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Comparison between formaldehyde and salt solutions for preservation of human liver and brain slices

Arnon Jumlongkul*

Pongpon Traithepchanapai*

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Background : *Formaldehyde exposure leads to increased risk of cancer. Recent studies reveal that salt solution can preserve human and animal tissues for analysis with high quality equal to formaldehyde.*

Objective : *To compare the effectiveness of formaldehyde and sodium chloride salt solutions in various concentrations for preservation by quantitative measurement of tissue discoloration, volume and weight of human liver and brain slices.*

Methods : *Sections of liver and brain were obtained from ten deceased subjects, and preserved in 10%, 20%, 26% sodium chloride salt solution (weight/weight) and 10% formaldehyde (volume/volume). The discoloration, volume and weight of each sample were quantitatively measured by colorimeter on the 1st day (before the preservation process), 3rd, 7th, 14th, 28th, and 56th day of the embalming.*

- Results** : *All the brain samples which were preserved in 10% sodium chloride salt solution, decomposed and only 4 samples in the 20% sodium chloride salt solution sample group survived until the 56th day of preservation. There was no statistically significant difference on the 56th and the 1st day of preservation between 26% sodium chloride salt solution and formaldehyde. The liver samples which were preserved in 10% and 20% sodium chloride salt solutions tended to decompose. There was a statistically significant difference between 26% sodium chloride salt solution and formaldehyde in A (red-green) and L (lightness) color (P - value < 0.001). This means the liver slices preserved in 26% sodium chloride salt solution showed less discoloration than those in formaldehyde.*
- Conclusions** : *It is shown that 26% sodium chloride salt solution is an appropriate alternative choice for human liver and brain slice preservation, since it is a non-carcinogenic substance and shows less discoloration than formaldehyde.*
- Keywords** : *Salt solution, preservation techniques, formaldehyde, formalin, colorimeter, decomposition.*

Correspondence to : Traithepchanapai P. Department of Forensic Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.

E-mail address: pongpon.t@chula.ac.th

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อานนท์ จำลองกุล, ปองพล ไตรเทพชนะภัย. การเปรียบเทียบการเก็บรักษาตับและ
สมองมนุษย์ระหว่างฟอร์มาลดีไฮด์และสารละลายเกลือ. จุฬาลงกรณ์เวชสาร 2560 ม.ค. - ก.พ.;
61(1): 17 - 30

- เหตุผลของการทำวิจัย** : การสัมผัสฟอร์มาลดีไฮด์นับเป็นความเสี่ยงต่อการเกิดโรคมะเร็งใน
มนุษย์ มีการศึกษาก่อนหน้านี้ระบุว่าสารละลายเกลือสามารถนำมา
ใช้เก็บรักษาเนื้อเยื่อมนุษย์และสัตว์โดยให้คุณภาพที่ใกล้เคียงกับ
การใช้ฟอร์มาลดีไฮด์
- วัตถุประสงค์** : เพื่อเปรียบเทียบประสิทธิผลระหว่างการใช้ฟอร์มาลดีไฮด์และ
สารละลายเกลือในการเก็บรักษาเนื้อเยื่อ โดยทำการวัดเชิงปริมาณ
ถึงสีที่เปลี่ยนแปลงไป ปริมาตร และน้ำหนักของตับและสมองมนุษย์
- ตัวอย่างและวิธีการศึกษา** : ตับและสมองมนุษย์จากศพจำนวน 10 ศพ ถูกนำมาหั่นแบ่งเพื่อดอง
ในสารละลายเกลือเข้มข้นร้อยละ 10, 20, 26 และฟอร์มาลดีไฮด์
เข้มข้นร้อยละ 10 อวัยวะแต่ละชิ้นถูกนำมาวัดสี ปริมาตร และน้ำหนัก
โดยใช้เครื่องวัดสี ปีกเกอร์ และตราชั่งดิจิตอล ตามลำดับ ในวันที่ 1
(ก่อนเริ่มกระบวนการดอง), 3, 7, 14, 28, และ 56 ของการดอง
- ผลการศึกษา** : ตัวอย่างสมองที่ดองด้วยสารละลายเกลือเข้มข้นร้อยละ 10 เน่า
ทั้งหมด มีสมองจากศพเพียง 4 ศพที่ดองด้วยสารละลายเกลือเข้มข้น
ร้อยละ 20 แล้วยังไม่เน่าเมื่อถึงวันที่ 56 ของการทดลอง ไม่พบ
ความแตกต่างอย่างมีนัยสำคัญทางสถิติในทุกตัวแปรของสารละลาย
เกลือเข้มข้นร้อยละ 26 และฟอร์มาลดีไฮด์ เมื่อเปรียบเทียบผลต่าง
ระหว่างวันที่ 56 และวันที่ 1 ของการดอง ส่วนตับที่ดองด้วยสาร
ละลายเกลือเข้มข้นร้อยละ 10 และ 20 มีแนวโน้มที่จะเน่า พบความ
แตกต่างอย่างมีนัยสำคัญทางสถิติของสี A และ L (P -value < 0.001)
ของผลต่างระหว่างวันที่ 56 และวันที่ 1 เมื่อทำการดองด้วยสาร
ละลายเกลือเข้มข้นร้อยละ 26 และฟอร์มาลดีไฮด์ แปลผลได้ว่า
การดองตับด้วยสารละลายเกลือความเข้มข้นร้อยละ 26 ให้สีที่
เปลี่ยนแปลงไปจากเดิมน้อยกว่าการดองด้วยฟอร์มาลดีไฮด์

