



Effects of Cadaver Fixation Solutions on Soft and Hard Tissue

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Abstract:

Objective: In this study, it was aimed to determine a suitable solution for cadaver embalming by evaluating the fixation effects of Citric acid, Kaiserling 4%, Jores, Formalin 10%, Klotz, Modified Larssen and Saturated Sall solutions on liver and lung tissue.

Methods: The solutions were prepared taking into account the literature information. Three months later, the lung and liver tissues kept in the solutions were taken for histological analysis.

Results: As a result of histological analysis, Jores, Klotz and Kaiserling 4% solutions gave very good analysis results in terms of preserving tissue integrity in lung tissue, and Jores solution gave very good analysis results in liver tissue.

Conclusion: The use of cadavers both in anatomy education and in surgical courses in recent years further increases the importance of the embalming solutions used to preserve them. Cadaver embalming is of great importance, especially in terms of the decrease in donors after the Covid-19 pandemic and the embalming solution of donated cadavers to preserve the cadaver for a long time.

Keywords: Cadaver Embalming, Fixation, Anatomy Education, Surgical Anatomy

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Introduction

Cadaver embalming has been among the most important topics in the field of anatomy since history(1). Because cadavers are perishable materials and the shortage of cadaver donations in some countries in recent years has increased the number of solution trials for long-term use of cadavers (2).

Especially after the covid-19 pandemic, the hygienic properties of cadaver solutions are extremely important for laboratory safety (3). Formalin is a solution that has been used in cadaver embalming for years and provides very good protection against most infectious agents (except prions) (4). However, its odor and irritating effect on the eyes and mucous membranes cause some problems in its use. It also

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significantly fades the color of the tissue in the cadaver (5).

In today's technology, the search for an effective solution that both protects the cadaver in terms of color and histology and resists infectious agents is an issue that remains on the agenda to a great extent.

In this study, histological examination of seven different cadaveric fixation solutions was performed on liver and lung tissues, using literature information. The results obtained from the study can bring an updated perspective to the subject of cadaver embalming, and the solutions with histological analysis can be tried in cadaver embalming in anatomy departments around the world.

Material And Methods

In order to observe the effects of seven types [Jores (JJJ) solution (6), Citric acid (CS) solution (7), Kaiserling 4% (K4) solution (8), Klotz (KLO) solution (9), Formalin 10% (FA 10%) solution (10), Saturated sall (SSS) solution (10), Modified Larssen (MLS) solution (11)] of cadaver embalming solutions (prepared according to the data of the article, 49 sheep livers and lungs obtained from the slaughterhouse were placed in storage containers filled with embalming at room temperature (25°C). At the end of three months, both liver and lung tissues were analyzed to compare with each other.

Histological analysis

After 90 days of fixation, cadaveric fixation using Modified Larssen (MLS), Jores (JJJ), 4% Kaiserling (K4), Saturated Sall (SSS), Klotz (KLO), Citric acid (CS), Formalin 10% solution (FA 10%) Liver and lung tissue samples taken from the solutions were washed under running water.

It was then passed through increasing degrees of alcohol to dehydrate the tissue and left in the alcohol overnight. Afterwards, the tissue was blocked by embedding it in metal cassettes in xylol-paraffin series. 5 micron thick sections were taken from tissue samples. The sections taken were stained with Hematoxylin-Eosin dye.

Statistical analysis

Analysis of the data obtained in our study (color measurements) was made using SPSS® Statistic Version 25 (IBM®, USA). Comparisons between groups were made with the Oneway ANOVA test. Post-hoc Tukey analysis was performed to compare multiple groups. As a result of the analysis, p value <0.05 was considered statistically significant.

Results

When lung tissue samples are examined, it is observed that FA 10% fixative preserves the cellular structure, but slight degenerations are observed in the bronchiolar structure. The cellular structure was also preserved in K4, KLO and JJJ fixatives. However, when these fixatives are compared to FA 10% fixative, degenerative damage, apoptotic and necrotic cells are observed more frequently. When CS and SSS fixatives are examined, it is observed that the cellular structure decreases and bronchiolar damage is exacerbated. The most severe damage to lung tissue is observed with MLS fixative, with loss of cellularity and tissue integrity.

