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Evaluation of the Fixation Effect of Citric Acid, Kaiserling 5% and Jores Solutions on Heart Tissue

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Abstract:

Objective: By evaluating the fixation effect of citric acid, kaiserling 5% and Jores solutions on heart tissue, it was aimed to determine a suitable solution for cadaver embalming. Because nowadays, carrying out cadaver embalming with a solution that is as strong as the infection-breaking effect of formalin but also preserves color and flexibility has become one of the most important issues of the entire anatomy community.

Methods: The solutions were prepared with the knowledge of the literature. The heart organs were photographed freshly with a millimetric scale on them. Three months later, the heart organs were removed from the solutions, a millimetric scale was placed on them again and they were photographed. In addition, the solutions were examined microbiologically every week. Three months later, after photographs were taken, histological examination was performed on sections taken from three heart tissues.

Results: As a result of color measurements, it was observed that the color was preserved best in Jores solution. In histological examination, it was determined that cell and tissue integrity was best preserved in Kaiserling 5% solution. But Jores solution also gave results close to 5% Kaiserling solution in histological examination. In the microbial analysis, no gram (+) and gram (-) bacteria were detected in all three solutions. Additionally, the solutions are good in that they do not cause yeast growth within three months in the room temperature.

Conclusion: Cadaver embalming solutions are becoming more and more important day by day.Considering the results obtained, the microbial, histological and color preservation effects of Kaiserling 5% solution can be disseminated as a cadaver embalming solution. In addition, it has been revealed that the Jores solution gives similar results to the Kaiserling 5% solution. Taking anatomy laboratory courses in a cadaver environment embalmed with a solution that does not smell of formalin and has no irritating effects will greatly affect the course quality and learning.

Keywords: Cadaver Embalming, Fixation, Kaiserling 5% Solution, Jores Solution, Citric Acid Solution

Introduction

Cadaver is the most valuable treasure that human beings offer to medical education. The importance of cadavers in health faculties is increasing day by day [1]. While the cadaver is such an important educational tool, its preservation methods have become a very valuable topic in the literature in recent years [2].

When cadaver is mentioned, another concept that comes to mind with the word 'cadaver' for years has been the word 'formaldehyde'. Because in most countries in the world, formalin solution has been used in cadaver preservation for years. Thus, the harmful effects of formaldehyde came to the fore and cadaver personnel, trainers and students were exposed to the irritant effects of formalin [3].

New solutions are being tried day by day due to the irritant effects of formalin on cadaver fixation. When we look at the literature, it is mentioned that the trial results of a lot of saturated sall solution [4,5,6]. In addition, 'Thiel solution' is recommended as the most popular and effective solution in recent years in the literature [7].

In this study, it is aimed to evaluate 3 different fixative solutions in terms of histological and microbiological aspects with the color they create in the heart tissue. Jores, citric acid and Kaiserling 5% solutions were prepared according to the solution articles in the literature and their effects were examined. Bringing the results of the study to the literature can provide great benefits to the literature on cadaver fixation. In addition, testing solutions over longer periods may be an important issue for further studies.

Material and Methods

The three solutions [Jores solution (8), Citric acid solution (9), Kaiserling 5% solution (10)]. used in the study were prepared in the light of literature knowledge.

Color Analysis:

In order to observe the effect of cadaver fixation solutions on the heart tissue, 21 sheep hearts obtained from the slaughterhouse were photographed by placing the metric before being put into the solutions with a camera. Color analysis of fresh heart organs was performed with Image J software. All solutions were prepared according to the literature description and placed at room temperature (25°C) in storage containers. Then the heart organ (sheep heart) was placed in all solution containers (21 solution containers were prepared). For three months, all solution containers were opened once a week, the heart organs were examined, and the color and odor of the solutions were subjectively examined. At the end of three months, metrics were placed on the heart organs and their photos were taken and color measurements were made with the help of Image J software program. Color measurement was examined in two ways as the difference between the solutions at the end of three months and the difference between them with fresh tissue at the end of three months. The resulting measurement values were interpreted by comparing with the fresh tissue color. In addition to color analysis, histological and microbiological analysis were performed.

