Effectiveness of May Grunwald Giemsa staining on tissues fixed with sugar cane molasses

Research Article

ABSTRACT

Formaldehyde commonly used in laboratories for fixation of tissues is found in many areas, including industry, household materials, dentistry coatings manufacturin. Sugar cane molasses, a very viscous product with a long shelf life, is normally produced by boiling the water obtained from sugar cane without adding any other additives. In this study, we aimed to compare the potential impact of the both fixation solusions of buffered formol-saline and low-cost sugar cane molasses on rat biosystem tissues stained with May Grunwald Giemsa. Liver, spleen, kidney, skin, testicle, small intestine, large intestine, brain, cerebellum and lung tissue samples of 4 healthy adult rats of both genders were used as materials. Tissue samples were divided into two parts and fixed in 30% sugarcane molasses (Group A) and 10% buffered formal-saline (Group B) for 24 hours at room temperature. The collected tissues evaluated in terms of chromatin distribution, nucleus separation and cytoplasm staining. As a result, intense cell loss was observed in the skin and small intestines. Considering the chromatin distribution, nucleus separation and cytoplasm staining in other tissues (liver, spleen, kidney, testis, large intestine, brain, cerebellum and lung), tissues fixed with sugarcane molasses showed similar properties to tissues fixed with buffered formal-saline.

Keywords: Fixation, formaldehyde, histology, May Grunwald Giemsa, sugar cane molasses

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Article info

Submission: 10-06-2024
Accepted: 18-08-2024
Online First: 20-08-2024
Publication: 22-08-2024

e-ISSN: 2548-1150
doi prefix: 10.31797/vetbio
http://dergipark.org.tr/vetbio

INTRODUCTION

Formaldehyde (CH_2O), an important member of the aldehyde family, is obtained as a liquid from the oxidation of methanol. Formaldehyde is a colorless, pungent, irritating, low molecular weight poisonous gas with a highly reactive property due to its strong electrophilic property, which can rapidly turn into gas at room temperature, burn, dissolves very well in water (Shaham et al., 1996; Smith, 1992). Formaldehyde taken into the organism from outside is not stored in the body. Formaldehyde is metabolized to formic acid in the liver and erythrocytes via the dehydrogenase (FDH) enzyme, and 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 their vitality (Bolt, 1987; Heck and Casanova, 1999; Usanmaz et al., 2002).

Molasses (Grape molasses), a traditional Turkish food, is produced from fresh or dried grapes, as well as mulberry, fig, apple, plum, carob,

How to cite this article

Yaren Kuloğlu H., Yahyazadeh A., (2024). Effectiveness of May Grunwald Giemsa staining on tissues fixed with sugar cane molasses. *Journal of Advances in VetBio Science and Techniques*, *9*(2), 157-165. https://doi.org/10.31797/vetbio.1499149

watermelon, sugar cane and sugar millet. Although the composition elements of molasses vary in comparsion with the fruit composition, the fundamental component is carbohydrate (Simsek and Artık, 2002). Molasses is a great source of vitamins such as thiamine (B1), pyridoxine (B6) and niacin (B3), which are necessary for healthy life (Batu, 1993).

In addition to its global utilization in the sugar industry, sugar cane molasses is also manufactured within our country, particularly in the Adapazarı province. Due to its high sugar concentration, it is easier to extract molasses from sugar cane because of (Batu, 2006). Sugar cane molasses has also been found to possess a high amounts of sugar, minerals and organic acids (Batu, 1993; Batu, 2001).

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, there is a significant need to find healthy and natural alternatives to the use of formaldehyde. Numerous studies have proven that honey can replace formaldehyde in routine histochemical and immunohistochemical staining procedures (Lalwani et al., 2015; Nerune et al., 2018; Pandiar et al., 2017; Priyadarshi et al., 2022; Rahma and Bryant, 2006; Sah et al., 2022; Singh et al., 2015). However, honey is not widely available all over the world, so its high cost makes it unsuitable for practical use. Therefore, due to the high cost of formaldehyde and honey, it is important to search for substances that can overcome these shortcomings. Various studies conducted to date on honey, sugar and sugarcane reveal that these natural fixatives fulfill almost all the requirements of an ideal fixative (Patil et al., 2013; Patil et al., 2015).

