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Fixative properties of honey solutions as a formaldehyde substitute in cardiac tissue preservation

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Abstract: Objectives: To evaluate the properties of natural sweetener solutions in whole organ preservation and assess their influence on the dimension, weight and shape of cardiac tissue samples in stated time intervals, up to a one-year period of observation.
Background: Tissue fixation is essential for biological sample examination. Many negative toxic effects of formaldehyde-based fixatives have forced us to seek alternatives for formaldehyde based solutions. It has been demonstrated that natural sweeteners can preserve small tissue samples well and that these solutions can be used in histopathological processes. However, their ability to preserve whole human organs are unknown.
Methods: A total of 30 swine hearts were investigated. Three study groups (n = 10 in each case) were formed and classified on the type of fixative: (1) 10% formaldehyde phosphate-buffered solution (FPBS), (2) 10% alcohol-based honey solution (ABHS), (3) 10% water-based honey solution (WBHS). Samples were measured before fixation and in the following time points: 24 hours, 72 hours, 168 hours, 3 months, 6 months and 12 months.
Results: The WBHS failed to preserve heart samples and decomposition of tissues was observed one week after fixation. In half of the studied parameters, the ABHS had similar modifying tendencies as compared to FPBS. The overall condition of preserved tissue, weight, left ventricular wall thickness, right ventricular wall thickness and the diameter of the papillary muscle differed considerably.

C o n c l u s i o n s: The ABHS may be used as an alternative fixative for macroscopic studies of cardiac tissue, whereas the WBHS is not suited for tissue preservation.

Key words: formalin, heart anatomy, fixation, tissue preservation, natural sweeteners.

Introduction

Tissue fixation is an essential step for the proper assessment and preservation of biological samples. The most commonly used fixative worldwide for both clinical and research purposes is formalin — a 4% formaldehyde solution [1]. The main purpose of preserving tissues in formaldehyde based solutions is to prevent their putrefaction — this is accomplished by creating covalent chemical bonds linking the endogenous proteins with the fixative and inhibiting hydrolysis [2]. Fixatives containing formaldehyde have many advantages — they are easy to use, commercially available and inexpensive. However, they also have many known toxic effects; their irritating fumes can cause a burning sensation of the eyes, nose, and throat, coughing, wheezing, nausea, skin irritation and their cancerogenic properties have caused researchers to consider other fixatives to fulfil the dreams of a formaldehyde-free laboratory [3, 4].

Formaldehyde solutions are not the only substances used for chemical tissue preservation. Other substances such as: ethanol, methanol, acetone, Zenker's fixative, zinc-based, shellac alcoholic solution are also good preserving solutions, although their use is often limited to small histopathological sample fixation [5]. For macroscopic anatomical studies, where large organs or entire bodies need to preserved, the range of available fixatives is limited. Formaldehyde substitutes are usually ethanol based fixatives, which show relatively good results [6]. However, both use of formaldehyde and alcohol-based fixatives implies specific changes in morphological architecture and tissue dimensions, which can be problematic in morphometrical studies [7].

Recently, there have been investigations surrounding the fixative properties of natural sweeteners such as jaggery and honey to see whether they could be used as alternatives to formaldehyde [8]. Thanks to their dehydrating, protective and antibacterial properties, natural sweeteners were shown to efficiently preserve small tissue samples in histopathological processes with similar efficiency as formalin [9–11]. However, whether these fixatives could preserve large human organs remained to be seen. The aim of this study was to evaluate whether natural sweetener solutions where able to preserve whole organs, and to understand their effects on the dimension, weight and shape of cardiac tissue samples with respect to time intervals (leading up to a one year's observation period).

Materials and Methods

Samples collection

This study was performed on 30 whole porcine hearts (*Sus scrofa f. domestica*). The authors wish to stress that all the samples were originally destined for use in the food industry; thus no animals have suffered exclusively for the purpose of this study. The animal slaughter was performed according to current reference standards (Council Regulation (EC) No. 1099/2009) [12]. Animals were electrically stunned by head tongs at a high frequency and low voltage (60–80 V) to produce unconsciousness. After stunning, each pig was placed in a horizontal position, and deep incisions into the carotid arteries and jugular veins helped bleed out the animals [13]. Within one hour of the commercial slaughter, the hearts were routinely dissected from the thorax cavity. After their removal from the body, they were washed in saline solution to get rid of any remaining blood. The hearts were dissected by incising from the apex of the heart, near the interventricular septum, along the long-axis of the heart. The right and left atria were also opened by the same incision [7, 14].

Measurements

Just like in our previous studies, each heart sample had permanently tagged measurement points with pins and sutures [7, 14]. The following points were marked: the left ventricular free wall thickness (midway point between the apex and the left atrioventricular ring), the right ventricular free wall thickness (in the middle of its length), the diameter of the papillary muscle located in the left ventricle and measured at its base, the length of the chordae tendineae, the length on the internal septum surface marked between two pins, the inner diameter of the left anterior descending artery and the angle marked by three pins on the epicardial surface of the left ventricle (to help estimate the amount of tissue rotation).

