World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Storage solutions containing tea tree oil may be alternative cadaver detection and storage solution

Selinay BORAZAN [1], Murat Sırrı AKOSMAN [2], Recep KARA [3,*] and Abdurrahman Fatih FİDAN [1]

[1] *Department of Biochemistry, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Türkiye.*
[2] *Department of Anatomy, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Türkiye.*
[3] *Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Türkiye.*

World Journal of Advanced Research and Reviews, 2025, 25(03), 1120-1129

Publication history: Received on 05 February 2025; revised on 11 March 2025; accepted on 14 March 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.25.3.0825

Abstract

Cadaver education in health sciences is very important for the professional development of the student. Dead human or animal bodies are used for cadaver training. It is aimed to be used for many years by exposing the bodies to various chemicals immediately after they die, purifying them from microbes and ending autolysis. The chemical formaldehyde is the most common and most easily applicable chemical for this process today. Although widely used, formaldehyde has negative effects on students, employees and the environment. These problems have pushed scientists into different pursuits. In this study, both a cadaver fixation and a cadaver storage solution that can be used in a healthy way instead of formaldehyde were investigated. As a result of the literature review, a solution composition consisting of a mixture of borax, nitrate, nitrite, glycerin, alcohol and thyme oil was created. Half of the seven kidneys, which were brought from the slaughterhouse and divided into two equal halves longitudinally, were left in this solution, and the other half was placed in the 10% formaldehyde solution used for cadaver storage for fixation for one month. When the kidneys were examined after one month, it was observed that the microbial growth in the solution remained low enough not to cause any deterioration. While color changes were at better levels than formaldehyde, texture profile analysis values were found to be closer to fresh kidney tissue. Although the tissue can be recognized at any magnification in histological examinations, it was observed that the cell nuclei received less dye in some sections at x100 magnification. As a result, it was concluded that this solution can be used as a cadaver fixation and storage solution, but more research is needed on it.

Keywords: Cadaver; Fixation; Formaldehyde; Tea tree oil

1. Introduction

It is desired that the cadaver to be used in the training process has the tissue softness of a living body, does not contain microbial risks and can maintain its form for a long time. Therefore, solutions used for cadaver detection are expected to provide these properties. The quality of a cadaver is evaluated based on criteria such as hardness, color, odor and structure, and it is desired that it be perfect and receive full marks in terms of these features. These criteria not only show how close the cadaver detection is to real tissue, but also reinforce the quality of the cadaver [1].

Formaldehyde does not fully meet the desired properties of a cadaver and problems such as discoloration, bad odor and hardness may occur in cadavers. The most effective feature of formaldehyde is that it stops microbial growth and tissues preserved with formaldehyde can remain intact for many years. Formaldehyde is also widely used in histological

* Corresponding author: Recep KARA

staining to enable observation of tissue structure. The use of formaldehyde is still a common choice because cadavers can be preserved for many years without deterioration, even though they turn into a harder tissue.

Formaldehyde is a colorless and pungent aldehyde that was first discovered by August Wilhelm von Hofmann in the 1800s. It is a chemical that dissolves very well in water. It shows its effect mainly by cross-linking to the proteins that make up the tissue. In this way, microorganisms cannot adhere to the tissue and support the decay process [2].

Thanks to its sterilization, protection and stabilization properties, formaldehyde has a wide range of applications not only in medical education and research, but also from resins to construction, wood processing to textile industry and chemical industry [3,4]. Since it is such an important chemical, its production is at the level of 46 billion pounds/year on a global basis [5].

When the formaldehyde density rises above 0.5 mg/m3 in an environment, tears occur in the eyes. When this value is above 0.6 mg/m3, coughing begins. Symptoms such as shortness of breath, cough, chest tightness, and headache occur at concentrations between 12 and 24 mg/m3.Decongestant. at concentrations higher than 60 mg/m3, serious health problems such as pneumonia, emphysema and even death can develop.