In this study, the staining properties of tissues fixed with low-cost sugar cane molasses, which is a natural sugar and produced organically in Turkey, shown by May Grunwald Giemsa (MGG) staining method were compared with the staining properties of tissues fixed with buffered formol-saline.

MATERIALS AND METHODS

Experimental Procedure

In this study, experimental animals were obtained from Selçuk University Experimental Medicine Application and Research Center (dated 21.03.2017; decision no. 2017-14). Laboratory studies were carried out at Selçuk University Central Research Laboratory. Liver, spleen, kidney, skin, testicle, small intestine, large intestine, brain, cerebellum and lung tissue samples of 4 healthy adult rats of both genders were used as materials. Tissue samples were divided into two parts and fixed in 30% sugarcane molasses (Group A) and 10% buffered formal-saline (Group B) for 24 hours at room temperature. The collected tissues will be evaluated in terms of chromatin distribution, nucleus separation and cytoplasm staining.

Preparation of Fixative Solutions

Group A - 30% Sugar Cane Molasses - Organically produced sugar cane molasses from the market was diluted with distilled water to a concentration of 30%. The sugar content of the product used was examined in Konya Food Control Laboratory and it was reported that it only naturally contains fructose and glucose.

Group B - 10% buffered formal saline - 10% buffered formal-saline solution with a pH of 7 was prepared with 0.1 M phosphate buffer.

Histological Procedure

After 24 hours of fixation, tissue samples were washed, dehydrated and polished with known histologic techniques and blocked in paraffin. May Grunwald Giemsa (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 (Olympus, CX23).

RESULTS

Sugarcane Molasses Solution (30%)

Liver: Pale staining was observed. However, hepatocytes were clearly distinguished (Figure 1a).

Spleen: Pale staining was observed. Red and white pulp areas were distinguished from each other. Cell nuclei were prominent at high magnifications (Figure 2a).

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

Skin: Pale staining was observed. Cellular details in the hair follicle and epithelial tissue were not well defined (Figure 4a).

Testicle: Tubules were well stained. However, Leydig cells could not be distinguished. The spermatozoa in the lumen were quite prominent. Cytoplasm, nucleus and chromatin distribution in the cells were well distinguished (Figure 5a).

Small intestine: The lamina epithelialis layer of the vilus intestinalis was not clear. Tissue integrity was disrupted. However, cell nuclei were well differentiated in non- macerated tissues (Figure 6a).

Large intestine: Tissue integrity was preserved and staining quality was quite good. However, cellular loss was observed in the connective tissue. The cytoplasm and nuclei were well stained and chromatin distribution was evident (Figure 7a).

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

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

Cerebellum: Nerve wires were clearly observed. Purkinje cells were easily distinguished. The appearance of the layers was distinct. Str. Granulosum layer was well stained (Figure 10a).

Lung: A very pale staining was observed. However,bronchi and bronchioles were easily distinguished from each other. Cell nuclei were evident at high magnifications (Figure 11a).

10% Buffered Formal-Saline Solution

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

Spleen: The tissue was well stained. Red and white pulp areas were well differentiated from each other (Figure 2b).

Kidney: Pale staining of the cytoplasm was observed, but cell nuclei were quite prominent. Chromatin distribution was well differentiated (Figure 3b).

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

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

Small intestine: Although disintegration was observed in some villi, the tissue was generally well stained (Figure 6b).

Large intestine: Tissue integrity and cellular lines were quite evident (Figure 7b).

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

Brain: The layers were quite distinct and nerve cells and nuclei were well differentiated (Figure 9b).

Cerebellum: Purkinje cells and nerve fibers were well differentiated. Cell nucleus and chromatin distribution was evident (Figure 10b).