- สรุป** : สารละลายเกลือเข้มข้นร้อยละ 26 สามารถใช้เป็นทางเลือกในการดองตับและสมองมนุษย์ เนื่องจากเป็นสารไม่ก่อมะเร็งและให้สีที่เปลี่ยนแปลงไปจากเดิมน้อยกว่าการดองด้วยฟอร์มาลดีไฮด์
- คำสำคัญ** : สารละลายเกลือ, เทคนิคการเก็บรักษา, ฟอร์มาลดีไฮด์, ฟอร์มาลีน, คัลเลอร์มิเตอร์, การเนา.

One essential process in preparing a human anatomy sample for teaching medical and related health science students is fixation. In gross anatomy teaching, organ slices are essential for health science students as they need to learn all of the external and internal details of the organs and their relationships. This step preserves tissue and organ structure by inhibiting the decomposition process, and uses several agents, for example Ethanol, Acetic acid, Trichloroacetic acid, Potassium dichromate, Osmium tetroxide, or Formaldehyde. The latter has been used extensively in medical schools for that purpose. ^(1,2)

An important property of Formaldehyde (CH_2O , Methyl aldehyde, Methylene glycol, Methylene oxide, Formalin) is the inhibition of microbial growth and suppression of the process of putrefaction. ^(1,3) As a consequence, it is used as a preservative agent in sample preparation for medical purposes, for instance, in gross dissection in the anatomy classroom, histopathological analysis or the forensic or anatomy museum. This agent is also used in other commercial applications, for example the leather industry, the automobile industry and the photographic industry. ^(2, 4) However, formaldehyde is a carcinogen in animals and humans. It can cause cancer of the nasopharynx, nasal cavities, lung, brain, prostate, pancreas, and lymphohematopoietic system. Nasopharyngeal cancer in humans, is an especially high risks of formaldehyde exposure. ^(2, 5) As a consequence, the European Parliament and the Council of the European Union have issued directions to reduce formaldehyde use for the safety of workers. ⁽⁶⁾ Recently, other preservative agents such as shellac, Thiel solution and saturated salt solution ^(2, 7, 8) have been investigated extensively for the purpose of reducing the use of formaldehyde.

