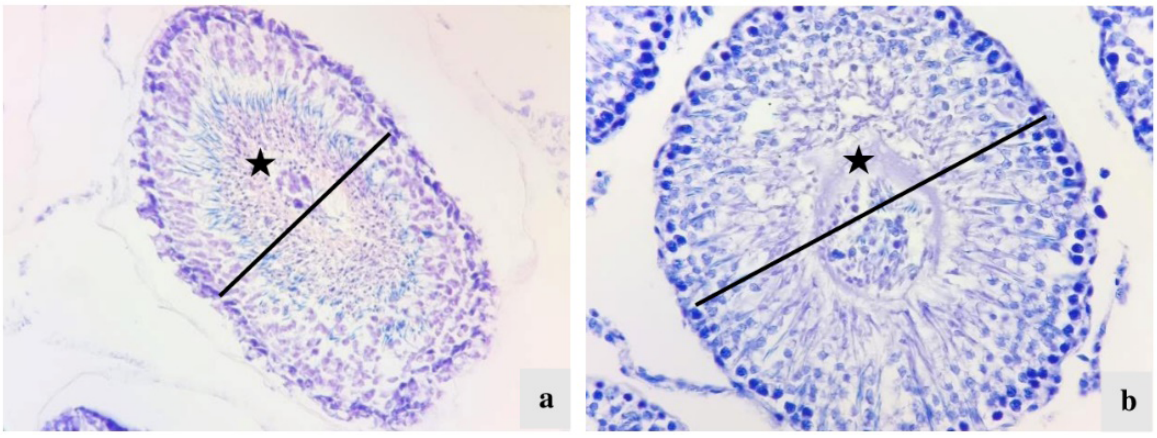

Figure 5. Testicle, MGG staining method

a) The tissue fixed with date molasses solution (30%), b) The tissue fixed with formol solution (10%)

Notse: Tubulus seminiferus contortus: Stars.

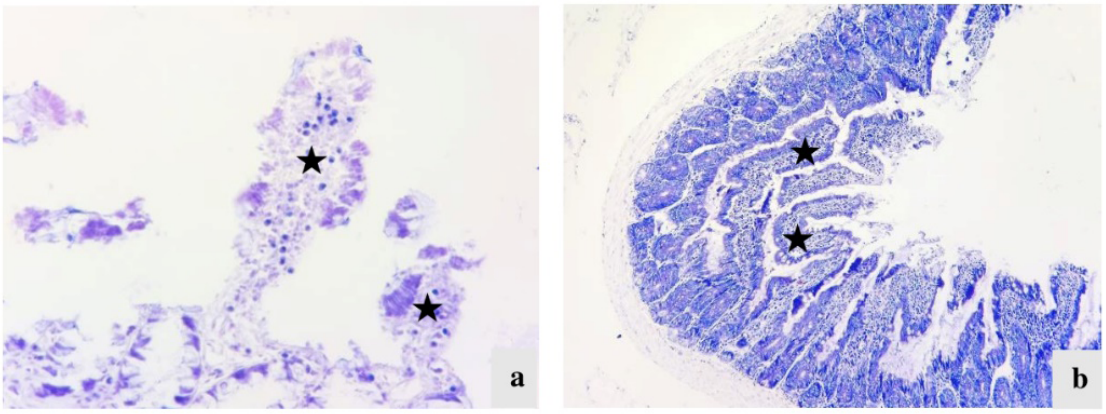

Figure 6. Small intestine, MGG staining method

a) The tissue fixed with Date Molasses Solution (30%), b) The tissue fixed with formol solution (10%)

Notes: Villus intestinalis: Stars.