Lung: Good staining was observed. Bronchial and bronchiolar epithelia were well stained. Cell nucleus and chromatin distribution were evident (Figure 11b).

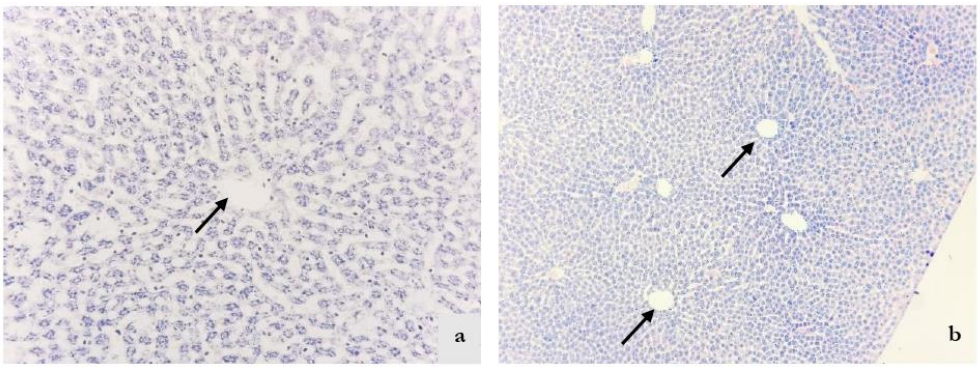

Figure 1. Liver, My-Grünwalt Giemsa (MG) staining method. a) The tissue fixed with Sugarcane Molasses Solution (30%), X40, b) The tissue fixed with Formol Solution (10%), vena centralis (arrows), X10.

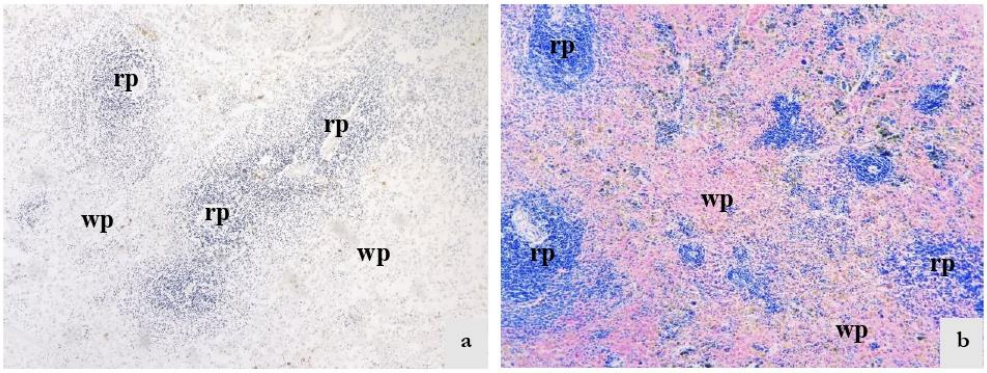

Figure 2. Spleen, My-Grünwalt Giemsa (MG) staining method. a) The tissue fixed with Sugarcane Molasses Solution (30%), X40, b) The tissue fixed with Formol Solution (10%), Red pulp (rp), white pulp (wp) areas, X40.