After the measurements of the aforementioned points, every heart was also weighed and numbered. Then the samples were randomly assigned to one of three study groups (n = 10 each), differing only in the fixative substance to be used:

1) 10% formaldehyde phosphate-buffered solution (FPBS) (n = 10),
2) mixture obtained by dissolving 10% natural bee honey in absolute ethanol — alcohol-based honey solution (ABHS) (n = 10),
3) mixture obtained by dissolving 10% natural bee honey in distilled water — water-based honey solution (n = 10).

All the heart samples were immediately immersed in their respective fixative solution (fixative to tissue ratio = 40:1) and then stored in closed containers at room temperature (21°C).

Subsequently, every heart sample was periodically weighed and measured at fixed time intervals: 24 hours, 72 hours, one week, 3 months, 6 months and 12 months from the beginning of the initial immersion in the fixative. Linear measurements were performed using 0.03 mm precision electronic calipers (YATO YT-7201, Poland) and angle measurements were taken using a 1-degree precision half-circle protractor. All the measurements of marked structures were performed by two independent researchers to minimize human error. If a difference in values between two measurements exceeded 5%, the measurements were repeated and the mean of the two new measurements was reported [7, 14].

Statistical analysis

Data was presented as median values with corresponding lower and upper quartiles. It also included relative (percentage) changes. Friedman's nonparametric test was used to evaluate whether parameters in a certain group had significantly changed over time. If the Friedman's test results were statistically significant, a post hoc analysis was performed to determine the value change between specific time points. The differences (D) in specimen change were compared between the baseline measurements prior to fixation and measurements gathered after 12 months of immersion in the fixative (D = baseline measurement — 12 months measurement) for each solution and for each specific parameter using the Mann-Whitney or t-test. Statistical analyses were conducted using STATISTICA v13.3 (StatSoft, Inc., Tulsa, OK, USA). A p-value of less than 0.05 was considered statistically significant.

Results

The water-based honey solution failed to preserve heart samples — decomposition of tissues was noted one week after fixation. As a result, we excluded this group from our study and only FPBS and ABHS were further evaluated. Overall, the ABHS fixed hearts were more brittle and more prone to deformities than those fixed in FPBS. Moreover, the color of the tissue was markedly different depending on the studied fixatives: it was beige for FPBS and burgundy for ABHS immersed hearts.

Table 1 presents the median values of given heart parameters measured before fixation and after specific time intervals in the two types of fixatives used in our study (FPBS and ABHS). Table 2 and Fig. 1 show percentage changes of mentioned measurements relative to time.

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Table 1. Median values of measured heart parameters before fixation and at consecutive time points for samples preserved in two different fixatives.