Histological Analysis:

After 90 days of fixation, heart tissue samples from cadaver fixation solutions named citric acid, 5% Kaiserling and Jores were washed under running water. Subsequently, it was passed through a series of increasing degrees of alcohol to dehydrate the tissue and left overnight in alcohol. Then, it was blocked by embedding in metal cassettes in xylol-paraffin series in order to make the tissue transparent and embed. Sections of 5 micron thickness were taken from tissue samples. Sections taken were stained with Hematoxylin-eosin stain.

Microbiological Analysis:

Solutions containing 21 heart organs used in the study were kept at room temperature (25 degrees) during the study. For bacterial isolation, 100 µl of the solutions containing the samples were taken and superficial fluid swab samples were taken, from heart tissue at the end of each month and these samples inoculated on Eosine Methylene Blue agar (EMB), Mac Conkey agar, and blood agar containing 7% sheep blood. Plates were incubated in aerobic and microaerobic environments at 37°C for 24-48 hours. Sabouraud Dextrose agar was used for fungal isolation and incubations were done at room temperature (18-22°C) and 37°C.

Statistical Analysis

The analyzes of the data (color measurements) obtained in our study were performed using SPSS® Statistic Version 25 (IBM®,USA). Comparisons between groups were made with the Oneway ANOVA test. Post-hoc Tukey analysis was performed for comparisons of multiple groups. As a result of the analysis, $p<0,05$ value was considered statistically significant.

Results

Color Analysis Findings:

Three groups of solutions were compared with each other and with their fresh images after three months (Table 1-2).

As a result of the comparison with each other at the end of three months, when the average of the red-blue-green colors was taken, it was determined that the jores solution was the closest to the fresh tissue. Kaiserling 5% is a solution that approaches fresh tissue after jores solution. The citric acid solution, on the other hand, could not catch the fresh color in the tissue and is quite unsuccessful in terms of color compared to the other two solutions.

Another evaluation was made after taking the differences between the color values in the photographs of the groups in which they were fresh tissue that had not yet entered the solution and the color values after waiting in the solutions for three months. Another evaluation was made after taking the differences between the color values in the photographs of the groups in which they were fresh tissue that had not yet entered the solution and the color values after waiting in the solutions for three months. In this difference, the average of red-blue-green colors is parallel to the average values above. In other words, while there is a slight difference in the Jores solution, the difference in the Kaiserling 5% solution is slightly higher than the Jores solution. The highest difference (i.e. fresh tissue and tissue remaining in solution for three months) is in the heart tissue remaining in citric acid solution. As can be seen from the photographs, the tissue color has almost turned yellow.

When the two tables (Table 1-2) obtained were examined, a significant difference was found between all three solutions in both evaluations ($P<0.001$; Oneway ANOVA).

Table 1. Comparisons between groups at 3 months.

Groups	Red Difference mean (SD)	Green Difference mean (SD)	Blue Difference mean (SD)	(K+Y+M)/3 Difference mean (SD)
Jores Solution	146.51 ± 0.94^{a}	97.29 ± 1.08^{a}	81.14 ± 0.67^{a}	108.45 ± 0.69^{a}
Citric Acid Solution	202.28 ± 0.71^{b}	198.36 ± 1.02^{b}	157.37 ± 0.95^{b}	186.28 ± 0.65^{b}
Kaiserling 5% Solution	171.20 ± 0.69^{c}	137.32 ± 1.08^{c}	109.29 ± 1.44^{c}	139.09 ± 1.54^{c}
F	8726.424*	16021.500*	9033.951*	9763.173*
P	<0.001	<0.001	<0.001	<0.001

$p<0.05$ (Oneway ANOVA). F: F value. SD: standard deviation. *: There are differences between groups. Different superscript characters (a,b,c) in the same column indicate statistical differences between groups.

Table 2. Difference between groups (3 Months - Fresh) comparisons.

Groups	Red Difference Mean (SD)	Green Difference Mean (SD)	Blue Difference Mean (SD)	(K+Y+M) /3 Difference Mean (SD)
Jores Solution	-22.43 ± 0.94^{a}	16.17 ± 1.08^{a}	20.06 ± 0.67^{a}	4.73 ± 0.69^{a}
Citric Acid Solution	33.33 ± 0.71^{b}	117.24 ± 1.02^{b}	96.29 ± 0.95^{b}	82.56 ± 0.65^{b}
Kaisering 5% Solution	2.25 ± 0.69^{c}	56.20 ± 1.08^{c}	48.21 ± 1.44^{c}	35.37 ± 1.54^{c}
F	8726.424*	16021.500*	9033.951*	9763.173*

P	<0.001	<0.001	<0.001	<0.001

p<0.05 (Oneway ANOVA). F: F value. SD: standard deviation. *: There are differences between groups. Different superscript characters (a,b,c) in the same column indicate statistical differences between groups.