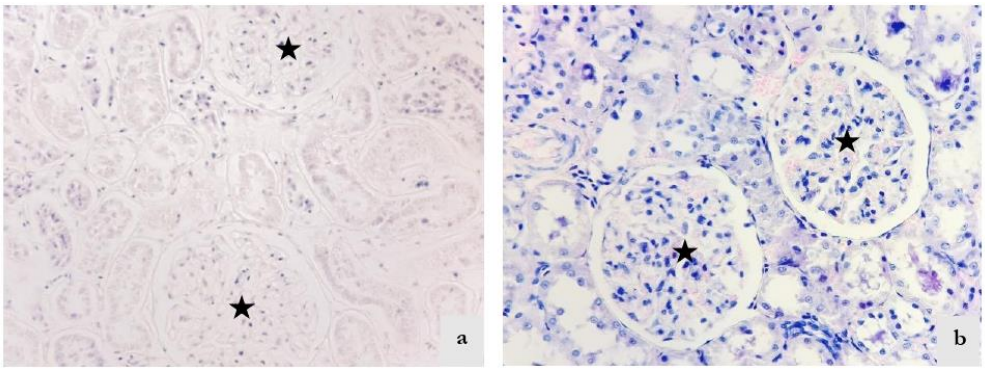

Figure 3. Kidney, My-Grünwalt Giemsa (MG) staining method. a) The tissue fixed with Sugarcane Molasses Solution (30%), X40, b) The tissue fixed with Formol Solution (10%), Renal corpuscle (stars), X40.

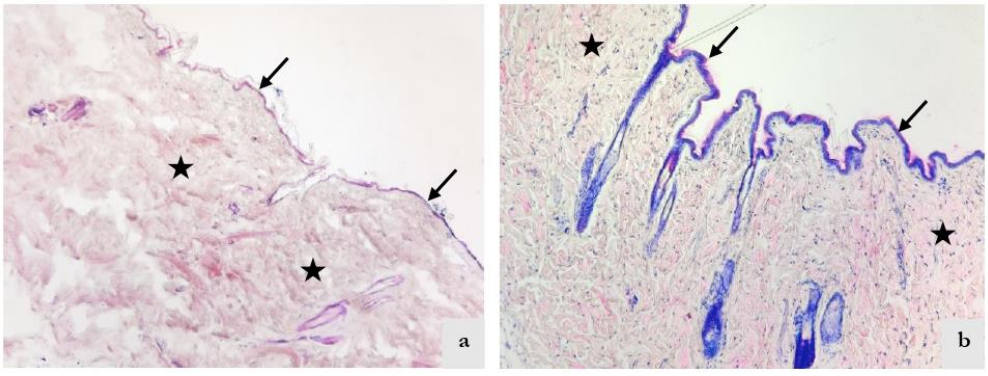

Figure 4. Skin, My-Grünwalt Giemsa (MG) staining method. a) The tissue fixed with Sugarcane Molasses Solution (30%), X4, b) The tissue fixed with Formol Solution (10%), Epidermis (arrows), hypodermis (stars), X4.

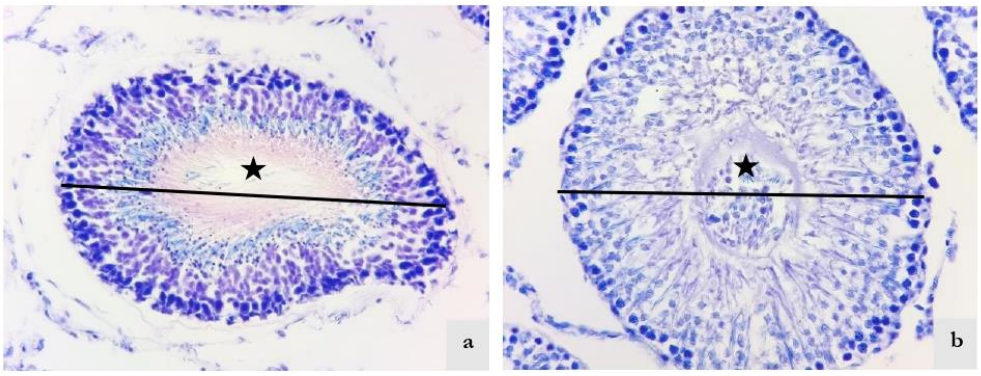

Figure 5. Testicle, My-Grünwalt Giemsa (MG) staining method. a) The tissue fixed with Sugarcane Molasses Solution (30%), X10, b) The tissue fixed with Formol Solution (10%), Tubulus seminiferus contortus (stars), X10.