It has been known for many years that formaldehyde has harmful effects on the nervous, digestive and reproductive systems; it has been reported that exposure to formaldehyde can disrupt testicular morphology and lead to fertility problems [6]. In addition, it is an important carcinogenic substance that causes health problems such as skin rashes, allergic reactions, respiratory tract problems, as well as harming the environment [2, 7]. The International Unit for Research on Cancer (IARC) has approved formaldehyde 1. it has been classified as a class carcinogenic substance [5]. Therefore, alternative solutions that can be used as cadaver storage solution instead of formaldehyde are being seriously investigated [7]. The need for new and alternative cadaver detection and storage solutions is related not only to the undesirable effects on health, but also to the fact that cadavers detected with formaldehyde do not have the color, softness and flexibility that a fresh cadaver has. In cadavers detected with formaldehyde, natural features such as the flexible structure of the heart and veins and the expansion of the lungs cannot be exhibited [2]. Due to such effects of formaldehyde, different detection solutions are being tried to be developed. In addition to formaldehyde, chemicals such as glycerin, ethyl alcohol and phenol are also used in cadaver detection. It has been determined that these chemicals perform the detection process without hardening the tissues and preventing joint movements [7]. Formaldehyde cross-links nucleic acids and proteins by forming methylene bridges between reactive groups to detect tissues. Alcohol, on the other hand, removes water from the tissues, enabling the formation of protein-aqueous clots and carrying out the fixation process. However, excessive shrinkage and microscopic deterioration have been observed in tissues kept in alcohol for a long time [8,9]. The addition of food additives such as nitrite and nitrate to the storage solution can ensure that cadavers retain their red color. These additives also have a bacteriostatic effect [1]. Although boron is seen as a mineral with antimicrobial properties that has attracted a lot of attention in recent years, new searches continue as it is understood that boric acid causes degeneration in muscles [10,11,12].

This study is designed to find a cadaver identification and storage solution that is suitable for human health and the environment. The secondary goal is to eliminate the undesirable effects of boron mineral and to utilize it in these processes.

2. Material and methods

2.1. Tissues

A total of 7 sheep kidneys were used in this project, which was carried out with the aim of producing a detection and storage solution close to the appearance of a healthy and fresh cadaver. Sheep kidneys were obtained from the slaughterhouse and brought to the laboratory without wasting time. The kidneys brought to the laboratory were divided longitudinally into two equal halves.

2.2. Solutions

While 7 half sheep kidneys were placed in 10% formaldehyde solution, the other 7 halves were placed in the experimental solution prepared by our research team. Borax, nitrate, nitrite, glycerin, alcohol and tea tree oil were used to prepare this solution. First, 200 g of borax, 5 g of nitrate and 5 g of nitrite were dissolved in 2 l of distilled water. Subsequently, 50 ml of glycerin was dissolved in 1 l of 96% ethanol and the prepared solutions were combined by adding 50 ml of concentrated tea tree oil. The pH of the solution we prepared was adjusted to 7.5 using glacial acetic acid and the kidneys were placed in the solution.

2.3. Analysis

The kidneys in both groups were kept in the solutions for one month. During this period, the tissues were constantly checked and examined for the presence of any deterioration or degeneration. At the end of the study, the kidneys in all groups were removed from the solutions and photographed together with the fresh kidney brought from the slaughterhouse. Comparisons were made in terms of hardness and color differences between the cadavers before and after fixation. For texture profile analysis and determination of color changes, 3cmx3cm sized samples were taken from the kidneys in both solutions and the fresh kidney brought from the slaughterhouse that day and placed in the devices mentioned below.

With the texture profile analysis (TPA), hardness (hardness-N), elasticity (springiness), cohesiveness (cohesiveness) and gumminess (gumminess-N) properties of samples (Microstable TA.XT Plus, USA) was determined, the color change was made using CIE L * (blackness), a* (redness) and b* (jaundice) values, Hunter-Lab ColorFlex (A60-1010-615 Model Colorimeter, HunterLab, Reston, VA). Before the measurements, the spectrocolormeter was calibrated with white and black reference colors and L*, a*, b* values were obtained with three different readings. pH analysis The pH value of the storage solutions was evaluated by measuring them with a standardized pH meter (Orion 420A, USA) in buffer solutions with pH 4 and pH 7.

In the study, samples were also taken from kidney tissue and histological tissue follow-up was performed. For histological examination, tissue samples were examined under a light microscope. For routine histological follow-up, tissues were passed through graded alcohols and xylol and embedded in paraffin. Paraffin-embedded tissues were sliced with a microtome at a thickness of 5 micrometers and the sections were stained with hematoxylin-eosin dye. Photographs of the stained tissue samples were taken under a light microscope.

The solutions used in the study were examined for microbiological growth. For this purpose, 9 ml of sterile peptone water was added to 1 ml of samples taken from the solutions. Then, serial dilutions were prepared from the 1:10 diluted sample and planting *was done instead of the medium.*

2.4. Statistics

In the research, multiple readings with 3 repetitions were performed to perform variance analysis from the samples taken from the tissues for color and texture analysis. The data obtained in the research were analyzed with ANOVA test. Statistical significance for the difference between groups was determined as 0.05.