Some recent articles propose that salt solution can preserve human and animal tissues. A human brain, which was placed in 26% sodium chloride (NaCl) salt solution at room temperature, has not been destroyed by decomposition processes for 30 years. Putrefactive change in fresh water is faster than in seawater, since the high salinity retards bacterial growth. ⁽⁹⁾ An embalming mixture of low concentration formaldehyde with high salt content has shown an excellent cadaver dissection, free of smell, safe and with the micro-structural tissue preserved, as proved by histological examination. ⁽¹⁾ Saturated sodium chloride solution has been used as a fixative for the liver, kidney and spleen of rabbits, and compared with neutral buffered formalin solution and distilled water for histological sections. The tissue sections, which were preserved in saturated sodium chloride solution, exhibited histological features the same as the formalin fixation. ⁽¹⁰⁾ Saturated salt solution has been chosen to embalm soft cadavers for surgical skills training, and compared with Thiel solution and formalin solution. The results show that salt embalming has sufficient antibiotic effects, preserves high quality tissues and produces flexible joints suitable for surgical skills training. ⁽⁷⁾

Saturated salt solution is suitable for the preparation of fixative and preservative agents. However, no previous studies have been conducted regarding the minimum concentration of sodium chloride which can effectively preserve human organs slices. Furthermore, a quantitative study of tissue discoloration, volume and weight changes for various preservative agents has not yet been carried out. Therefore, the main purpose of the study was to compare the effectiveness of preservation techniques

using formaldehyde and NaCl salt solutions. The second objective of the study was to explore the minimum NaCl concentration which can effectively preserve human brain and liver slices. The brain and liver slices that are suitably preserved might be used as teaching materials for medical and related health science students.

Materials and Methods

Subjects

Deceased subjects, who died unnatural deaths in October 2015, were sent to the Department of Forensic Medicine, Faculty of Medicine, King Chulalongkorn Memorial Hospital, Bangkok, Thailand for medico-legal autopsy. They were selected according to the following inclusion criteria:

1. The cadavers were pronounced dead within 24 hours of the autopsy;
2. There was no sign of liver or brain decomposition when examined by gross morphology.

Exclusion criteria were as follows:

1. Either the liver or brain showed gross decomposition;
2. Either the liver or brain presented pathological or traumatic findings when examined by gross morphology.

The researchers selected 10 deceased subjects which were labeled cadaver No. 1 to cadaver No. 10. Eight cadavers were male (age range from 24 to 76 years, mean age 51.38 years) and two cadavers were female (aged 25 and 81 years, mean age 51.7 years). The autopsies were performed at the Forensic Department, Faculty of Medicine, King Chulalongkorn Memorial Hospital. This study included

brain and liver slices since the brain usually presents pathological and traumatic findings and is suitable for exhibition in forensic museums as the slices show greater internal details of abnormality, while the liver represents solid organ slices which decompose more slowly.

The brain and liver slices were collected within 2 hours of the autopsy. All parameters were measured immediately before preservation and measurements were repeated on the third, seventh, fourteenth, twenty-eighth and fifty-sixth day of preservation.

Each brain was cut in coronal sections, and the liver was cut in the horizontal plane, with approximately 1 cm thickness. Eight samples (4 brain sections and 4 liver sections) were obtained from each cadaver.

This research was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (Certificated Approval Number 583/2015). Informed consents were signed by the next of kin of the donors.

Preservative agents

The study used 99% Sodium Chloride (NaCl) weight/weight (w/w) diluted to 10%, 20% and 26% NaCl salt solutions (w/w). A solution of 8% formaldehyde in 70% alcohol is classified as a high level of formalin that can destroy putrefactive organisms⁽¹¹⁾, therefore, this study used 10% formaldehyde volume/volume (v/v) since recent study⁽¹²⁾ suggests this concentration gives minimal alteration of the organ size.

All the preservative agents were diluted, and the volume of the preservative agent for each sample

was approximately 700 ml, which is the minimum volume to cover the largest tissue slice. The duration of the preservation procedure was 56 days, at room temperature.

Measuring tools

The tissue discoloration was measured by high-quality portable colorimeter, model NR20XE, 3nh[®], P. R. China, accompanied with 3nh Color Quality Controller System CQCS3 supporting software. This equipment has a 20 mm diameter extended aperture for calculating color values.

