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IS KAISERLING SOLUTION A CONVENIENT FIXATIVE FOR MAMMALIAN ORGAN SPECIMENS? EVALUATION OF MORPHOMETRIC, COLORIMETRIC AND VOLUMETRIC PROPERTIES

C. BAKICI[1], R. O. AKGÜN[1], O. EKIM[1], Ç. OTO[1], D. ÖZEN[2] & M. BILSAY[3]

[1]Department of Anatomy Faculty of Veterinary Medicine, Ankara University, Turkey [2] Department of Biostatistics, Faculty of Veterinary Medicine, Ankara University, Turkey; [3]Faculty of Veterinary Medicine, Ankara University, Turkey

Summary

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Fixative solutions used for the preparation of anatomical specimens as for education materials show a great variety. This variation derives from the different chemical components of the fixative solutions and the proportions of these components in the solutions as well. In this study, it was aimed to make quantitative analyses to the mammalian organ specimens fixed with Kaiserling solutions prepared at different concentrations and with the formalin solution, which has frequently being used for anatomical studies. Based on our quantitative analyses Kaiserling solution was found to be more effective to preserve the natural morphometric and colorimetric values of the organs. Considering the obtained results, it is planned to develop or modify fixative solutions not only healthy for human health but also minimal morphometric, colorimetric and volumetric alterations on specimens prepared for the anatomy education.

Key words: colorimetry, fixation, Kaiserling, morphometry, preservation, shrinkage

INTRODUCTION

Dissection of the pre-prepared or fresh cadavers by the veterinary students is one of the effective education methods in anatomy practices (Eisma *et al.*, 2013; Brenner, 2014). For this reason, a proper dissection plays an important and critical role (Natekar & Desouza, 2012). However, various fixative solutions should be applied to obtain convenient specimens for education (Balta *et al.*, 2015; Elnady, 2016). With the invention of formaldehyde about 150 years ago by August Wilhelm von Hofmann, the development in fixative solutions for cadavers and organs accelerated (Balta *et al.*, 2015). Immediately after that the risks of formaldehyde on human health had been detected. Besides, morphometric and colorimetric changes on the specimens was remarkable. As a result, dimension, volume or colour differences in the tissues caused by fixative agents were discussed for so long

by the researchers (Boonstra *et al.*, 1983; Quester & Scröder, 1997; Jonmarker *et al.*, 2006; Turan *et al.*, 2017). It has been noted that different chemicals were used to prevent colour changes on tissues (Natekar & Desouza, 2012). Various fixative solutions were developed to eliminate the negative effects on the tissues. Researchers stated that Kaiserling was one of the effective solutions among the fixative agents to protect the natural colour of the tissues. In addition to that, formaldehyde concentration is very low in this solution. The specimens are prepared with 3 consecutive stages; fixation, colour restoration and storage (Pulvertaft, 1950; Boushey & Stultz, 1983; Patil *et al.*, 2013). The aim of the study was to compare the quantitative data obtained from morphometric, volumetric and colorimetric measurements of the mammalian organ specimens prepared with two different Kaiserling solutions and the formalin solution that is frequently used in the anatomy education.

MATERIAL AND METHODS

Sheep kidney, heart and spleen were used for this study. Twenty four pieces of each organ were fixed. Ethical permission was obtained from the Ankara University Animal Experiments Local Ethics Board for all procedures (Approval No: 2017-13-106). Dimensions, with digital caliper, volumes, with Archimedes method, and colour measurements, with colorimeter device (Konica Minolta CR-400, Tokyo, Japan) and its software (SpectraMagic NX), were performed on the organs before fixation procedures. Each organ were randomly assigned to one of three study groups (n=8 for each organ type in groups) that differed in terms of concentration and fixative type: 1) 4% Kaiserling solution contains 170 g potassium acetate (Merck, Darmstadt, Germany), 90 g potassium nitrate (Merck, Darmstadt, Germany) and 9600 mL 4% formalin solution (Sigma, Steinheim, Germany), 2) 10% Kaiserling solution contains 170 g potassium acetate, 90 g potassium nitrate and 9600 mL 10% formalin solution, 3) 10% formalin solution. First 2 groups, with Kaiserling solutions, were passed to next step, colour restoration. These organs were placed into 80% alcohol (Sigma, Steinheim, Germany). Then these organs were finally put into storage solution (400 g potassium acetate, 600 mL glycerin (Emir Kimya, Ankara, Turkey), and 1800 mL tap water. First two groups were kept in fixation step for ten days, colour restoration step for thirty minutes and storage step for ten days. The 3rd group, with formalin solution, was kept 20 days to equalise the total duration of Kaiserling solution groups. At the end of the fixation procedure, previous morphometric, volumetric and colorimetric measurements taken before were repeated. Therefore, alterations on the organs by the effect of fixation processes were evaluated. Descriptive statistics for each variable were calculated and presented as mean ± SEM.