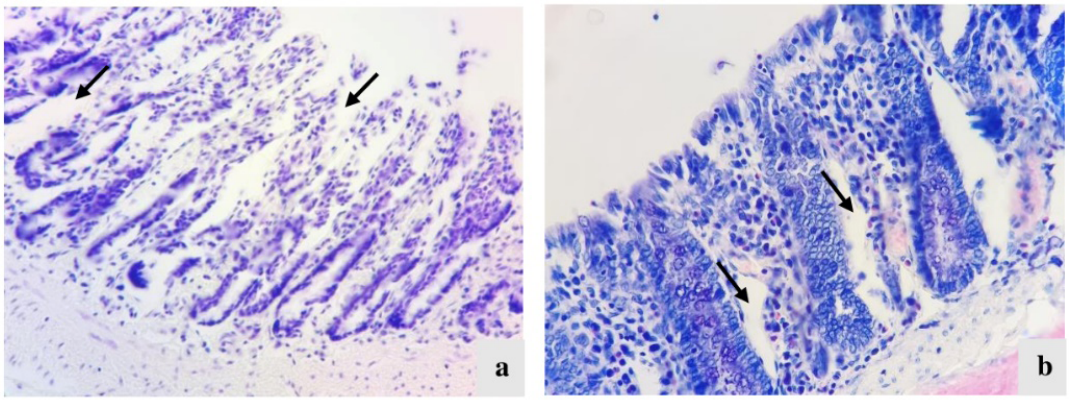

Figure 7. Large intestine, MGG staining method

a) The tissue fixed with date molasses solution (30%), b) The tissue fixed with formol solution (10%)

Notes: Intestinal crypts: Arrows.

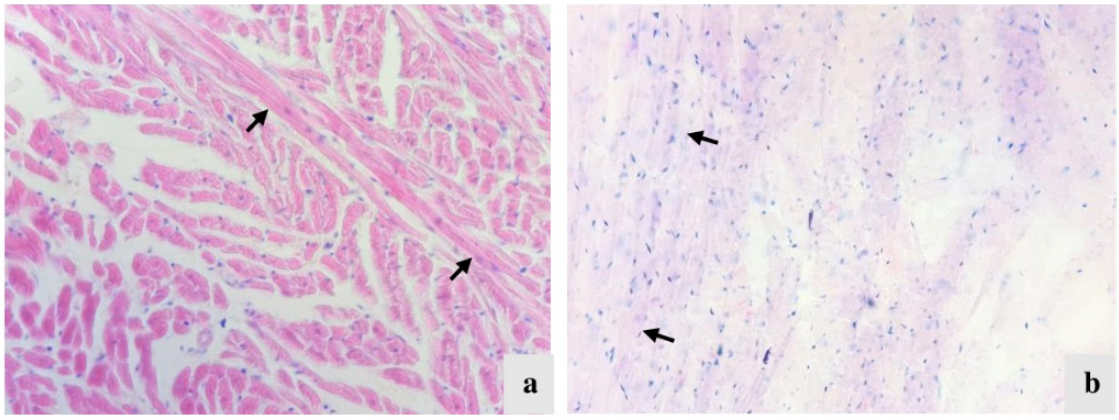

Figure 8. Heart, MGG staining method

a) The tissue fixed with date molasses solution (30%), b) The tissue fixed with formol solution (10%)

Notes: Transverse bandings: arrows.