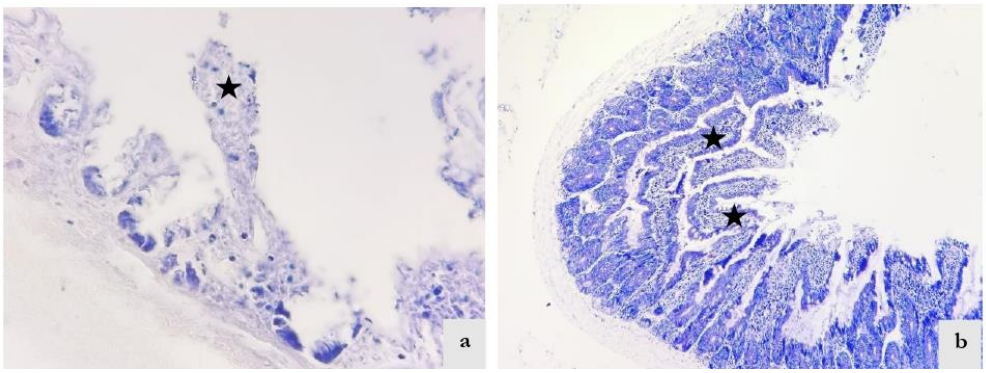

Figure 6. Small intestine, My-Grünwalt Giemsa (MG) staining method. a) The tissue fixed with Sugarcane Molasses Solution (30%), X10, b) The tissue fixed with Formol Solution (10%), villus intestinalis (stars), X10.

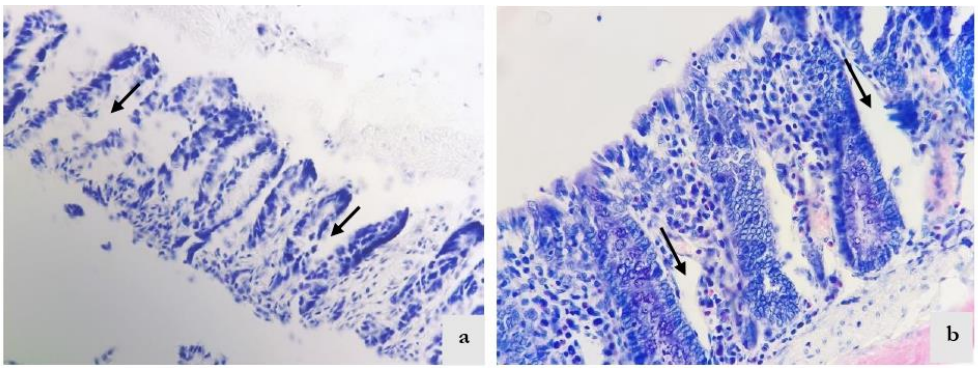

Figure 7. Large intestine, My-Grünwalt Giemsa (MG) staining method. a) The tissue fixed with Sugarcane Molasses Solution (30%), X10, b) The tissue fixed with Formol Solution (10%), Intestinal crypts (arrows), X10.

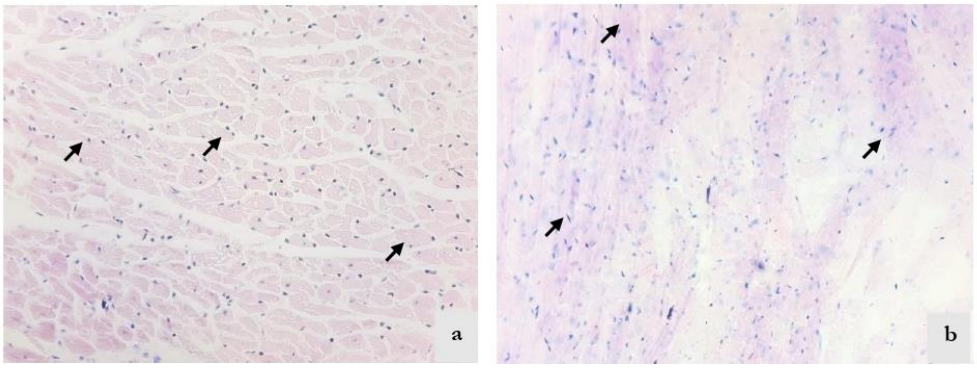

Figure 8. Heart, My-Grünwalt Giemsa (MG) staining method. a) The tissue fixed with Sugarcane Molasses Solution (30%), X10, b) The tissue fixed with Formol Solution (10%), transverse and longitudinal muscle fibers (arrows), X10.