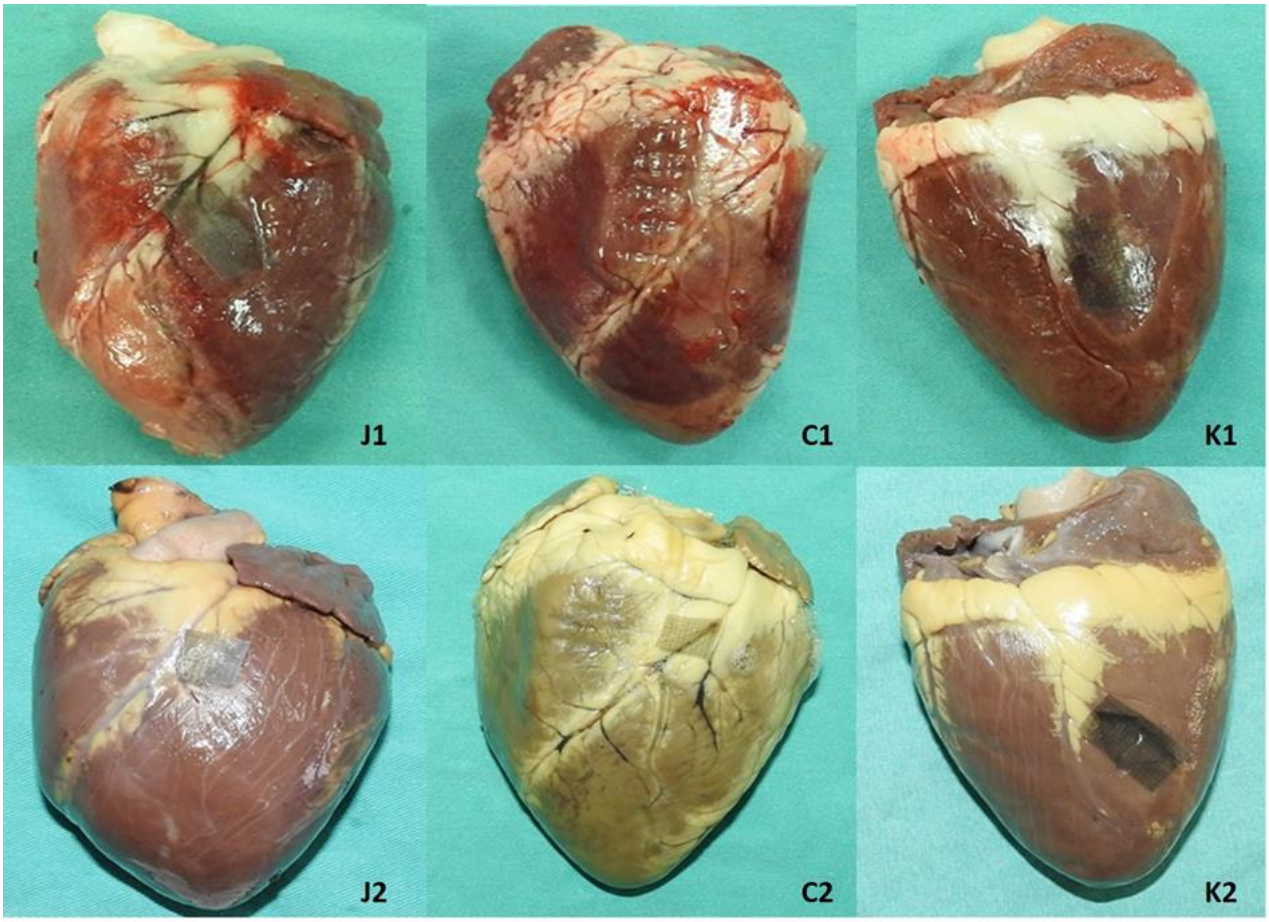

Figure 1.

J1; Fresh heart that has not yet entered the Jores solution,

J2; Heart that remained in Jores solution for three months,

C1; Fresh heart that has not yet entered the Citric acid solution,

C2; Heart that remained in Citric acid solution for three months,

K1; Fresh heart that has not yet entered the Kaiserling %5 solution,

K2; Heart that remained in Kaiserling %5 solution for three months.