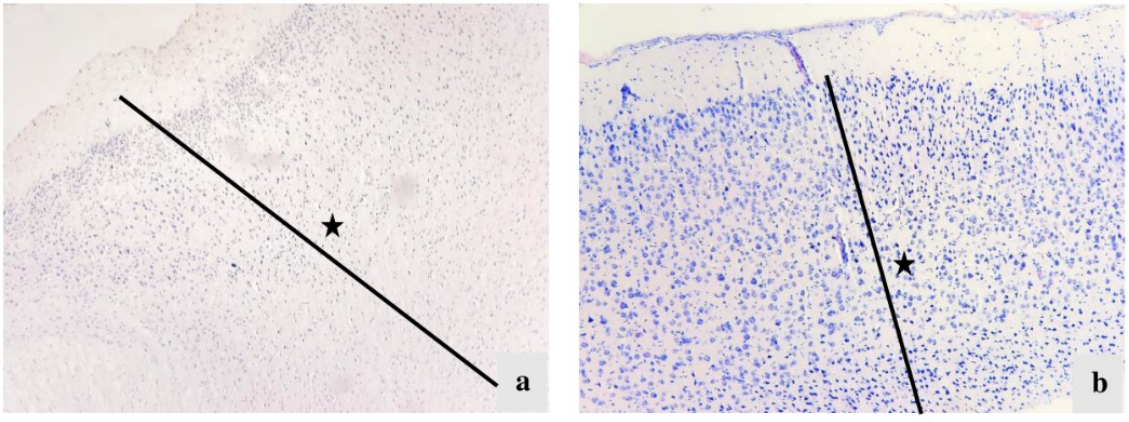

Figure 9. Brain, MGG staining method

a) The tissue fixed with date molasses solution (30%), b) The tissue fixed with formol solution (10%)

Notes: Neurons arranged in layers: Stars.

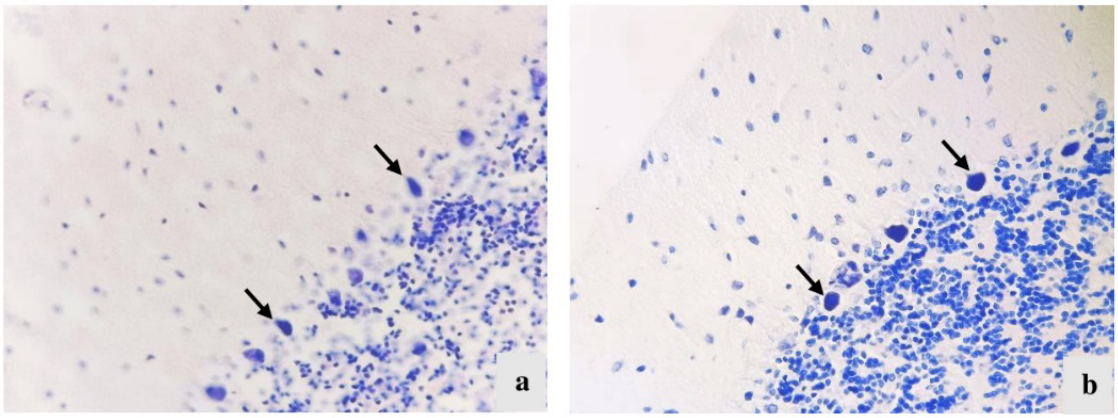

Figure 10. Cerebellum, MGG staining method

a) The tissue fixed with date molasses solution (30%), b) The tissue fixed with formol solution (10%)

Notes Purkinje cells: Arrows.

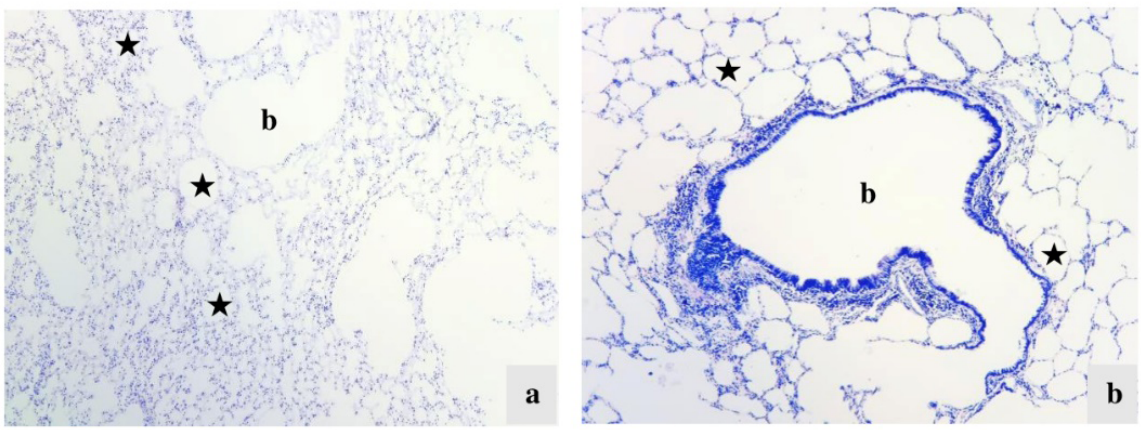

Figure 11. Lung, MGG staining method

a) The tissue fixed with date molasses solution (30%), b) The tissue fixed with formol solution (10%)

Notes: Bronchiole: b; Alveolus: Stars.