3. Results and discussion

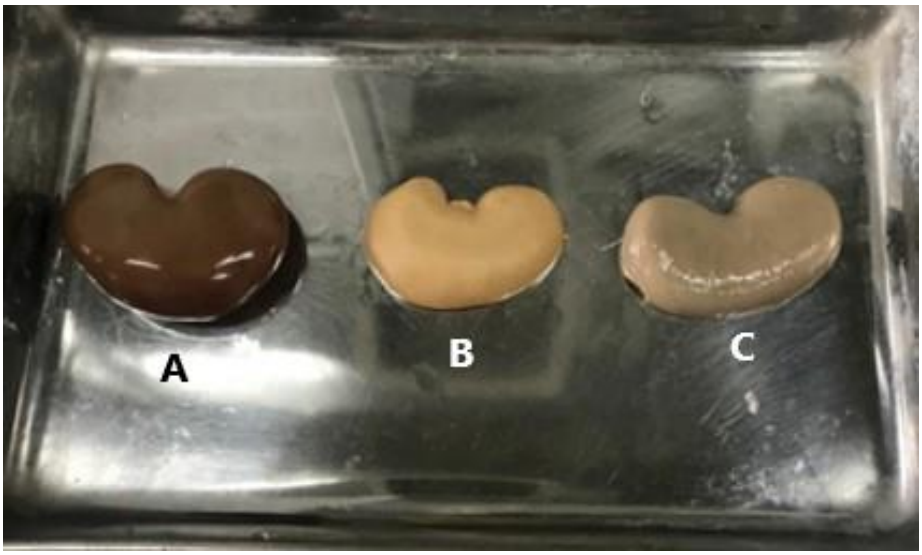

Figure 1 Comparison of kidneys at the end of the study. A) Fresh kidney cadaver B) Kidney cadaver fixed in solution C) Kidney cadaver fixed in formaldehyde

The kidneys used in the study were taken from slaughterhouses, from freshly slaughtered animals. When removed, the kidneys were vibrant, shiny brown, and had a soft texture. Tissues placed in the solutions were monitored day by day.

At the end of the study, the tissues were removed from the solutions and their condition was photographed and compared with the kidney tissue brought fresh from the slaughterhouse that day (Figure 1).

It was observed that the cadavers in formaldehyde solution had a disturbing odor of formaldehyde when they were taken out of the container, causing watery eyes and difficulty breathing. In the solution group, no bad odor was noticed that made one not want to approach the cadaver.

When examined in terms of color; It was observed that the kidney tissue in the solution group turned black (L*) more than the fresh cadaver and fixed cadaver in formaldehyde, the redness (a*) had the red color of the fresh cadaver, and the yellowing (b*) was higher than the fresh cadaver. In the formaldehyde group, it was determined that the blackness rate of the tissues was closer to the fresh cadaver than to the solution group, the redness was lower than both the fresh cadaver and the solution group, and the jaundice was closer to the fresh cadaver (Table 1).

Table 1 Color and pH analysis results of kidney samples (n=3). L* (blackness); a* (redness); b* (yellowness) There is a statistical difference between groups marked with different letters in the same column ($p<0.05$).

Groups	**L***	**a***	**b***	**pH**
Fresh	39.82 ± 1.12^{c}	9.86 ± 0.25^{a}	17.80 ± 0.20^{b}	6.54 ± 0.07^{a}
Formaldehyde	52.92 ± 0.38^{b}	5.19 ± 0.10^{b}	16.42 ± 0.18^{c}	5.41 ± 0.09^{b}
Solution	60.66 ± 0.21^{a}	9.23 ± 0.15^{a}	25.64 ± 0.07^{a}	6.50 ± 0.04^{a}
P	0.000	0.000	0.000	0.000

When the kidneys were compared in terms of texture, it was seen that the kidneys fixed in formaldehyde were considerably harder compared to the fresh cadaver. While there was no statistical difference in the tissues in terms of elasticity, it was observed that the adhesion values had a similar statistical difference with the solution. It was observed that the gumminess values of the tissues fixed in formol were quite different from both the solution and the freshly fixed cadaver (Table 2).

Table 2 Texture Analysis Results of Kidney Samples (n=3). There is a statistical difference between groups marked with different letters on the same line ($p<0.05$).