Histological Findings:

When the heart tissue samples are examined, in all three samples; decrease in cellular structure, changes in cell nuclear structures, increased eosinophilia, separations in muscle fibers and endomysium structure surrounding heart fibers draw attention.

When the tissue samples are evaluated among themselves, it is observed that the cellular structure and tissue integrity are better preserved compared to other fixatives, as well as degenerative changes in the heart tissue fixed with 5% Kaiserling fixative.

Tissue integrity and cellular structure were preserved in Jores solution. However, according to Kaiserling, the rate of tissue damage is slightly higher.

When citric acid fixations are evaluated, it is observed that cellularity decreases as a result of citric acid fixation, karyolysis increases, degenerative damage increases in the epicardium and myocardium, and the destruction of the vessel wall structure is more severe.

Histopathological evaluation of three different fixative solutions; it is observed that the best fixation in tissues is provided with 5% Kaiserling, Jores and Citric acid, respectively (figure 2).

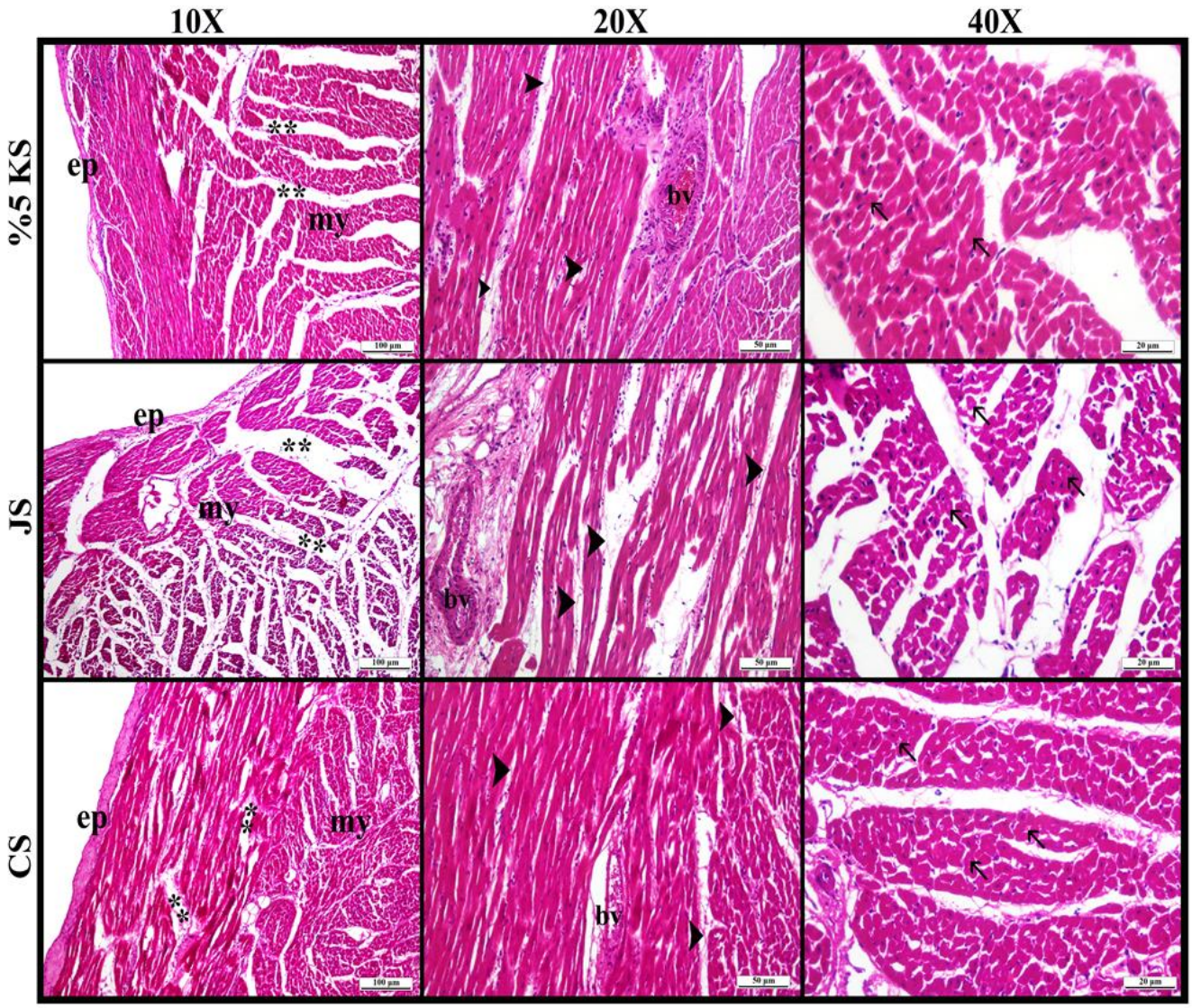

Figure 2. ep: epicardium, my: myocardium, ** : muscle fiber separations, arrowheads: separations in endomysium, arrow: karyolysis, bv: blood vessel areas.

Microbiological Findings:

No gram (+) or gram (-) microorganisms or yeast growth were found in the samples taken from Kaiserling 5%, citric acid and Jores solutions every week for three months.

Discussion

The most important element of anatomy education is the cadaver. However, providing a cadaver as well as preserving it is a very laborious process [11].

Looking from past to present, formaldehyde, the active ingredient of cadaver preservation solution, still maintains its popularity. Although the harmful irritant and carcinogenic effects of formaldehyde are known, its use has not been completely abandoned. Because formaldehyde is a solution that provides very good protection against microbial infection [12]. However, the real tissue image on cadavers fades when it comes into contact with formaldehyde solution and even begins to darken and dry after years of use. Unfortunately, this situation causes cadavers to become educational materials that move away from reality [5].

In recent years, various fixative solutions for cadaver preservation have been published in the literature. The search for this solution still continues today and is a subject of future research [13]. The most important feature of a solution is

its good protection against infectious agents[14,15]. A fter this feature, the solution is able to protect the color and texture of the cadaver in a flexible way and does not have an irritating odor [13,16].

In this study, as a result of keeping the heart organ in three different solutions, no microbial growth was observed in all three solutions (at 25°C), indicating that these solutions can be used in cadaver tanks in the laboratory. In histological analysis, 5% kaiserling followed by Jores solution, where tissue integrity and cellular structure are best preserved, may provide an advantage in embalming, especially in cadavers where microanatomical structures must be carefully preserved. Unfortunately, citric acid solution did not provide very good tissue integrity in terms of both color and histological analysis.