Lung

Normal staining properties were observed. Cell nuclei and chromatin distribution were observed very well (Figure 11b).

Discussion

Fixation is an initial and important step in preparing the tissue for microscopic examination. The primary purpose of fixation is to keep tissues closest to vitality, prevent bacterial destruction, prevent autolysis, and increase the index of better examination of the tissue.

In recent years, scientists have conducted several studies on the fixative properties of honey and jaggery. Generally, fixatives with low pH do not preserve cytoplasmic organelles; however, they act as suitable nuclear fixatives. For centuries, honey has been known to have anti-bacterial, acidic and dehydrating properties. The anti-autolysis and tissue hardening, wound healing and anti-bacterial properties of honey have been emphasized in studies (Sabarinath et al., 2014). These findings show that honey is an excellent fixative and a good preservative. Lalwani et al. compared the fixative properties of formaldehyde with processed and unprocessed honey in oral tissues (Lalwani et al., 2015). The fixation and staining quality of processed and unprocessed honey were evaluated in terms of staining efficiency in parallel with neutral buffered formaldehyde. The staining quality of the nucleus and cytoplasm and evaluation of tissue morphology were determined as 100%, 92% and 75%, respectively. The study results indicate that processed

and unprocessed honey are safe to use as an alternative to formaldehyde (Lalwani et al., 2015). Singh et al. (2015) analyzed the fixation efficiency on cytological smear samples using ethanol and 20% unprocessed honey and compared the efficiency between the two fixatives. The results showed that the honey-fixed smear was adequately fixed compared to the ethanol-fixed smear. They concluded that both ethanol-fixed and honey-fixed smears were equal and that honey could be used safely to replace ethanol (Singh et al., 2015). Sabarinath et al. (2014) conducted a study to determine the effectiveness of honey as a fixative by comparing honey and formaldehyde. The study showed that nuclear details in honey and formaldehyde-fixed samples were similar, with no difference in staining and microscopic morphology. However, cytoplasmic staining was sufficient to ensure the integrity of the tissue. No changes in the cytoplasm of epithelial cells and connective tissue cytoplasm showed good staining by H&E with a complete homogenization effect on collagen fibers.

Patil et al. (2013) compared the tissue fixation properties of 20% honey, 20% sugar syrup and 30% jaggery (dark brown unrefined sugar obtained from date) with 10% buffered formaldehyde by staining with hematoxylin-eosin (H&E). They found that the fixation of jaggery was excellent, and tissue sections showed good overall morphology, nuclear-cytoplasmic details, and staining in clearly distinguishable cellular outlines. In another study, Patil et al. (2015) examined the fixative properties of 30% jaggery and 20% honey for 6 months (10% buffered formaldehyde was used as control). They stained the results with H&E, periodic acid schiff (PAS) and Masson-trichrome (MT). They evaluated the suitability of the fixatives. At the end of 6 months, they reported that all three stained sections (H&E, PAS, MT) had the same staining quality as formaldehyde-fixed tissues. Numerous studies have proven that honey replaces formalin in routine histochemical and immunohistochemical staining procedures (Rahma & Bryant, 2006).

A study done by Pandiar et al. (2017) reported a better clarity of staining with honey-fixed smears. When nuclear and cytoplasmic staining characteristics were compared, they found honey slightly better than ethanol and jaggery. However, there was no overall significant difference between the three fixatives. The study done by Sona et al. on buccal smears also showed no statistically significant differences ($P>0.05$) between the two fixatives based on the above-mentioned cytological parameters (Sona & Preethamol, 2017).

Nerune et al. (2018) compared the fixative properties of 95% ethyl alcohol and 20% honey on buccal mucosa. They concluded that 20% processed honey could be used efficiently in cytological smear fixation to preserve cellular details.