Parameter	Study group	Before fixation	24h day	72h days	one week	3 months	6 months	12 months	
		Me (Q1; Q3)	Me (Q1; Q3)	Me (Q1; Q3)	Me (Q1; Q3)	Me (Q1; Q3)	Me (Q1; Q3)	Me (Q1; Q3)	p[a]
Heart weight (g)	ABHS (n = 10)	315.0 (301.0; 370.0)	269.0 (249.0;309.0)	241.0 (230.0; 286.0)	233.0# (220.0; 278.0)	233.5† (214.0; 275.0)	233.0‡ (212.0; 276.0)	236.0§ (213.0; 276.0)	<0.001
	FPBS (n = 10)	230.0 (210.0; 237.0)	227.0 (212.0; 237.0)	227.0 (210.0; 237.0)	2270. (209.0; 234.0)	218.0† (201.0; 223.0)	219.0‡ (194.0; 241.0)	217.0§ (200.0; 222.0)	<0.001
Angle (°)	ABHS (n = 10)	90 (90; 90)	89.5 (88.0; 96.0)	93.0 (89.0; 94.0)	92.0 (88.0; 94.0)	91.5 (89.0; 97.0)	88.5 (84.0; 95.0)	89.5 (88.0; 92.0)	**0.326**
	FPBS (n = 10)	90 (90; 90)	89.0 (84.0; 97.0)	93.0 (85.0; 97.0)	89.0 (84.0; 94.0)	88.0 (86.0; 91.0)	91.0 (89.0; 93.0)	90.0 (86.0; 92.0)	**0.613**
Length on interatrial septum surface (mm)	ABHS (n = 10)	25.2 (22.2; 27.2)	23.7 (21.5; 25.9)	22.5 (21.6; 26.0)	22.9 (21.5; 25.5)	22.7(21.6; 25.7)	23.2 (21.8; 25.6)	23.6 (22.2; 26.4)	0.010
	FPBS (n = 10)	26.0 (25.3; 26.5)	26.1 (24.2; 27.8)	25.2 (24.0; 26.8)	25.5 (25.0; 26.5)	25.5 (24.4; 27.2)	25.6 (23.7; 26.1)	24.9 (24.0; 26.1)	0.039
Coronary artery diameter (mm)	ABHS (n = 10)	3.6 (2.9; 3.9)	2.8 (2.5; 3.6)	2.1** (1.9; 2.7)	2.2# (1.6; 2.4)	2.2† (1.5; 2.4)	2.2 (1.9; 2.6)	2.2§ (1.9; 2.5)	0.001
	FPBS (n = 10)	3.3 (3.1; 3.5)	3.2 (2.9; 3.4)	3.6 (3.4; 3.8)	3.7 (2.8; 4.0)	2.7 (2.3; 3.1)	2.7 (2.1; 2.9)	2.2§ (1.9; 2.9)	0.001
Chordae tendineae length (mm)	ABHS (n = 10)	17.8 (13.7; 25.1)	19.5 (14.9; 24.5)	16.3 (13.2; 19.1)	17.0 (13.1; 19.8)	15.7 (12.6; 20.4)	19.0 (14.5; 20.4)	20.5 (16.1; 24.7)	<0.001
	FPBS (n = 10)	11.7 (6.7; 13.3)	10.1 (7.4; 13.2)	9.8 (7.0; 11.8)	8.8 (7.7; 13.8)	10.9 (9.8; 12.7)	10.4 (8.5; 14.9)	14.1 (9.0; 15.9)	**0.095**
Left ventricle thickness (mm)	ABHS (n = 10)	17.9 (16.2;19.2)	17.9 (15.4; 19.1)	16.9** (14.3; 17.4)	16.8 (14.3; 17.4)	16.3† (14.7; 17.7)	16.9‡ (14.1; 17.5)	17.2 (14.8; 17.9)	0.001
	FPBS (n = 10)	18.5 (16.5; 20.1)	20.5* (17.8; 21.3)	20.0 (17.7; 21.1)	19.7 (17.5; 20.4)	19.2 (18.5; 20.9)	20.4‡ (18.6; 21.3)	19.9 (17.0; 21.5)	0.001
Right ventricle thickness (mm)	ABHS (n = 10)	5.9 (4.5; 6.4)	4.9 (3.7; 6.4)	4.7 (3.6; 5.7)	4.4# (2.9; 5.5)	4.6† (3.1; 5.5)	5.5 (3.3; 6.2)	4.5 (3.7; 6.2)	0.002
	FPBS (n = 10)	5.8 (5.5; 7.3)	6.8 (6.5; 8.0)	6.3 (5.5; 7.5)	6.2 (5.5; 7.4)	7.3† (6.5; 8.5)	7.4 (6.7; 8.2)	6.8 (6.3; 7.9)	0.001
Papillary muscle diameter (mm)	ABHS (n = 10)	5.6 (5.0;7.2)	5.8 (4.4; 7.4)	5.8 (5.1; 6.9)	5.3 (5.0; 7.5)	5.5 (4.9; 6.7)	5.6 (4.8; 7.5)	6.1 (4.1; 7.0)	**0.969**
	FPBS (n = 10)	6.4 (5.6; 8.6)	7.1 (6.3; 9.6)	6.6 (5.8; 9.8)	6.3 (5.1; 9.5)	6.9 (6.3; 10.1)	7.5‡ (6.6; 10.6)	7.4§ (6.9; 10.1)	<0.001

Notes. ABHS — alcohol based 10% honey solution; FPBS — 10% formaldehyde phosphate-buffered solution; Me — median; n — number of samples; Q1 and Q3 — lower and upper quartiles.

[a]Friedman test

* Repeated-measures analysis of variance evaluating the 24-h time point score compared with the baseline score, $p < 0.05$.

** Repeated-measures analysis of variance evaluating the 72-h time point score compared with the baseline score, $p < 0.05$.

Repeated-measures analysis of variance evaluating the one-week point score compared with the baseline score, $p < 0.05$.

† Repeated-measures analysis of variance evaluating the 3-month time point score compared with the baseline score, $p < 0.05$.

‡ Repeated-measures analysis of variance evaluating the 6-month time point score compared with the baseline score, $p < 0.05$.

§ Repeated-measures analysis of variance evaluating the 12-month time point score compared with the baseline score, $p < 0.05$.

Table 2. Relative (percentage) changes in the measured heart parameters at consecutive time points before the preservation process (A) and between specific time intervals (B).