Groups	**Hardness**	**Elasticity**	**Stickiness**	**Gumminess**
Fresh	2001.00 ± 10.53^{c}	0.80 ± 0.03	0.61 ± 0.04^{b}	1403.83 ± 29.58^{c}
Formaldehyde	13044.09 ± 47.32^{a}	0.91 ± 0.02	0.81 ± 0.03^{a}	10931.94 ± 41.99^{a}
Solution	3437.13 ± 232.32^{b}	0.85 ± 0.02	0.73 ± 0.01^{a}	2703.83 ± 34.41^{b}
p	0.009	0.130	0.000	0.000

After this stage, small tissue samples were taken from the kidneys and histologic tissue follow-up was performed. No structural difference was observed between the tissues examined under light microscopy. All formations in the kidneys could be easily observed. However, in some sections, cell nuclei did not take hematoxylin-eosin stain as well as the formaldehyde group.

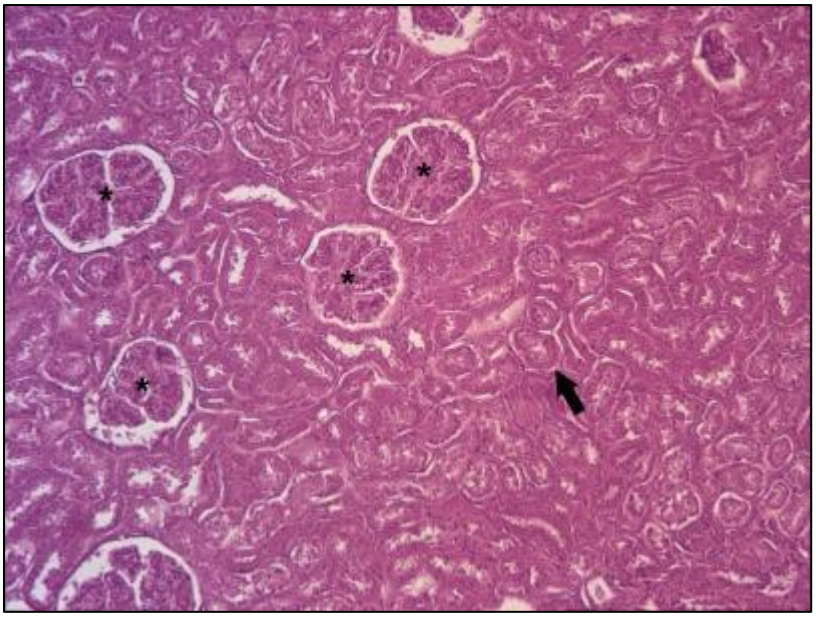

Figure 2 Solution group kidneys. * Glomerulus Arrow: Tubules

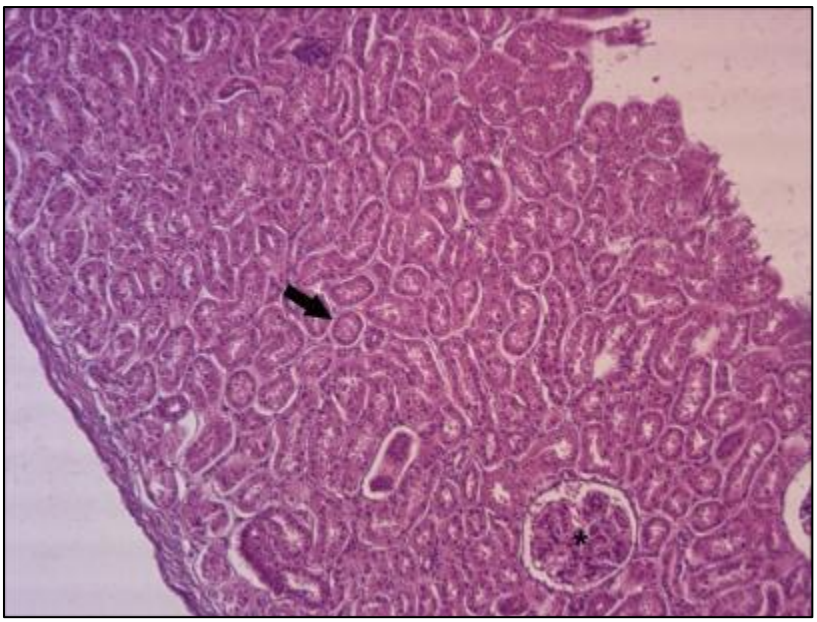

Figure 3 Solution group kidneys. * Glomerulus Arrow: Tubules

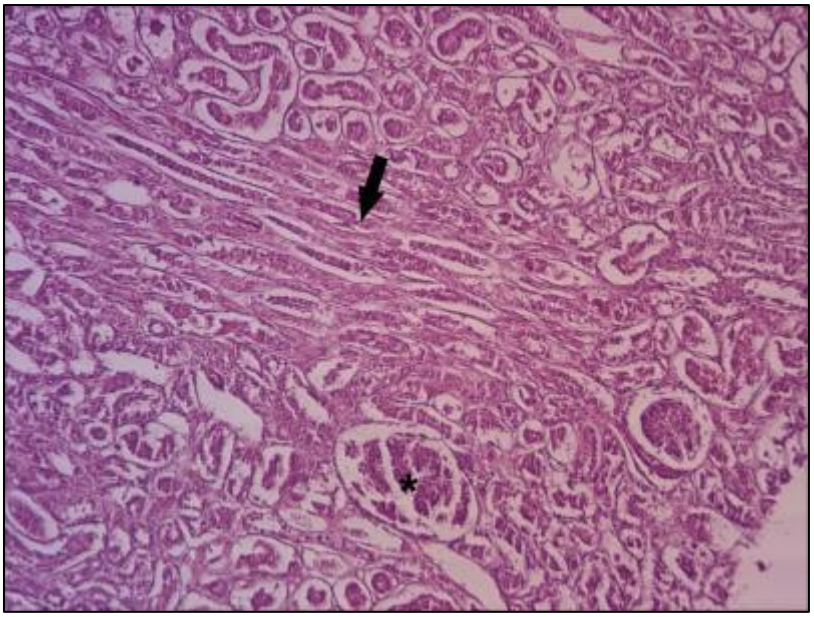

Figure 4 Solution kidney tissue. * Glomerulus Arrow: Tubules

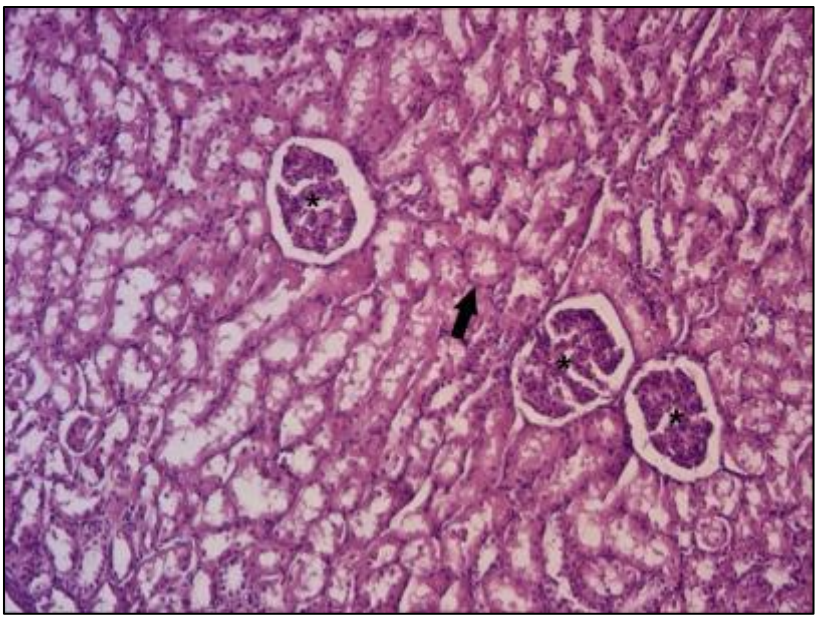