Priyadarshi et al. (2022) compared smears fixed in 20% honey as a cytological fixative with 95% ethyl alcohol and found a strong agreement between both fixatives (kappa value varying between 0.896 and 0.942) and a $P<0.05$.

In their study to evaluate the effectiveness of 20% honey and 20% jaggery as a fixative for oral exfoliative cytology, Sah et al. (2022) reported that a low concentration of honey is an excellent alternative to ethanol (95%) and jaggery as a fixative for oral exfoliative cytological samples.

In this study, the tissues fixed with 30% date molasses and those fixed with buffered formal saline were examined histologically using the MGG staining method. The use of date molasses as a fixative was not found in the literature. Again, in the study, it was seen that the data obtained from the tissues fixed with formalin-saline were in agreement with the data in the control groups of previous studies (Al-Aaraji & Addi Ali, 2022; Almuttairi, 2023; Hamza Fares et al., 2022; Othman et al., 2023).

In this study, the MGG staining quality of tissues fixed with 30% date molasses was compared with those fixed with buffered formal-saline. When all tissues were evaluated anatomically, the general morphology of the tissues showed preserved tissue integrity. There was no color change in the tissues. In addition, tissue hardness was at the required level, supporting previous studies.

Conclusion

As a result, in terms of chromatin distribution, nucleus separation, and cytoplasm staining, the tissues fixed with date molasses and the tissues fixed with buffered formal saline showed similar properties, except for the tissues subjected to intense maceration (skin and small intestines). Pale staining was observed in some tissues fixed with buffered formal saline (liver, skin, heart), but the nucleus and cytoplasm structures were better distinguished at higher magnifications. Considering the chromatin distribution, nuclear separation, and cytoplasm staining in other tissues (spleen, kidney, testicle, large intestine, brain, cerebellum and lung), tissues fixed with date molasses showed similar properties to tissues fixed with buffered formal-saline. For researchers interested in the

subject, we recommend extending the fixation period (6 months) and comparing the fixation effect on the tissues with formalin in this long period because a good fixative is expected to preserve tissues well for long periods.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of Selcuk University Experimental Medicine (Code: 2017-14).

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Conflict of interest

The author declared no conflict of interest.

References

Al-Aaraji, A. S., Addi Ali, B. (2022). Effect of pomegranate peels aqueous extract on the histological structure of small intestine of local male rabbits (Oryctolagus cuniculus). *Archives of Razi Institute, 77*(5), 1935-1943. [DOI:10.22092/ari.2022.358197.2176]

Almuttairi, R. S. (2023). The effects of metformin treatment on diabetic albino rats pancreas, liver, and kidney histology. *Razi Vaccine & Serum Research Institute, 78*(1), 459-463. [DOI:10.22092/ARI.2022.359289.2394]

Bolt H. M. (1987). Experimental toxicology of formaldehyde. *Journal of Cancer Research and Clinical Oncology, 113*(4), 305–309. [DOI:10.1007/BF00397713] [PMID]

Hamza Fares, B., Abdul Hussain Al-tememy, H., Mohammed Baqir Al-Dhalimy, A. (2022). Evaluation of the toxic effects of aluminum hydroxide nanoparticles as adjuvants in vaccinated neonatal mice. *Razi Vaccine & Serum Research Institute, 77*(1), 221-228. [DOI:10.22092/ARI.2021.356418.1839]

Heck, H., & Casanova, M. (1999). Pharmacodynamics of formaldehyde: Applications of a model for the arrest of DNA replication by DNA-protein cross-links. *Toxicology and Applied Pharmacology, 160*(1), 86–100. [DOI:10.1006/taap.1999.8764] [PMID]

Lalwani, V., Surekha, R., Vanishree, M., Koneru, A., Hunasgi, S., & Ravikumar, S. (2015). Honey as an alternative fixative for oral tissue: An evaluation of processed and unprocessed honey. *Journal of Oral and Maxillofacial Pathology, 19*(3), 342–347. [DOI:10.4103/0973-029X.174641] [PMID] [PMCID]