Parameter	Study group	A/B	24h	72h
			Me (Q1; Q3)	Me (Q1; Q3)
Heart weight (g)	ABHS (n = 10)	A	–16.9% (–18.5;–13.6)	–24.2% (–25.8; –21.9)
		B	–	–8.9% (–11.2; –7.6)
	FPBS (n = 10)	A	0.0% (–0.4; 1.0)	0.0% (–0.4; 0.0)
		B	–	0.0% (–0.5; 0.0)
Angle (°)	ABHS (n = 10)	A	–0.6% (–2.2; 6.7)	3.3% (–1.1; 4.4)
		B	–	0.6% (–3.0; 6.9)
	FPBS (n = 10)	A	–1.1% (–6.7; 7.8)	3.3% (–5.6; 7.8)
		B	–	1.2% (0.0; 3.6)
Length on interatrial septum surface (mm)	ABHS (n = 10)	A	–0.6% (–6.1; 1.6)	–0.2% (–12.9; 1.1)
		B	–	–1.5% (–4.1; 0.5)
	FPBS (n = 10)	A	3.0% (–4.3; 6.7)	–0.3% (–6.4; 1.9)
		B	–	–2.1% (–5.6; –0.8)
Coronary artery diameter (mm)	ABHS (n = 10)	A	–8.3% (–25.7; 3.4)	–24.5% (–46.2; –15.6)
		B	–	–11.7% (–30.8, 0.0)
	FPBS (n = 10)	A	–5.3% (–7.1; –1.7)	16.8 (1.4; 18.6)
		B	–	15.8% (6.2; 26.8)
Chordae tendineae length (mm)	ABHS (n = 10)	A	8.5% (–0.9; 16.0)	–5.6% (–21.0; –1.9)
		B	–	–14.0% (–20.6;–10.6)
	FPBS (n = 10)	A	2.6% (–17.8; 15.4)	4.0% (–16.7; 15.0)
		B	–	–5.9% (–18.0; 0.0)
Left ventricle thickness (mm)	ABHS (n = 10)	A	–1.4% (–11.8; 1.7)	–9.4% (–13.3; –5.6)
		B	–	–5.6% (–8.9; –1.4)
	FPBS (n = 10)	A	9.9% (3.2; 13.7)	8.1% (2.4; 10.9)
		B	–	–4.9% (–8.7; 0.6)
Right ventricle thickness (mm)	ABHS (n = 10)	A	–7.9% (–26.0; 0.0)	–9.9% (–26.0; –1.8)
		B	–	–3.5% (–18.2; 2.5)
	FPBS (n = 10)	A	11.3% (8.3; 17.6)	2.2% (–3.2; 7.6)
		B	–	–7.6% (–18.9; –3.9)
Papillary muscle diameter (mm)	ABHS (n = 10)	A	0.7% (–13.7; 5.8)	–2.0% (–4.3; 1.7)
		B	–	–5.1% (–9.6; 25.0)
	FPBS (n = 10)	A	11.6% (10.9; 20.1)	5.9% (2.7; 18.7)
		B	–	–3.4% (–13.4; –2.2)

ABHS — alcohol based 10% honey solution; FPBS — 10% formaldehyde phosphate–buffered solution; A — relative (percentage) changes of parameters in particular time intervals, always compared with results baseline-12 months.

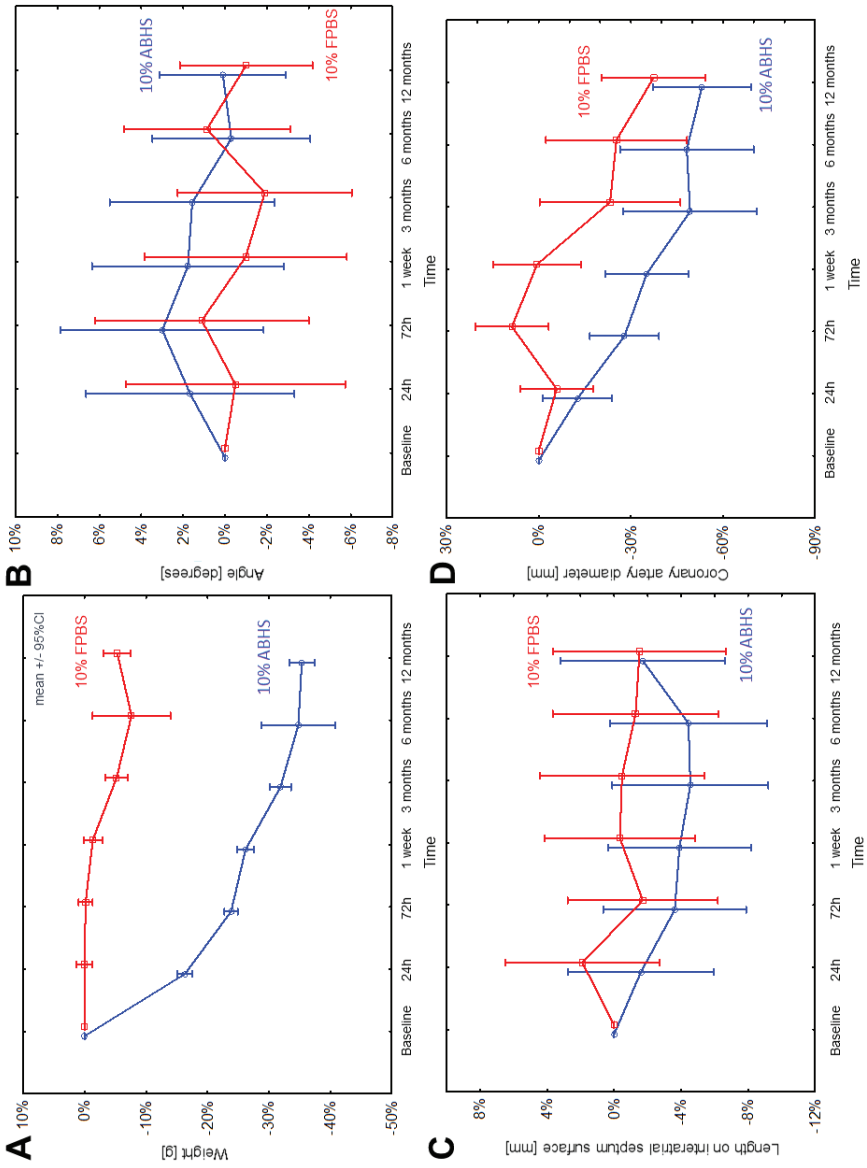
B — relative (percentage) changes of parameters in time intervals in the point-to-point comparison: 24h–72h,