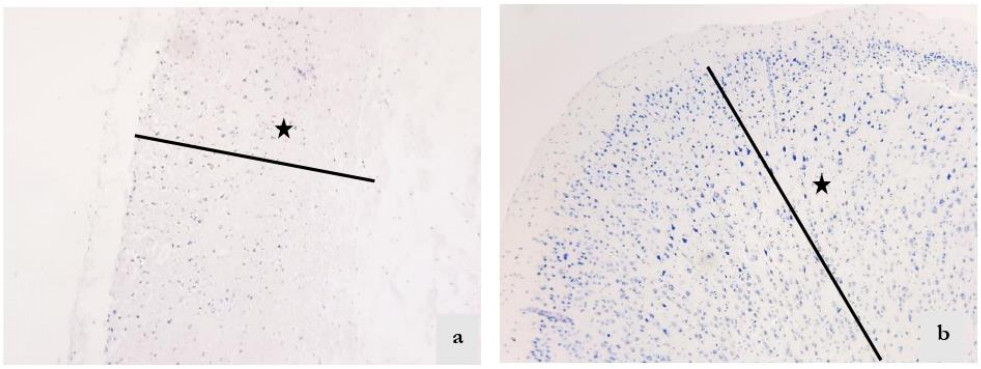

Figure 9. Brain, My-Grünwalt Giemsa (MG) staining method. a) The tissue fixed with Sugarcane Molasses Solution (30%), X10, b) The tissue fixed with Formol Solution (10%), Neurons arranged in layers (stars), X10.

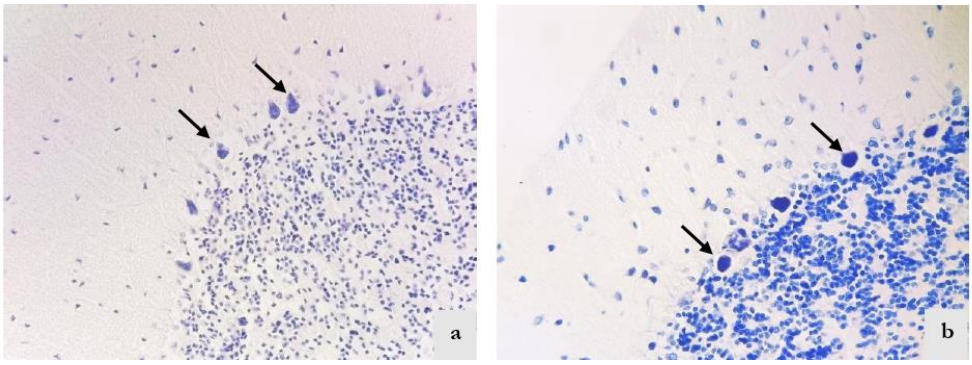

Figure 10. Cerebellum, My-Grünwalt Giemsa (MG) staining method. a) The tissue fixed with Sugarcane Molasses Solution (30%), X40, b) The tissue fixed with Formol Solution (10%), Purkinje cells (arrows), X40.