Nerune, S. M., Khan, M. N., Potekar, R. M., Patil, V. (2018). Natural versus synthetic fixative in oral cytological smears-A double blind study. *Indian Journal of Pathology and Oncology, 5*(4), 663-6. [DOI:10.18231/2394-6792.2018.0126]

Othman, G. Q., Mustafa, T. A., Ismael, H. D., Ali, S. S., Abdullah, H. H., & Abdullah, B. U. (2023). A comparative investigation applying testicular fine needle aspiration cytology and open testicular biopsy histology for the diagnosis of azoospermia and severe oligospermia. *Archives of Razi Institute, 78*(4), 1343–1348. [DOI:10.32592/ARI.2023.78.4.1343] [PMID] [PMCID]

Patil, S., Premalatha, B., Rao, R. S., & Ganavi, B. (2013). Revelation in the field of tissue preservation-A preliminary study on natural formalin substitutes. *Journal of International Oral Health, 5*(1), 31–38. [PMID]

Patil, S., Rao, R. S., Ganavi, B. S., & Majumdar, B. (2015). Natural sweeteners as fixatives in histopathology: A longitudinal study. *Journal of Natural Science, Biology, and Medicine, 6*(1), 67–70. [DOI:10.4103/0976-9668.149089] [PMID] [PMCID]

Pandiar, D., Baranwal, H. C., Kumar, S., Ganesan, V., Sonkar, P. K., & Chattopadhyay, K. (2017). Use of jaggery and honey as adjunctive cytological fixatives to ethanol for oral smears. *Journal of Oral and Maxillofacial Pathology, 21*(2), 317. [DOI:10.4103/jomfp.JOMFP_224_15] [PMID] [PMCID]

Priyadarshi, A., Kaur, R., Issacs, R. (2022). Honey as a cytological fixative: A comparative study with 95% alcohol. *Cureus, 14*(8), 2-11. [DOI:10.7759/cureus.28149]

Rahma, A. M., Bryant, P. (2006). The Effectiveness of honey as a substitute for formalin in the histological fixation of tissue. *Journal of Histotechnology, 29*(3), 173-6. [DOI:10.1179/014788806794775946]

Sabarinath, B., Sivapathasundharam, B., Sathyakumar, M. (2014). Fixative properties of honey in comparison with formalin. *Journal of Histotechnology, 37*(1), 21-25. [DOI:10.1179/2046023613Y.0000000037]

Sah, K., Janardhana Amaranath, B. J., Chandra, S., Ahmad, S. (2022). Utilization of processed honey and jaggery as an oral cyto-fixative. *Indian Journal Dental Science, 14*(1), 6-10. [DOI:10.4103/IJDS.IJDS_180_20]

Shaham, J., Bomstein, Y., Meltzer, A., Kaufman, Z., Palma, E., & Ribak, J. (1996). DNA--protein crosslinks, a biomarker of exposure to formaldehyde--in vitro and in vivo studies. *Carcinogenesis, 17*(1), 121–125. [DOI:10.1093/carcin/17.1.121] [PMID]

Singh, A., Hunasgi, S., Koneru, A., Vanishree, M., Ramalu, S., & Manvikar, V. (2015). Comparison of honey with ethanol as an oral cytological fixative: A pilot study. *Journal of Cytology, 32*(2), 113–117. [DOI:10.4103/0970-9371.160563] [PMID] [PMCID]

Smith A. E. (1992). Formaldehyde. *Occupational Medicine, 42*(2), 83–88. [DOI:10.1093/occmed/42.2.83] [PMID]

Sona, M., Preethamol, S. (2017). A comparative study on the efficacy of honey and ethanol as cytological fixative. *International Journal of Advances in Medicine, 4*(3), 762-7. [DOI:10.18203/2349-3933.ijam20172268]

Usanmaz, S. E., Akarsu, E. S., & Vural, N. (2002). Neurotoxic effects of acute and subacute formaldehyde exposures in mice. *Environmental Toxicology and Pharmacology, 11*(2), 93–100. [DOI:10.1016/s1382-6689(01)00109-0] [PMID]

This Page Intentionally Left Blank