for samples preserved in two different fixatives. The data are presented and compared both with values

	one week	3 months	6 months	12 months
	Me (Q1; Q3)	Me (Q1; Q3)	Me (Q1; Q3)	Me (Q1; Q3)
	–26.0% (–27.5; –24.8)	–31.0% (–33.8; –29.8)	–33.8% (–36.6; –32.1)	–34.1% (–36.1; –33.0)
	–3.1% (–3.8; –2.3)	–0.2% (–1.4; 0.4)	0.2% (–0.9; 0.5)	0.7% (0.3; 1.4)
	–0.5% (–1.3; –0.4)	–5.3% (–5.5; –4.3)	–4.8% (–6.4; –4.7)	–5.0% (–5.9; –4.8)
	–0.5% (–0.5; 0.0)	–4.3% (–5.0; –3.9)	–0.5% (–0.8; 0.5)	0.0% (–0.5; 0.4)
	2.2% (–2.2; 4.4)	1.7% (–1.1; 7.2)	–1.6% (–6.5; 5.2)	–0.6% (–2.2; 2.2)
	–1.1% (–3.1; 1.15)	0.5% (–2.3; 1.1)	0.0% (–3.3; 2.2)	–0.6 (–2.1; 3.6)
	–1.1% (–6.7; 4.4)	–2.4% (–4.1; 1.0)	1.1% (–1.2; 3.0)	0.0% (–4.7; 2.1)
	–1.3% (–2.0; 0.0)	–1.0% (–3.2; 3.5)	4.5% (–2.1; 5.8)	0.0% (–2.2; 0.0)
	–0.6% (–11.4; 0.0)	–1.4% (–13.6; 0.5)	–0.9% (–11.6; 0.4)	1.5% (–11.2; 4.2)
	–0.5% (–1.2; 0.0)	0.5% (–1.8; 1.0)	0.0% (–0.7; 2.0)	2.9% (0.9; 3.7)
	–1.1% (–3.9; 2.2)	0.3% (–3.6; 4.1)	–0.3% (–6.6; 2.5)	0.2% (–7.1; 1.7)
	–0.5% (–1.2; 4.5)	0.8% (–1.2; 1.0)	–0.4% (–2.3; 0.0)	–0.4% (–0.8; 1.6)
	–34.7% (–46.2; –29.0)	–40.1% (–51.3; –33.3)	–46.0% (–55.6; –15.8)	–50.0% (–66.7; –37.5)
	–11.1% (–15.8; 12.5)	0.0% (–16.7; 7.3)	9.5% (0.0; 26.7)	0.0% (–13.6; 10.5)
	–12.0% (–20.9; 17.5)	–26.6% (–35.9; –10.6)	–27.9% (–39.3; –11.1)	–43.7% (–46.4; –22.3)
	–5.9% (22,0; 3.1)	–20.0% (–27.2; –13.2)	–5.7% (–12.9; 0.0)	–9.5% (–17.3; –3.7)
	–6.4% (–12.1; 3.6)	–16.4% (–23.8; –5.5)	2.3% (–3.2; 8.2)	10.4% (3.6; 30.7)
	3.7% (–4.1; 8.6)	–6.5% (–16.7; 7.3)	19.0% (0.0; 43.5)	11.4% (0.8; 17.6)
	–11.5% (–24.4; 4.9)	4.2 (–6.5; 18.8)	17.9% (–15.3; 15.8)	24.4% (2.5; 27.5)
	0.9% (–9.4; 16.1)	18.1% (–10.9; 31.0)	3.1% (–10.9; 15.0)	0.0% (–1.8; 7.4)
	–8.7% (–11.3; –4.2)	–6.4% (–15.2; –3.3)	–10.1% (–14.0; –3.6)	–7.6% (–11.1; –1.1)
	0.3% (0.0; 1.8)	0.0% (–5.2; 1.9)	0.3% (–2.1; 4.0)	0.7% (–1.3; 4.7)
	6.1% (–4.9; 10.4)	8.7% (1.4; 10.9)	12.1% (9.6; 14.1)	10.7% (4.5; 11.4)
	–1.1% (–4.4; 0.0)	3.9% (–0.6; 6.3)	2.5% (1.0; 4.0)	–1.8% (–6.6; 1.2)
	–19.4% (–34.5; –14.1)	–26.4% (–51.4; –17.1)	–14.2% (–45.9; 55.9)	–14.7% (–40.0; 9.4)
	–9.7% (–16.2; –3.5)	–3.4% (–9.6; 7.3)	10.0% (5.1; 32.3)	–2.3% (–23.2; 30.2)
	–1.9% (–5.1; 5.8)	16.4% (13.8; 19.4)	13.4% (11.6; 16.7)	9.3% (–1.9; 17.6)
	0.0% (–3.9; 3.6)	16.8% (6.9; 18.7)	–3.5% (–4.5; –2.4)	–3.7% (–12.2; 0.0)
	–6.0% (–10.4; 2.7)	–2.8% (–8.9; 2.6)	–3.5% (–9.1; 7.7)	–2.7% (–19.2; 14.0)
	–1.9% (–8.5; 4.2)	1.6% (–4.9; 6.0)	2.0% (–2.6; 6.9)	–6.2% (–13.9; 16.9)
	3.2% (–7.6; 8.7)	15.5% (12.4; 19.4)	16.5% (7.6; 26.8)	15.9% (15.2; 29.1)
	–2.5% (–9.4; 0.0)	9.9% (9.0; 18.8)	4.8% (0.0; 6.9)	3.0% (–1.3; 4.5)