The meanings of each color (Figure 1) are as follows ⁽¹³⁾:

A (red-green) - red for positive values, green for negative values and 0 is neutral (color scale from -100 to 100).

B (blue-yellow) - yellow for positive values, blue for negative values and 0 is neutral (color scale from -100 to 100).

C (chroma) - 0 is low colorfulness and 100 is high colorfulness.

L (lightness) - 0 is black and 100 is white.

This research measured brain and liver slice areas which had homogeneous color. In brain slices, only white matter was measured, whereas in liver

slices, parenchymal tissue, which did not contain hepatic bile duct, was measured. The experiments were repeated at the same site as the first time measurement. The volume of tissue was measured by displacement of water in a measuring cup which has a precision of ± 10 cubic centimeters (cm^3). The weight of tissue was evaluated using a digital weighing scale, model HS-33, Nakata[®], Taiwan, which has a precision of ± 0.005 kilograms (kg). The color and weight were measured 3 times, and the mean value for each sample was calculated. The volumetric testing was measured only once.

Statistical analysis

A total of 7,248 data points were analyzed using IBM SPSS statistical software (Version 20). Demographic data were analyzed using descriptive statistics. The differences in the 56th and 1st day embalming of tissue discoloration, volume and weight of the samples preserved in 10%, 20%, 26% NaCl salt solution, and formaldehyde were analyzed by comparison of 2 samples with paired sample *t*-tests, multi-samples were compared using repeated ANOVA, and *P* - value < 0.05 was considered statistically significant.

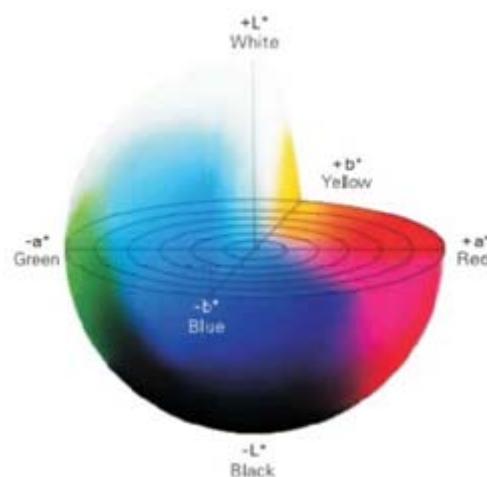


Figure 1. The CIE (Comission Internationale de l'Eclairage - International Commission of Illumination) system. ⁽¹⁴⁾

Results

Brain specimens

Once the autopsy had been performed, the samples were taken. Each brain section was between 0.06 and 0.25 kg in weight and 50 to 240 cm³ in volume, as there was a variation in the cerebral weight and volume of each cadaver, as the areas selected had to be appropriate for calculation by colorimeter in regards to homogeneous color. All the brain samples were preserved in 10% NaCl salt solution decomposed within the 56th day of embalming. There were 2 samples, however, could not be evaluated for all parameters after the 14th day, and the remainder showed decomposition on later days. Only 4 samples preserved in 20% NaCl salt solution survived to the last day of the study, and they began to show decay. Only the 26% NaCl salt solution and the formaldehyde preserved all the brain tissues (Figure 2 (A and B)

and Figure 3 (A and B)). The data showed no statistically significant difference on the 56th and 1st day of embalming between 26% NaCl salt solution and formaldehyde (Table 1).

Liver specimens

Each liver section was between 0.04 and 0.295 kg in weight and 40 to 250 cm³ in volume, as there was a variation in the liver weight and volume of each cadaver. Since the areas selected had to be appropriate for calculation by colorimeter in regards to homogeneous color. The tissues in 10% and 20% NaCl salt solutions had tended to decompose when they were observed at the end of the experiment. The samples of liver in 26% NaCl salt solution and formaldehyde are shown in Figure 2 (C and D) and Figure 3 (C and D), respectively.