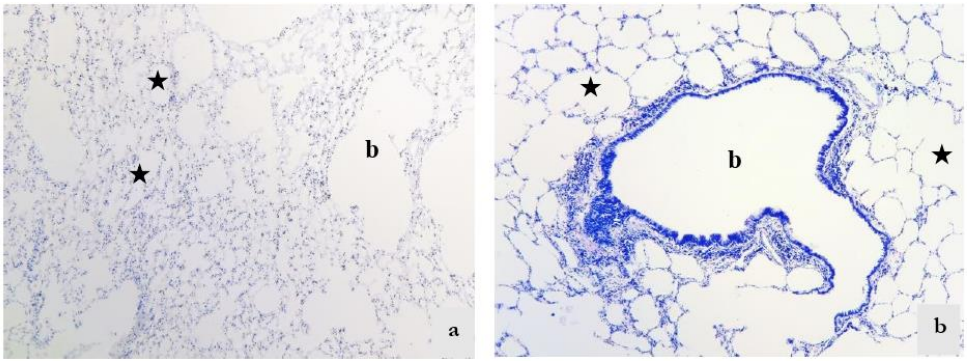

Figure 11. Lung, My-Grünwalt Giemsa (MG) staining method. a) The tissue fixed with Sugarcane Molasses Solution (30%), X10, b) The tissue fixed with Formol Solution (10%), Bronchiole (b), alveolus (stars), X40

DISCUSSION

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

In recent years, scientists have conducted a number of studies on the fixative properties of honey and sugar cane. Generally, fixatives with low pH do not support the preservation of cytoplasmic organelles; however, they act as a good nuclear fixative. Honey has been known to have anti-bacterial, acidic and dehydrating properties for several centuries. The anti-autolysis and tissue hardening, wound healing and anti-bacterial properties of honey have been emphasized in studies (Sabarinath et al., 2014). These show that honey is a very good fixative as well as 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, cytoplasm and evaluation of tissue morphology were determined as 100%, 92% and 75%, respectively. The results of the study indicate that processed honey and unprocessed honey are safe to use as an alternative for formaldehyde (Lalwani et al., 2015). Singh et al., (2015) analyzed the fixation efficiency on cytological smear samples using ethanol and 20% unprocessed honey. They also 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- and honey-fixed smears were equal to each other 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 results of the study showed that nuclear details in both 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 were seen in the cytoplasm of epithelial cells and connective tissue cytoplasm showed good staining by H&E with complete homogenization effect on collagen fibers (Sabarinath et al., 2014).

Patil et al., (2013) compared the tissue fixation properties of 20% honey, 20% sugar syrup and 30% sugar cane syrup (dark brown unrefined sugar obtained from sugarcane) with 10% buffered formaldehyde by staining with Hematoxylin Eosin (H&E). They found that the fixation of sugarcane molasses was excellent and tissue sections showed good overall morphology, nuclear, cytoplasmic details and staining in clearly distinguishable cellular outlines (Patil et al., 2013). In another study, Patil et al., (2015) examined the fixative properties of 30% cane sugar and 20% honey for 6 months (10% buffered formaldehyde was used as control) and stained the results with Hematoxylin Eosin (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 (Patil et al., 2015).

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

Priyadarshi et al., (2022) They 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 value of <0.05 (Priyadarshi et al., 2022).

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

In this study, May Grunwald Giemsa staining quality of tissues fixed with 30% sugar cane molasses was compared with tissues fixed with buffered formal-saline. When all tissues were evaluated anatomically, the general morphology of the tissues showed that they preserved tissue integrity and that there was no color change in the tissues. In addition, tissue stiffness was at the required level, supporting previous studies.

CONCLUSION

In line with the histological findings, considering chromatin distribution, nucleus separation and cytoplasm staining, tissues fixed with sugarcane

molasses showed similar properties to tissues fixed with buffered formal-saline (except for the skin and small intestine, where intense cellular loss was observed). We believe that this study will be a resource for researchers who will study this subject, as it is the first study conducted with this staining (May Grunwald Giemsa) in the literature.

ACKNOWLEDGMENT

Financial support: This study was carried out without support from any organization.

Conflict of interest: The authors have no conflicts of interest to report.

Ethical statement or informed consent: This study was carried out at Selçuk University Reserch Animals Application Center. This research was approved by Selçuk University Experimental Medicine Application and Research Center Animal Experiments Ethics Committee (SÜDAM, Ref No: 2017-14, Date: 03/2017).

Author contributions: The research idea, obtaining materials, various processes in the laboratory and evaluation of the results were carried out by HYK and AY.

Availability of data and materials: Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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