Me — median; n — number of samples; Q1 and Q3 — lower and upper quartiles.
before fixation (baseline): baseline-24h, baseline-72h, baseline-one week, baseline-3 months, baseline-6 months,

72h–one week, one week–3 months, 3 months–6 month and 6 months–12 months.


A
Weight [g]
mean +/- 95%CI
10% FPBS
10% ABHS
Baseline
24h
72h
1 week
3 months
6 months
12 months
Time
B
Angle [degrees]
10% ABHS
10% FPBS
C
Length on interatrial septum surface [mm]
D
Coronary artery diameter [mm]

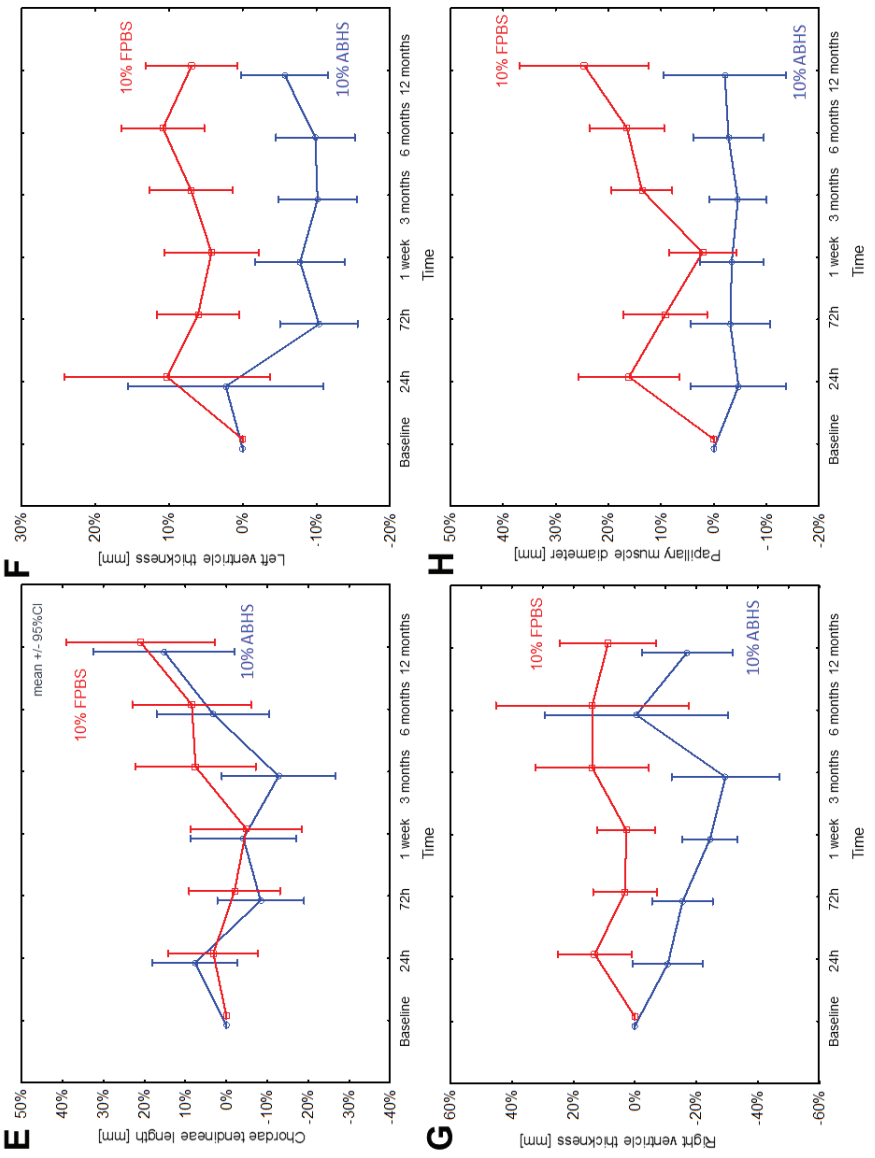


Fig. 1. Graphs of means with 95% confidence intervals (CIs) of the percentage changes of the measured morphometric parameters of the heart samples preserved in two different fixing solutions at stated time points: heart weight (A), the angle marked on the epicardial surface of the left ventricle (B), length on interatrial septum surface (C), anterior interventricular artery diameter (D), the chordae tendineae length (E), the left ventricle thickness (F), the right ventricle thickness (G), the papillary muscle diameter (H). 10% FPBS — 10% formaldehyde phosphate-buffered solution; 10% ABHS — alcohol-based honey solution.

Heart weight

For samples stored in the ABHS, the total net weight of the heart rapidly decreased during the first week of observations. It continued to slowly decrease up until the last measurement taken (the median change = –34.1%, $p < 0.05$). In contrast, the weight of hearts in FPBS did not change after the initial week; a decrease in mass began after the third month of fixation to reach a median change of –5.0% after one year's fixation time ($p < 0.05$) (Fig. 1A). Although the weight changes caused by FPBS were statistically significant, they were not nearly as pronounced as those observed in the ABHS preserved hearts ($p < 0.05$).

The angle marked on the epicardial surface of the left ventricle

Although the angle marked on the left ventricular epicardial surface did slightly fluctuate, there were no major changes observed during the entire observation period in both ABHS (median change = –0.6%, $p > 0.05$) and FPBS (median change = 0.0%, $p > 0.05$) heart specimens (Fig. 1B). During the last observation, there was no difference in percentage change of the measured angle between ABHS and FPBS samples ($p > 0.05$).