Figure 5 Formaldehyde group. * Glomerulus Arrow: Tubules

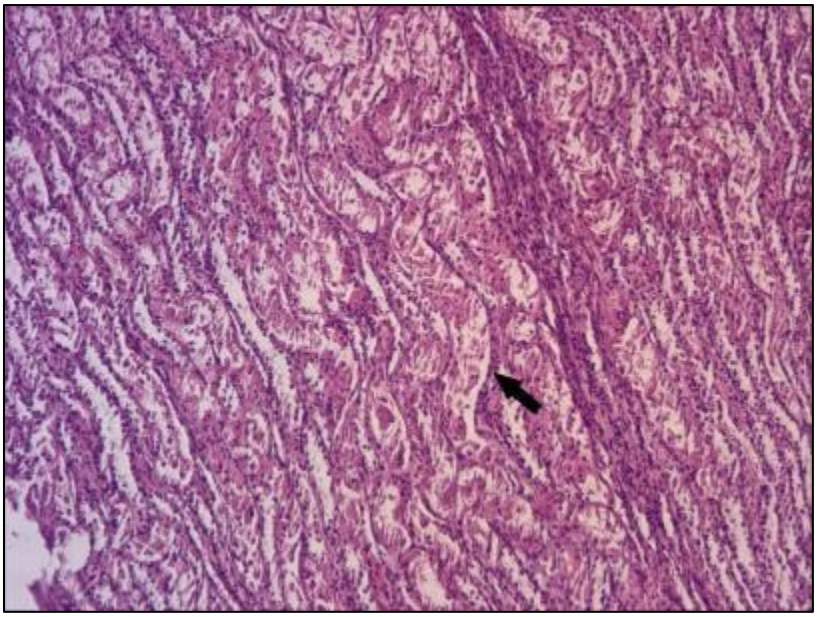

Figure 6 Formaldehyde group kidney tissue. Arrow: Tubules

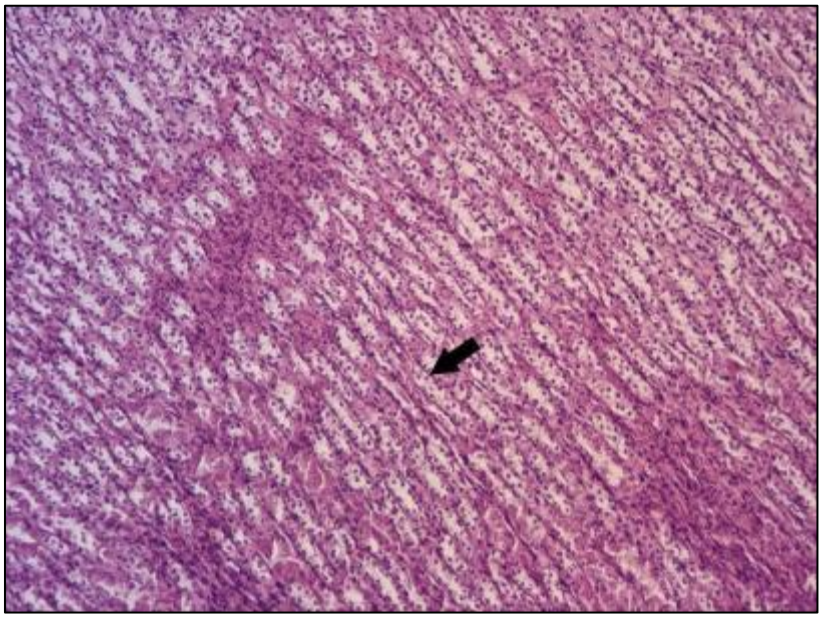

Figure 7 Formaldehyde group kidney tissue. Arrow: Tubules

In microbial analyses, it was observed that there was some microbial growth in the solution group compared to the formaldehyde group. However, this growth was not at a level that would damage the tissue of the cadaver (Table 3).

In the study, borax, which is the alkaline form of boron, was preferred instead of boric acid, which is the acidic form of boron. Because boric acid is the form of boron that is used in the content of solutions that are frequently preferred around the world instead of formaldehyde, such as Thiel's solution, but there are reports that it causes deterioration. In general, it was observed that cadavers fixed with Thiel's solution showed darkening, and it was suspected that boric acid might be the cause of this. Studies on this have found that boric acid causes color changes such as degeneration and darkening on the muscle layer [12]. Akosman et al. [13] tried borax solution instead of boric acid in the storage of muscle cadavers, and muscle tissues fixed in formaldehyde were kept in a solution containing borax for forty days. No difference was found in histological examination between muscle tissues kept in borax solution and muscle tissues kept in formaldehyde, no irritating odor was observed in muscle cadavers kept in borax solution, and microbiological growth remained in trace amounts [13]. In this study, the irritating odor of formaldehyde was not detected in kidney cadavers

kept in solution, and no structural deterioration was observed histologically between the tissues. It was also observed that microbial growth in tissues remained at an insignificant level. Considering the color and texture analysis of the tissues, it was observed that the tissues in the solution group containing tea tree oil differed from the fresh cadaver in terms of color, and their texture was close to the fresh cadaver.