5 different areas from each slide to facilitate evaluation between groups; Findings of apoptosis, necrosis and bronchiolar damage were scored as absent (-), mild (+), moderate (++) and severe (+++) (table 1). The results were evaluated statistically according to One Way Anova Tukey test (Fig.1,2).

Table 1. Cellular events in lung tissue.

Groups	Apoptosis	Necrosis	Bronchiole Damage
FA 10%	+/-	+/-	+
CS	++/+++	++	+++
K4	+	+	+/++
JJJ	++	+	+/+++
MLS	+++	+++	+++
KLO	+/+++	+/+++	+
SSS	++	++	++

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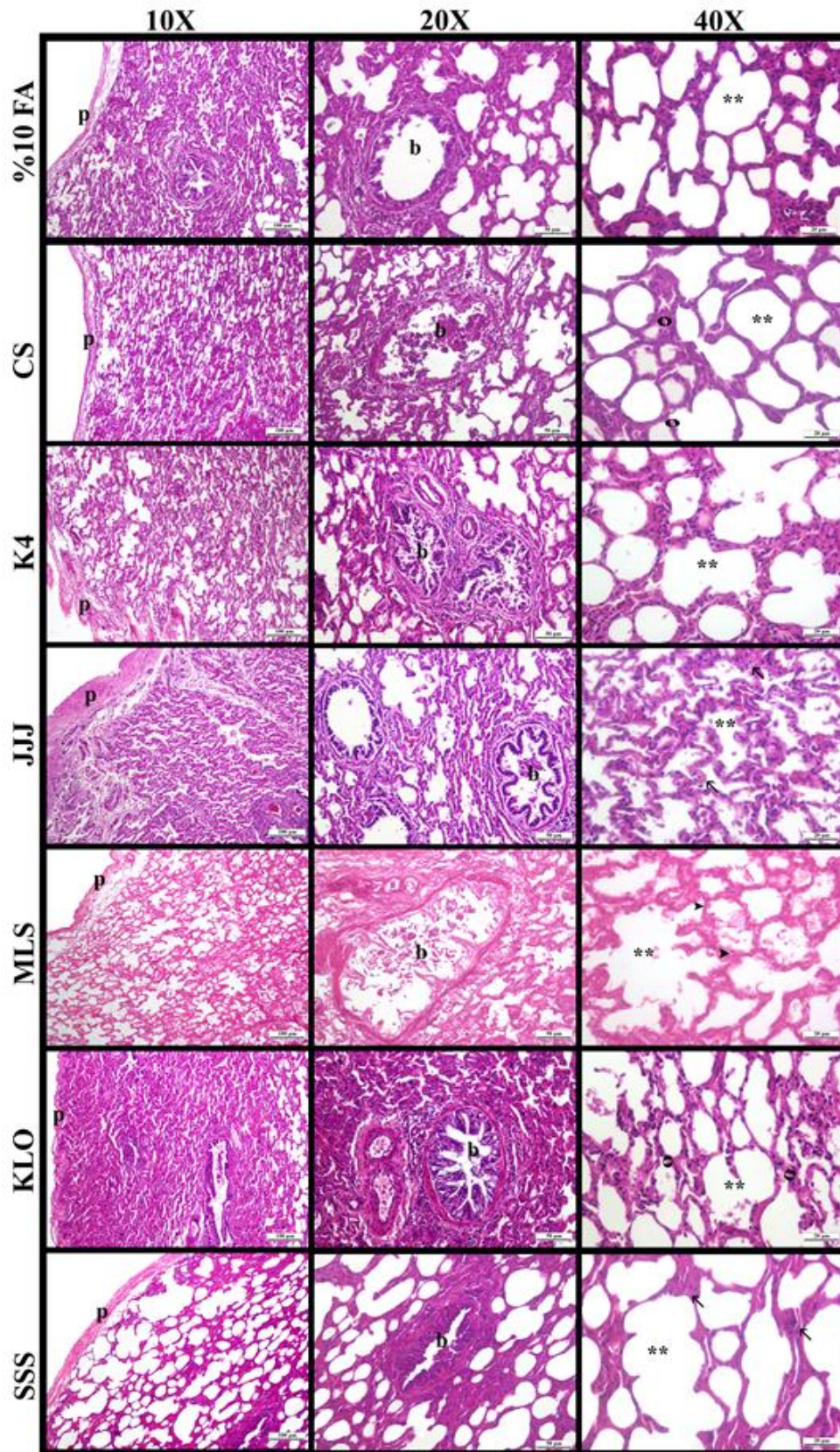