Length on the interatrial septum surface

The length of the interatrial septum surface in ABHS preserved hearts slightly decreased during the first three months but then increased in subsequent follow-ups. Consequently, there was little variation in the net measurement throughout the 12 month observation period (median change = 1.5%, $p > 0.05$). In FPBS samples, the length fluctuated minimally with median change of 0.2% after one year's observations ($p > 0.05$) (Fig. 1C). No difference was observed in terms of percentage change of measured lengths in the last observation point between ABHS and FPBS specimens ($p > 0.05$).

Anterior interventricular artery diameter

Hearts in the ABHS group demonstrated a gradual and significant decrease in the diameter of the coronary artery throughout the whole observation period. A median change of –50.0% was seen after one year's fixation ($p < 0.05$). Hearts in the FPBS group also experienced a net decrease in arterial diameter, which showed an annual median change of –43.7% ($p < 0.05$) (Fig. 1D). No difference in measured diameter was observed in the last observation point in terms of percentage changes between the two groups ($p > 0.05$).

The length of the chordae tendineae

The length of the chordae tendineae increased in both solutions during the 12 months of the preservation process, however this was shown to be statistically insignificant; ABHS median change = 10.4%, ($p > 0.05$) and FPBS median change = 24.4%, ($p > 0.05$) (Fig. 1E). No difference was observed in terms of percentage changes of measured lengths in the last observation point between ABHS and FPBS groups ($p > 0.05$).

The left ventricle wall thickness

The fixation in ABHS caused a reduction in left ventricular wall thickness (median change after one year = –7.6%, $p < 0.05$), while the fixation in FPBS had the opposite effect and thickened it (median change after one year = 10.7%, $p < 0.05$) (Fig. 1F).