In the study, heart organs were kept in three solutions at room temperature for only three months. For this reason, very different results may be obtained after one, two or three years. In this study, heart organs were taken out of the solution for control purposes and placed back in the solution every week. Thus, contact with air and surface is ensured. This contact situation is important for microbial activity. Because cadaver tissue remains out of solution in the laboratory for a long time while teaching. During this process, the contaminated microorganisms re-enter the tanks and solution liquid together with the cadaver. Therefore, importance was given to keeping the heart tissue out of solution for a certain period of time each week. Color analysis gave results close to real fresh tissue in three months of Jores and Kaiserling 5% solution, but will these solutions still have color protection properties after 1, 2, 3 or years? It is a subject that needs to be studied. In parallel with this, the results obtained in histological analysis years later are also very important.

For years, formalin has been the only important preservation solution for cadaveric training. However, unfortunately, this solution has very irritating and toxic effects in anatomy laboratories. The smell it emits is even enough to drive both students and instructors away from the anatomy laboratory. Therefore, in recent years, the search for new solutions to eliminate this situation has become the most basic and current issue in the field of anatomy.

Jores and Kaiserling 5% solutions are solutions that can be recommended for the preservation of cadavers. In this study, these two solutions were evaluated histologically and microbially and the results were quite positive. Flexibility tests and long-term (more than 1, 2, 3 years) protection effects of these two solutions can be examined. This study may be an important study that will shed light on future solution studies. Improving the study and examining it in terms of different parameters may benefit many solution studies.

Conclusion

Not only is it difficult to obtain a human cadaver, but its preservation is also an issue that requires special attention. Cadaveric fixation solutions are a very current research subject that continues to be tested both in the past and today. Especially if kaiserling 5% and jore solution are used in cadaver embalming, students' interest in the anatomy laboratory may increase. Because, unfortunately, the irritating effects of formalin solution, which is widely used in our country, drives students and instructors away from the anatomy laboratory. Therefore, the quality and efficiency of anatomy education make the choice of cadaver embalming solution important for a comfortable lesson environment.

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