Figure1. In the lung tissue stained with hematoxylin and eosin, p: pleura, b: bronchiole, ** sign: alveolar structure, arrowhead: necrotic cells, arrow sign: apoptotic cells, round: karyolysis.

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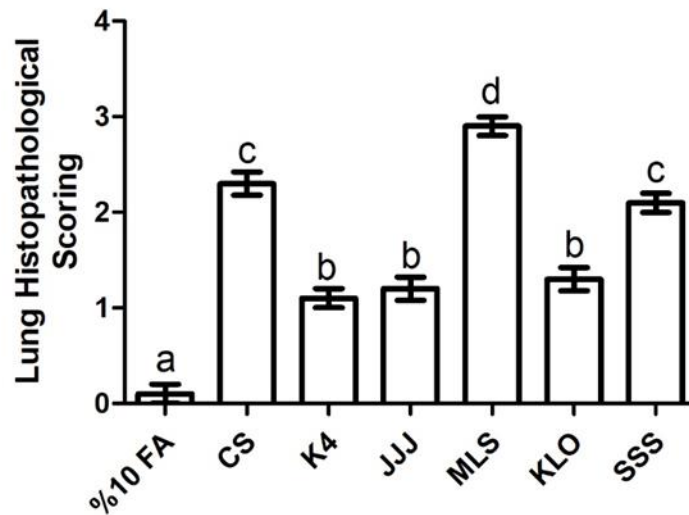


Figure2. Lung histopathological scoring Tukey test results

When lung tissue samples are evaluated statistically, it is seen that 10% FA fixative causes the least damage and MLS fixative causes the most damage. When KLO, JJJ and K4 fixatives are evaluated among themselves, no statistically significant difference is observed. It is observed that the cellular damage in SSS and CS fixatives is more than other fixatives except MLS fixative, but when evaluated among themselves, no significant difference is observed ($p < 0.05$).

When liver tissue samples are examined, it is observed that 10% FA fixative preserves tissue integrity and cellular structure. However, areas of slight dilatation are observed in the tissue. Tissue integrity, cellular structure, and portal area were preserved in JJJ fixative, but sinusoidal dilatations were higher compared to 10% FA fixative. In K4 and KLO fixatives, it is observed that tissue

integrity and cellularity decrease and degenerative damage in portal areas increases. When CS fixative is evaluated, degenerations due to damage in the capsule, severe decrease in cellularity, increase in sinusoidal dilatations and intense degenerative changes in the portal area structure are observed. Liver samples fixed with SSS and MLS fixatives show severe damage to cellularity and tissue integrity, and no nuclear structure is observed, especially in MLS fixative.

5 different areas of each slide to facilitate evaluation between groups; Findings of apoptosis, necrosis and sinusoidal dilation were scored as absent (-), mild (+), moderate (++) and severe (+++) (table 2). The results were evaluated statistically according to One Way Anova and Tukey test (Fig.3,4).