The right ventricle wall thickness

The thickness of the right ventricular wall in hearts fixed in ABHS see-sawed considerably throughout the observation period. During the first 3 months, there was a steady decrease in its value, after which came an increase followed by a subsequent decline. The median change one year after initial fixation was of –14.7% ($p < 0.05$). In the FPBS group, the right ventricular wall thickness fluctuated slightly, and the observed median change was 9.3% ($p < 0.05$) (Fig. 1G). The observed percentage change after 12 months of fixation was significantly higher in the ABHS category than in the FPBS subgroup ($p < 0.05$).

The diameter of the papillary muscle

The diameter of the papillary muscle of hearts preserved in ABHS remained relatively stable throughout the 12 months (median change after one year = –2.7%, $p > 0.05$), whereas the reported values in the FPBS subgroup oscillated a lot, resulting in an overall increase in the parameter at the end of the observation period (median change = 15.9%, $p < 0.05$) (Fig. 1H). When assessing the overall percentage change (before and after 12 months of preservation), it was found to be statistically higher in the FPBS group than in its counterpart ($p < 0.05$).

Discussion

In this study, we contrasted the preservative properties of natural honey solutions and 10% FPBS. The latter has been shown to be the best formaldehyde-based fixative for cardiac morphometric purposes [14]. Commonly used fixative solutions

can be divided into two main categories based on their effect on proteins: there are protein-denaturing agents (which include alcohols such as methyl alcohol, ethyl alcohol and acetic acid) and protein cross-linking agents (which are comprised of aldehydes such as formaldehyde or glutaraldehyde) [1, 15]. Formaldehyde, a cross-linking fixative, works mainly by inducing methylene bridges between amino acids and between amino acids and nucleotides [16]. On the other hand, bee honey is renowned for its many antibacterial and healing properties. Its dehydration capacity, low pH (~3–4) and high osmolality along with its tissue preserving abilities have hinted at its possible use as a fixative [11, 17]. Moreover, there exists a theory about its possible mechanism of fixation, according to which low pH enables the fructose present in honey to breakdown aldehydes. Then, these aldehydes cross-link with tissue amino acids (similarly as in formaldehyde) allowing for fixation to occur [18].

In their double-blind pilot study, Sabarinath *et al.* used a 10% water-based honey solution as a fixative for small pathomorphological samples derived from the human oral cavity. They concluded that this easily available solution with no known toxicities could be used as a nuclear fixative and was a suitable alternative to formalin[11]. Similar results were obtained by Patil *et al.*, where an aqueous solution of 30% jaggery and 20% honey showed satisfactory fixative features of goat buccal mucosa. These tissues, immersed in the solution for 6 months, showed viability in histological processing i.e. Hematoxylin & Eosin and special stains [8]. Moreover, Özkan *et al.* suggested that a 10% water-based honey fixative could be used as a safe alternative to formaldehyde in histopathological processing of relatively small segments from different types of human tissue (endometrium, breast, placenta, uterus, omentum, stomach and lung) [10]. Unfortunately, our study cannot confirm the reliability or feasibility of honey as a fixative for large tissue samples. It seems that aqueous solutions with relatively low concentrations of natural sweetener (around 10%) do not penetrate tissues quickly and have reduced preservative potential. As such, they can only be used as fixatives for small blocks of tissue.

In this study, we also used 10% ABHS to test its fixative properties in large samples. This ethanol-based solution proved to be similar to the formaldehyde fixative. However, changes in the biological samples did occur and varied with the type of fixative used (FPBS or ABHS) and time from initial immersion in the preservative solution. The ABHS induced significant shrinkage of cardiac structures (a decrease in weight and wall dimensions was noted), most likely due to the strong dehydration properties of both ethanol and honey. The question remains whether the fixative property of the 10% ABHS was due to the properties of honey, ethanol or of the union of these two substances. Previous studies have confirmed ethanol's reliability as an excellent fixative for morphologic research, but further studies are needed to better answer the previous question [19].

Our study is not without its limitations. Firstly, animal tissues were the subject of this investigation. Although we cannot be entirely sure how the research findings would translate in human organ preservation, we believe that our results can be extrapolated in their entirety because swine hearts are very similar to human tissues anatomically and histologically [20]. Secondly, we only used one concentration and one type of natural sweetener (i.e. 10% natural bee honey). Sweeteners such as jaggery, different types of honeys, corn syrups, etc. should be tested, with varying concentrations, dissolved in different solvents, in order to find the best alternative to formaldehyde-based fixatives. Finally, we suggest that future studies investigate the fixative properties of the above-mentioned substances in a wide variety of tissues.

Conclusions

In this study we have proved that a natural sweetener, honey, may be used for long-lasting whole organ preservation. The 10% ethanol solution of the natural bee honey turned out to be a good fixative with formalin-like effects, however some differences in size and overall condition were noticed between FPBS and ABHS. For macroscopic studies of cardiac tissue, the ethanol-based honey solution can be considered as an alternative to formaldehyde. The water-based natural bee honey solution is not a suitable option for tissue preservation.

Conflict of interest

None declared.

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