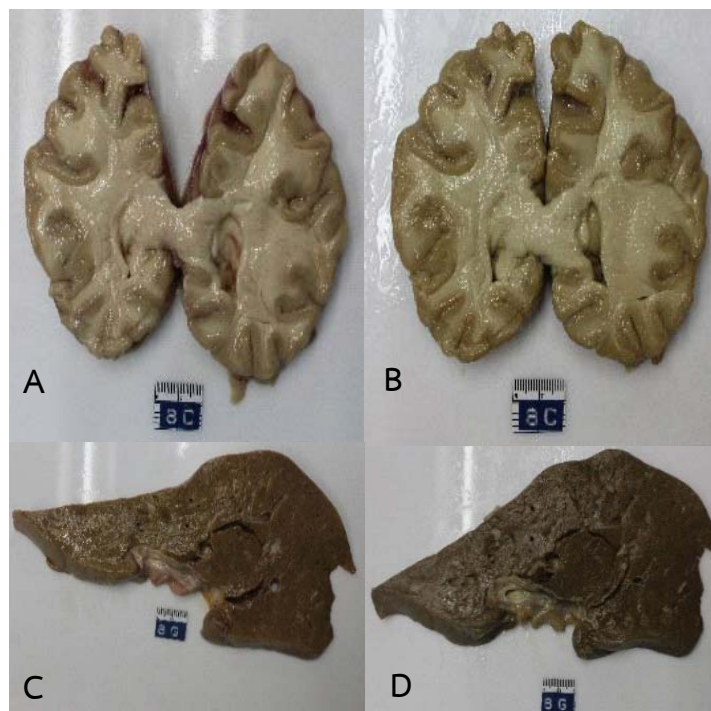


Figure 2. The organs of cadaver No. 8 (photographed by digital single lens reflex camera, Canon[®], model EOS 550D, lens zoom 18 mm, distance to object 25 cm, shutter speed 1/125, ISO 1600, white balance – auto. (A) the brain, 1st day in 26% NaCl salt solution before preservation, (B) the brain, 56th day in 26% NaCl salt solution, (C) the liver, 1st day in 26% NaCl salt solution before preservation, and (D) the liver, 56th day in 26% NaCl salt solution.