Table 3 Microbiological Analysis Results of Solutions (n:3, log10)*<log 2.00

Groups	**Total Number of Bacteria**	**Enterococci**	**Coliform**	**Yeast / Mold**	**Enterobacters**	**Staphylococci**	**E.coli**
Formaldehyde	-*	-	-	-	-	-	-
Solution	2,54	-	-	-	-	2,39	-

Menon et al. [14] prepared a solution consisting of ethanol, polyethylene glycol, chlorosylenol and sodium nitrite, applied the solution they prepared to the cadavers of various farm animals such as cats, dogs, sheep and goats, and observed it for color change, hardness and microbial growth for six months. They determined that redness increased except cat. They stated that the brown, red and purple colors seen in fresh cadavers were provided by oxymyoglobin, deoxymyoglobin and metmyoglobin, and that the color changes from red to pale brown in the prepared cadaver were due to postmortem oxidation and other biochemical changes caused by putrefaction. It has also been reported that this type of deterioration accelerates when the cadaver comes into contact with air during cadaver dissection [14]. In this study, it was observed that the redness in kidney tissues kept in a solution containing tea tree oil had values similar to those in fresh cadavers.

Menon et al. [14] stated that the solution they prepared slowed down the color change in the cadaver; They stated that the antioxidant sodium nitrite they used suppressed lipid peroxidation and that the nitrite combined with myoglobin in the meat (muscles) and improved the color. This process is explained as sodium nitrite, an antioxidant, prevents the deterioration process of the tissue by suppressing lipid peroxidation and ensures the preservation of color by interacting with myoglobin. However, nitrite also causes an increase in yellow color, especially in fatty tissues [14].

In the study, the blackness and yellowness parameters of the kidney tissues had different values from fresh cadavers and were observed to be more yellow and black. The red color remained at a value similar to fresh cadaver. It can be assumed that the added nitrite preserved this value. Another property of nitrite salts is that they cause yellowing in adipose tissues, so since nitrite salts increase the yellow coloration in the kidneys [14], this may be the reason for the more yellow cadaver in our study.

It is known that tea tree oil, which has been used externally and locally to support treatment of skin infections in recent years, has high antimicrobial activity [15]. Additionally, antimicrobial, antifungal, antiviral and antiprotozoal effects of tea tree oil have been reported [15]. In the solution group in our study, it was determined that staphylococci grew at a negligible level despite tea tree oil, but it had a completely inhibiting effect on the growth of enterococci, coliforms, yeasts, moulds, enterobacteria and escherichia coli.

Menon et al., [14], who investigated the effects of microbial growth in their study, did not encounter microbial growth except for one cadaver in their study, and stated that the solution they developed could be used safely for six months in cadaver detection and storage. Turan et al. [16] also mentioned the presence of bacterial growth in their study in which they used a similar solution, but observed that yeast and mold did not grow at all. In this study, cadavers were constantly taken outdoors and examined by students and researchers under laboratory conditions. In other words, cadavers were allowed to become contaminated from the environment and cause microbial contamination. Despite this, it was observed that microbial growth in the solutions was not at a significant level. This reproduction did not cause any visual signs of deterioration in the cadavers. This result was associated with the antioxidant effects of tea tree oil as well as its antimicrobial activity.

Queiroz et al. [1] developed a solution consisting of ethanol, glycerin, sodium chloride, nitrite and sodium nitrate to be used in short-term courses, dissection or surgical courses, stored cat cadavers subjected to this solution in vacuum packs at 0-40oC for seven days, and stated that the combination of glycerin and ethyl alcohol facilitated joint movements more than formaldehyde fixation. In the microbiological examination performed at the end of the seventh day, it was stated that aerobic and anaerobic bacteria grew, but this growth was not at a level that would cause deformations in the cadaver. It was also reported that cadavers kept in the solution gave results like fresh cadavers in stretching tests on the skin and intestine and did not statistically differ.

4. Conclusion

The cadavers produced and stored with the solution were well received by researchers, academics and students due to its non-irritating odor. Although the unique color of the kidney was not fully preserved, an effect similar to the formaldehyde group was observed in histological staining. It is noteworthy that the kidney fixed with this solution has a softness close to that of a fresh cadaver, despite the excessive hardening of the kidney tissue fixed with formaldehyde. It shows that the solution used in the study will be suitable for short-term use of cadavers and tissues and can be safely applied in processes requiring short-term fixation such as courses, seminars and workshops. In the tissues prepared and fixed with this solution, it was observed that the cell nuclei were noticeable and covered less paint. In cadavers kept in the solution we prepared, trace amounts of microbial growth were observed despite contact and contamination with the external environment. As a result, it was concluded that this solution, prepared by combining chemicals safer than formaldehyde, has a generally positive effect on cadavers and can be safely used in courses, especially in short-term courses and workshops.

Compliance with ethical standards

Acknowledgments

This study was supported by TÜBİTAK (Project No: 1919B012203121).