Data were subjected to two-way ANOVA (analysis of variance) using General Linear Model procedure. Post hoc testing was only carried out for significant interactions and was performed using simple effect analysis with Bonferroni adjustment. One way ANOVA was used for the differences among solutions for intra cardiac measurements. Tukey test was used as post hoc procedure for significant differences. A probability value of less than 0.05 was considered as significant, unless otherwise noted. SPSS 14.01 was used for statistical analysis.

RESULTS

The fixative solutions used in this study indicated various results on the morphometric and colorimetric parameters of organs as expected. Shrinkage values of length on kidneys were not statistically significant among the Kaiserling solutions. But shrinkage values of length for spleen were constantly increased and the differences were significant between solutions (Table 1). The shrinkage values of the kidney widths were statistically significant among all solutions and these values were at maximum for formalin solution. There was no statistical difference between shrinkage values of spleen widths for Kaiserling solutions (Table 1). Shrinkage values of volumes of kidneys and hearts were statistically significant be-

Table 1. Morphometric data of the organs (mean+SEM; n=8)

	Organ	n	Kaiserling (4%)	Kaiserling (10%)	Formalin
Length	Kidney	8	11.66 ± 0.35 $^{b.\,A}$	13.07 ± 0.37 $^{b.\,A}$	15.1 ± 1.08 $^{a.\,A}$
	Spleen	8	4.95 ± 0.26 $^{c.\,B}$	11.84 ± 0.28 $^{b.\,A}$	13.52 ± 0.27 $^{a.\,A}$
	Heart	8	3.03 ± 0.34 $^{b.\,C}$	7.47 ± 0.29 $^{a.\,B}$	8.86 ± 0.25 $^{a.\,B}$
Width	Kidney	8	10.14 ± 0.65 $^{c.\,A}$	12.89 ± 0.39 $^{b.\,A}$	20.24 ± 0.69 $^{a.\,A}$
	Spleen	8	4.37 ± 0.12 $^{b.\,B}$	4.7 ± 0.12 $^{b.\,B}$	6.61 ± 0.35 $^{a.\,B}$
Volume	Kidney	8	6.01 ± 0.37 $^{c.\,A}$	9.94 ± 0.46 $^{b.\,C}$	12.99 ± 0.67 $^{a.\,C}$
	Spleen	8	3.25 ± 0.33 $^{b.\,B}$	4.76 ± 0.2 $^{b.\,B}$	9.56 ± 0.71 $^{a.\,B}$
	Heart	8	7.7 ± 0.75 $^{c.\,A}$	12.53 ± 0.38 $^{b.\,A}$	15.34 ± 0.63 $^{a.\,A}$

^{a,b,c}: Different letters in the same line indicate statistically significant difference (P<0.05); ^{A,B,C}: Different letters in the same column indicate statistically significant difference (P<0.05).