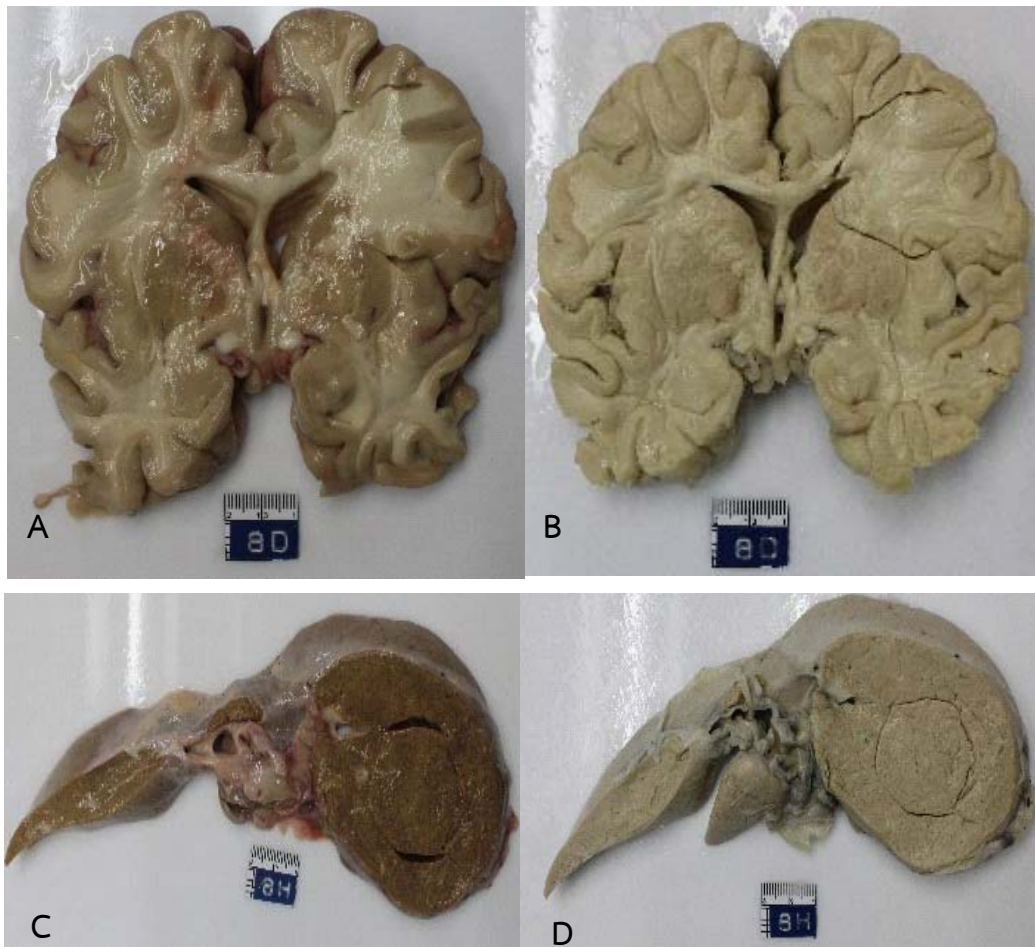


Figure 3. The organs of cadaver No. 8 (photographed by digital single lens reflex camera, Canon[®], model EOS 550D, lens zoom 18 mm, distance to object 25 cm, shutter speed 1/125, ISO 1600, white balance – auto. (A) the brain, 1st day in formaldehyde before preservation, (B) the brain, 56th day in formaldehyde, (C) the liver, 1st day in formaldehyde before preservation, and (D) the liver, 56th day in formaldehyde.

Table 1. Results of paired *t*-tests comparing the difference of brain tissues fixed in 26% NaCl salt solution and formaldehyde on the 56th and 1st day.

Parameters	Mean \pm SD		N (cadaver)	P-value (two-tailed)
	26% salt solution	10% formaldehyde		
A color	-4.151 \pm 2.200	-5.956 \pm 3.234	10	0.124
B color	8.120 \pm 4.0159	6.380 \pm 3.564	10	0.159
C color	4.850 \pm 3.908	2.602 \pm 4.400	10	0.064
L color	-13.310 \pm 2.387	-11.454 \pm 3.672	10	0.235
Weight (Kg)	-0.082 \pm 0.270	0.002 \pm 0.022	10	0.359
Volume (cm ³)	-3.000 \pm 20.976	2.000 \pm 20.440	10	0.377

The A color of the 26% NaCl salt solution and formaldehyde showed the most significant difference (A color Table 2; $P < 0.001$). This means that

formaldehyde can change hepatic tissue redness more than 26% NaCl salt solution (The average A color Figure 4).



Figure 4. The average results of 10 cadavers (brain in 20% NaCl salt solution results of 4 cadavers).

The B color of the 20% NaCl salt solution and formaldehyde showed a significant difference (B color Table 2; $P = 0.05$), suggesting that 20% NaCl salt solution can change tissue to yellowish discoloration more than formaldehyde (The average B color Figure 4).

The C color did not exhibit any significant difference (C color Table 2; $P > 0.05$) among the liver

samples (The average C color Figure 4).

The L color showed a significant difference in all the preservative agents, but formaldehyde caused the most discoloration, turning the tissue white, followed by the 20%, 10% and 26% NaCl salt solutions, respectively (L color Table 2; P of formaldehyde < 0.001) (The average L color Figure 4).

Table 2. Results of pairwise comparisons of the difference in liver tissues on the 56th and 1st day in 10%, 20%, 26% NaCl salt solutions and formaldehyde.

(a) liver-10% NaCl salt solution compared to anything, (b) liver-20% NaCl salt solution compared to anything, (c) liver-26% NaCl salt solution compared to anything, (d) liver-formaldehyde compared to anything.