Disclosure of conflict of interest

The authors declared that there is no conflict of interest

Statement of ethical approval

The present research work does not contain any studies performed on animals/humans subjects by any of the authors.

References

[1] Queiroz ABPS, Rodrigues A, Cardozo MV, Costa NTB, Soares LG, Fechis ADS, Oliveira FS. (2022) Biomechanical and microbiological analysis of embalmed cats - acute effect of conservation. An Acad Bras Cienc. Jan 7;94(1):e20201583. doi: 10.1590/0001-3765202120201583. PMID: 35019006.

[2] Balta JY, Cronin M, Cryan JF, O´Mahony SM. (2015). Human preservation techniques in anatomy: A 21st century medical education perspective. Clin Anat 28: 725-734.

[3] Tang X, Bai Y, Duong A, Smith MT, Li L, Zhang L. (2009). Formaldehyde in China: Production, consumption, exposure levels, and health effects. Environ Int 35:1210–1224.

[4] Duong A, Steinmaus C, McHale CM, Vaughan CP, Zhang L. 2011. Reproductive and developmental toxicity of formaldehyde: A systematic review. Mutat Res 728:118–138.

[5] IARC. 2006. Formaldehyde, 2-butoxyethanol and 1-tert-butoxypropan-2-ol. IARC Monogr Eval Carcinoq Risks Hum. 88:1–478.

[6] Han, S. P., Zhou, D. X., Lin, P., Qin, Z., An, L., Zheng, L. R., & Lei, L. (2015). Formaldehyde exposure induces autophagy in testicular tissues of adult male rats. Environmental toxicology, 30(3), 323–331. https://doi.org/10.1002/tox.21910

[7] Denis-Rodríguez E, Aguirre-Gutiérrez ÁA. (2018). Thiel Soft-Fix method for long term preservation. Rev Mex Med Forense, 3(2):91-98.

[8] Perry C, Chung JY, Ylaya K, Choi CH, Simpson A, Matsumoto KT, Smith WA, Hewitt SM. (2016) A Buffered Alcohol-Based Fixative for Histomorphologic and Molecular Applications. J Histochem Cytochem. Jul;64(7):425-40. doi: 10.1369/0022155416649579. Epub 2016 May 24. PMID: 27221702; PMCID: PMC4931761.

[9] Chung JY, Song JS, Ylaya K, Sears JD, Choi L, Cho H, Rosenberg AZ, Hewitt SM. (2018). Histomorphological and molecular assessments of the fixation times comparing formalin and ethanol-based fixatives. J Histochem Cytochem. Feb;66(2):121-135.

[10] Benkhadra M, Bouchot A, Gérard J, Genelot D, Trouilloud P, Martin L, Girard C, Danino A, Anderhuber F, Feigl G. (2011). Flexibility of Thiel's embalmed cadavers: the explanation is probably in the muscles. Surg Radiol Anat. 33:365-368.

[11] Brenner E. Human body preservation – old and new techniques. (2014). J Anat. 224:316-344.

[12] Türkmen, R., Demirel, H. H., Akarca, G., & Akosman, M. S. (2017). The investigation of potential preservative effect f boric acid on formalin fixed striated muscle tissues. Kocatepe Veterinary Journal, 10(4), 317-321.

[13] Akosman, M.S., Kara, R., Fidan, A.F. (2022). Preservation of muscle tissue with a formaldehyde-free borax solution for gross anatomy lessons. World Journal of Advanced Research and Reviews, 16(03), 390–395.

[14] Menon, P., Aldarwich, A., Hamdan, L., Hammoud, M., Aiyan, A.A. (2021). Novel formaldehyde-free embalming fluid formulation for long-term preservation of cadavers for anatomy teaching. Emirates Journal of Food and Agriculture. 33(9): 718-725

[15] Carson CF, Hammer KA, Riley TV. Melaleuca alternifolia (Tea Tree) oil: a review of antimicrobial and other medicinal properties. Clin Microbiol Rev. 2006 Jan;19(1):50-62. doi: 10.1128/CMR.19.1.50-62.2006.

[16] Turan, E., Gules, O., Kilimci, F. S., Kara, M. E., Dilek, O. G., Sabanci, S. S., & Tatar, M. (2017). The mixture of liquid foam soap, ethanol and citric acid as a new fixative-preservative solution in veterinary anatomy. Annals of anatomy = Anatomischer Anzeiger : official organ of the Anatomische Gesellschaft, 209, 11–17. https://doi.org/10.1016/j.aanat.2016.09.002