Table 2. Colorimetric data of the organs (mean+SEM; n=8)

	Organ	n	Kaiserling (4%)	Kaiserling (10%)	Formalin
dL	Kidney	8	6.81 ± 0.68 $^{b.\,A}$	5.18 ± 0.44 $^{b.\,A}$	16.02 ± 1.02 $^{a.\,B}$
	Spleen	8	4.28 ± 0.74 $^{c.\,B}$	6.56 ± 0.41 $^{b.\,A}$	19.27 ± 0.21 $^{a.\,A}$
	Heart	8	1.96 ± 0.08 $^{b.\,C}$	3.06 ± 0.08 $^{b.\,B}$	13.74 ± 0.12 $^{a.\,C}$
da	Kidney	8	–6.06 ± 0.45 $^{b.\,A}$	–6.01 ± 0.35 $^{b.\,B}$	–7.86 ± 0.28 $^{a.\,B}$
	Spleen	8	–2.8 ± 0.11 $^{b.\,B}$	–7.89 ± 0.47 $^{a.\,A}$	–8.82 ± 0.75 $^{a.\,B}$
	Heart	8	–2.1 ± 0.24 $^{c.\,B}$	–5.98 ± 0.19 $^{b.\,B}$	–10.41 ± 0.14 $^{a.\,A}$
db	Kidney	8	–0.04 ± 0.48 $^{b.\,A}$	0.11 ± 0.11 $^{b.\,A}$	2.1 ± 0.38 ^{a}
	Spleen	8	0.73 ± 0.36 $^{c.\,A}$	–1.02 ± 0.26 $^{b.\,AB}$	1.95 ± 0.2 ^{a}
	Heart	8	–2.28 ± 0.52 $^{b.\,B}$	–1.21 ± 0.36 $^{b.\,B}$	1.56 ± 0.11 ^{a}
dEab	Kidney	8	9.43 ± 0.79 $^{b.\,A}$	7.75 ± 0.5 $^{b.\,B}$	17.87 ± 1.02 $^{a.\,B}$
	Spleen	8	6.09 ± 0.46 $^{c.\,B}$	10.4 ± 0.4 $^{b.\,A}$	21.18 ± 0.42 $^{a.\,A}$
	Heart	8	3.65 ± 0.41 $^{c.\,C}$	6.88 ± 0.06 $^{b.\,B}$	17.5 ± 0.16 $^{a.\,B}$
dL	Myocardium	8	2.51 ± 0.33 ^{c}	5.53 ± 0.17 ^{b}	11.1 ± 1.09 ^{a}
da	Myocardium	8	–4.92 ± 0.25 ^{a}	–8.77 ± 0.05 ^{b}	–11.08 ± 0.7 ^{c}
db	Myocardium	8	0.02 ± 0.21 ^{b}	0.06 ± 0.35 ^{b}	1.95 ± 0.24 ^{a}
dEab	Myocardium	8	5.58 ± 0.34 ^{c}	10.45 ± 0.09 ^{b}	17.61 ± 1.28 ^{a}
	Heart	8	7.7 ± 0.75 $^{c.\,A}$	12.53 ± 0.38 $^{b.\,A}$	15.34 ± 0.63 $^{a.\,A}$

^{a,b,c}: Different letters in the same line indicate statistically significant difference (P<0.05); ^{A,B,C}: Different letters in the same column indicate statistically significant difference (P<0.05).

tween all solutions and calculated as maximum in 10% formalin solution and as minimum in 4% Kaiserling solution. But there was no statistical difference in the volumes of the spleens among the Kaiserling solutions (Table 1).

Although there was no difference in terms of colour parameters of brightness (dL) for the kidney and heart among Kaiserling solutions, it was significant for both organs fixated with formalin solution (Table 2). The difference between the yellow and blue variance of the colour (da) in the kidney was not significant in between the Kaiserling solutions. For the heart and spleen it was found significant (Fig. 1).

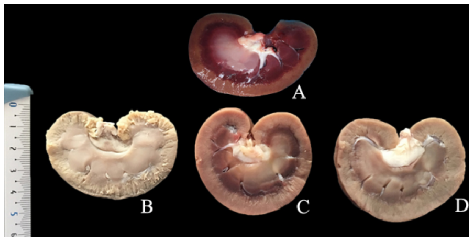

Fig. 1. Fixation colour effects on kidneys. **A**. No fixation, **B**. Formalin fixation, **C**. 4% Kaiserling fixation, **D**. 10% Kaiserling fixation.