Table 2. Cellular events in liver tissue

Groups	Apoptosis	Necrosis	Dilation
FA 10%	+/-	+/-	+
CS	++/+++	++/+++	++/+++
K4	+/>++	+/>++	++
JJJ	+	+	+/>++
MLS	+++	+++	+++
KLO	++	++	++/+++
SSS	++/+++	+++	+++

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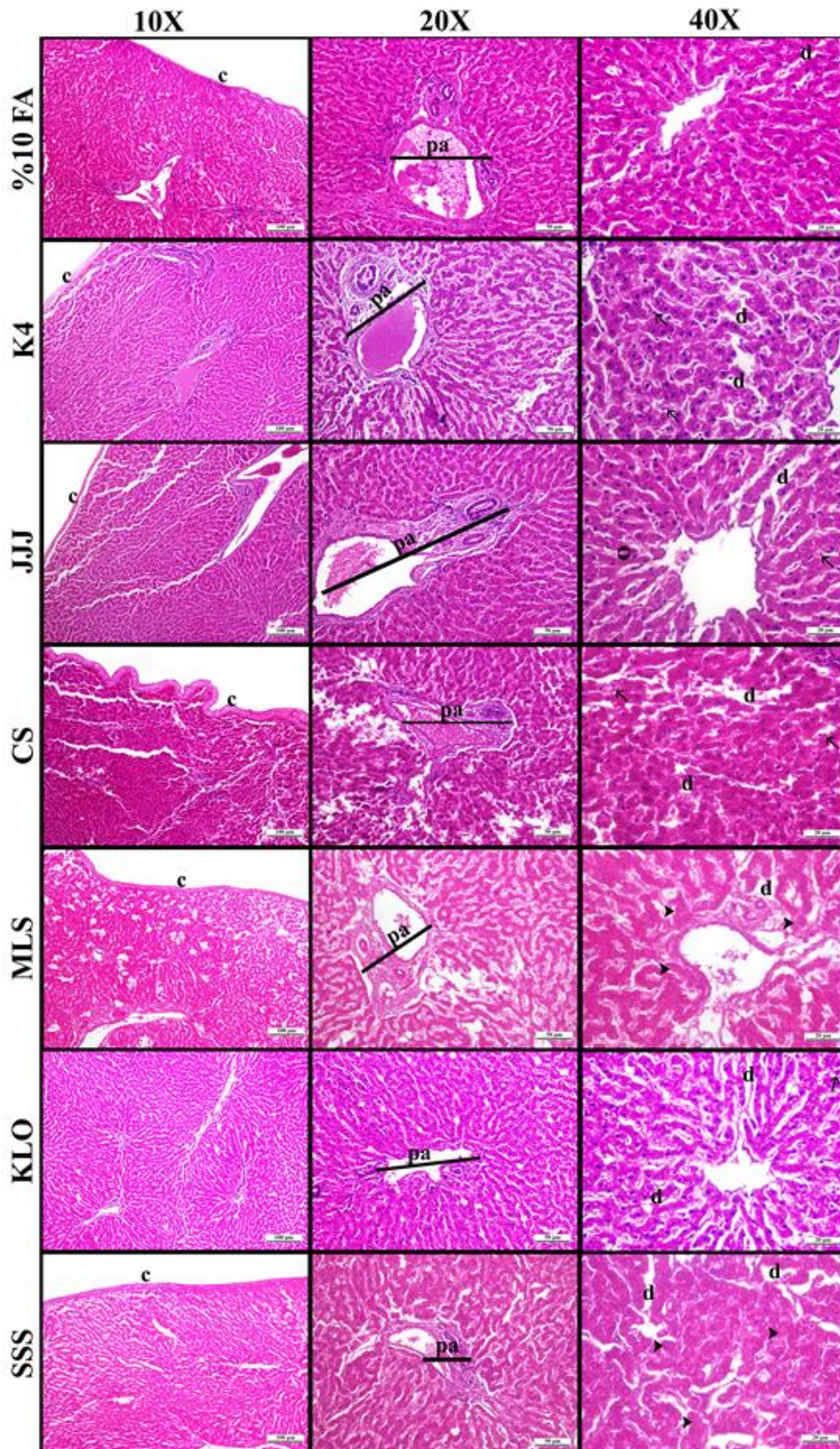


Figure 3. In the liver tissue stained with hematoxylin and eosin, c: capsule, pa: portan area, d: dilation areas, arrowhead: necrotic cells, arrow sign: apoptotic cells, round: karyolysis.

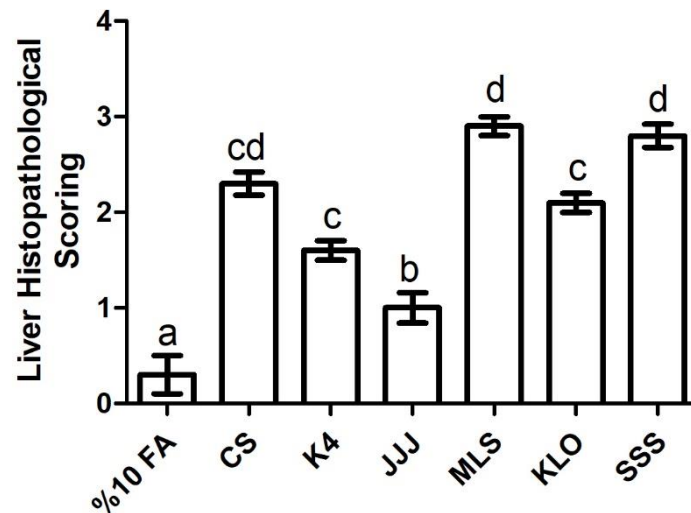


Figure 4. Liver histopathological scoring Tukey test results

When liver tissues are evaluated, it is seen that FA 10% fixative causes the least damage and SSS and MLS fixatives cause the most damage. When SSS and MLS are evaluated among themselves, no statistically significant difference is observed. While JJJ fixative provides the closest fixation to FA 10%, when CS, KLO and K4 fixatives are compared among themselves, it is observed that there is no significant difference between KLO and K4 fixatives and CS fixative causes more damage than KLO and K4 ($p < 0.05$).

Discussion

Cadavers, which are one of the most important ground materials for almost all branches of medical education, require a careful technical infrastructure for their protection and preservation (12). The cadaver infrastructure of the anatomy departments also provides the laboratory with a comfortable environment in teaching the course (cleanliness, the cadaver does not smell, it does not emit irritating odor or discharge, etc.) (13).

Another research topic brought about by cadaver training in recent years is cadaver embalming solutions. The quality of the cadaver embalming solution can significantly affect both the comfort of the lesson environment and the students' ability to understand the lesson (14) In addition, since cadavers have become an indispensable part of important surgical courses in recent years, the cadavers used in these courses must be both suitable for manipulation and hygienic. Therefore, in addition to the hygienic conditions required in the embalming solution, the ability of the solution

to provide flexibility while preserving the texture and color integrity can be listed as very important factors (14,15).

In this study, the histological effects of cadaver embalming solutions in preserving tissue integrity were investigated. Multiple solution comparative studies are rare in the literature. In addition, histological analysis of cadaver embalming solutions is not a frequently researched subject. Therefore, it is very important for the study to be included in the literature so that it can be tested in other studies on the solutions used.

Histologically, formalin is an excellent tissue-preserving solution. However, in the results of the study, it was observed that the solutions close to formalin in protecting the integrity of the lung tissue were KLO, JJJ and K4, while the solution that protected the liver tissue at least as much as formalin was found to be JJJ. In the findings section, the closeness of the solutions to each other is also supported by graphics.

Histological analysis is almost non-existent in studies conducted with MLS (8). This also applies to work with K4(11). The use of KLO and JJJ in solution trials is a rare study. Therefore, multiple histological comparisons of these seven solutions can shed light on many solution trials and can be tested in different organ tissues.

Conclusion

In the study, the effects of seven different embalming solutions on preserving tissue integrity were investigated in detail. These seven solutions

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can be tested in different organ tissues and may shed light on new studies planned to be carried out in terms of microbial, color and flexibility properties. At the same time, the results of extensive analyzes of these solutions can bring innovation to cadaver embalming solutions and be used worldwide.

Conflict of interest: The authors declare that there is no conflict of interest.

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Ethics Committee Approval: : Ethics committees approval was obtained from Kafkas University Animal Experiments Local Ethics Committee KAU-HADYEK 2023/096.

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