The colour change value of all organs between yellow and blue were close to blue in the formalin solution and found statically significant (Table 2). In all organs, the colour change between green and red (db) was found statically significant between Kaiserling and formalin solutions (Fig. 2). There was statistically significant difference between Kaiserling solutions in spleen but not for kidney and heart (Table 2). In all organs, the measured change of visible variation (dEab) from the colour parameters was found statistically significant between the Kaiserling and formalin solutions (Fig. 3). There was no significant difference in kidney between Kaiserling solutions for this parameter. This parameter was higher in 10% Kaiserling solution than 4% one. All myocardium colour parameters were found to be statistically significant in between the Kaiserling solutions and formalin solution (Table 2). It was seen that the pre-fixation values affected negatively after formalin fixation.

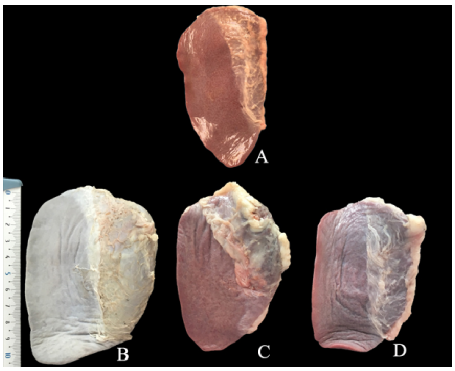

Fig. 2. Fixation colour effects on spleens. **A**. No fixation, **B**. Formalin fixation, **C.** 4% Kaiserling fixation, **D.** 10% Kaiserling fixation.

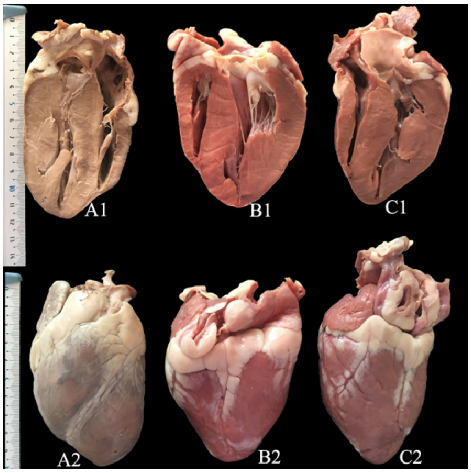

Fig. 3. Fixation colour effects on hearts. **A1-A2**. Formalin fixation, **B1-B2**. 4% Kaiserling fixation, **C1-C2**. 10% Kaiserling fixation.

DISCUSSION

A wide variety of fixation solutions have been used to preserve the tissues or organs. The most common one is the formalin solution. However, it causes remarkable morphometric and colorimetric changes on the organs (Quester & Scröder, 1997; Jonmarker *et al.*, 2006; Sandhymani *et al.*, 2005; Balta *et al.*, 2015; Kamath *et al.*, 2016). If the organ specimens are planning to be used as a teaching or presentation material, it is very important for these materials to show their natural properties. The aim of this study was to determine quantitatively morphometric, volumetric and colorimetric alterations on the kidney, spleen and heart. When length, width and volume shrinkage ratios were examined, 4% Kaiserling solution was close to the natural values in all organs than the other fixation solutions. Apart from the effect of fixation solutions on the morphometry, impact on human health is the most important property than long term storage or inhibition of bacterial growth. Formalin solution has a very bad fame about human health (Ulmer, 1994; Hayashi *et al.*, 2015; Elnady, 2016). In parallel with the previous studies, Kaiserling solution reduces the harmful effects on human health because of having little concentration of formaldehyde. Also containing glycerin allows storing the tissues for a long time and reducing the shrinkage. Preservation of natural colours of the specimens by the Kaiserling solution has been mentioned in previous studies (Sandhyamani *et al.*, 2005; Patil *et al.*, 2013). However, few researchers have quantitatively measured the effects of fixation solutions on specimens (Turan *et al.*, 2017). The effect of Kaiserling solution on the kidney, spleen and heart was quantitatively determined in this study. As a result, there was a little difference on organs between different concentrations of Kaiserling solutions. However, the organs prepared with formalin solution were rather different from their natural colours.

CONCLUSIONS

It was determined that 4% Kaiserling solution protected the natural colour of the organs more than both 10% Kaiserling and 10% formalin solution. In further studies, the plastination procedure combined with Kaiserling solution will be applied on the specimens and the morphometric and colorimetric alterations will be discussed either.

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