Parameter	Sample	Mean \pm SD	N (cadaver)	P - value
A color	10% salt solution	-11.488 \pm 5.246	10	> 0.05 _{b, c, d}
	20% salt solution	-12.172 \pm 6.520	10	> 0.05 _{a, c, d}
	26% salt solution	-8.987 \pm 4.241	10	< 0.001 _d
	Formaldehyde	-13.423 \pm 3.814	10	< 0.001 _c
B color	10% salt solution	-2.409 \pm 5.318	10	> 0.05 _{b, c, d}
	20% salt solution	-3.147 \pm 4.632	10	0.05 _d
	26% salt solution	-0.534 \pm 3.701	10	> 0.05 _{a, b, d}
	Formaldehyde	-0.237 \pm 3.517	10	0.05 _b
C color	10% salt solution	-9.592 \pm 7.161	10	> 0.05 _{b, c, d}
	20% salt solution	-10.548 \pm 7.163	10	> 0.05 _{a, c, d}
	26% salt solution	-6.441 \pm 5.264	10	> 0.05 _{a, b, d}
	Formaldehyde	-8.318 \pm 3.600	10	> 0.05 _{a, b, c}
L color	10% salt solution	-4.300 \pm 7.256	10	< 0.05 _b , < 0.001 _d
	20% salt solution	-8.709 \pm 7.288	10	< 0.05 _{a, c} , < 0.001 _d
	26% salt solution	-3.712 \pm 6.219	10	< 0.05 _b , < 0.001 _d
	Formaldehyde	21.819 \pm 5.013	10	< 0.001 _{a, b, c}
Weight (kg)	10% salt solution	-0.009 \pm 0.030	10	< 0.05 _b
	20% salt solution	0.008 \pm 0.026	10	< 0.05 _a
	26% salt solution	0.007 \pm 0.022	10	> 0.05 _{a, b, d}
	Formaldehyde	-0.006 \pm 0.008	10	> 0.05 _{a, b, c}
Volume (cm ³)	10% salt solution	-6.000 \pm 26.750	10	> 0.05 _{b, c, d}
	20% salt solution	2.000 \pm 23.944	10	> 0.05 _{a, c, d}
	26% salt solution	2.000 \pm 22.755	10	> 0.05 _{a, b, d}
	Formaldehyde	1.500 \pm 10.014	10	> 0.05 _{a, b, c}

The hepatic weight of the 10% and 20% NaCl salt solutions showed a significant difference (Weight Table 2; $P < 0.05$) in that the 10% NaCl salt solution had tended to decrease. However, another showed the adverse result (Weight Figure 4). There was no significant difference in tissue volume (Volume Table 2; $P > 0.05$) (The average volume Figure 4).

Discussion

Formaldehyde has been used as a fixative and preservative agent for a long time. Since researchers discovered that formaldehyde is a carcinogenic substance, experiments have been conducted for the purpose of discovering agents which can substitute the formaldehyde use. ^(1, 2, 7, 8) Saturated salt solution is an option for solving this problem, since it can interfere with microbial osmosis, resulting in water loss and finally cell death or growth retardation. ⁽¹⁵⁾ Previous studies have focused on saturated salt solution for human cadaver preservation ^(7, 8) but only few researchers have reported the appropriate concentration of NaCl salt solution for cadaver and tissue preservations.

The authors applied a quantitative measurement tool for objective and reliable results. Colorimeter was chosen, since the outcomes can be revealed in a ratio scales. This tool has also been used in forensic medicine experiments for postmortem interval evaluation, by observing the change in postmortem hypostasis ⁽¹⁶⁾, and more recently to define cause of death by detecting discoloration of the skull. ⁽¹⁷⁾ However, no study with application of a colorimeter for evaluation of tissue preservation has occurred at the time the authors conduct this experiment.

In this study, 26% NaCl salt solution has been shown to preserve the human liver and brain samples for approximately 2 months without any sign of decomposition. However, 10% and 20% NaCl salt solution showed different results. From this experiment, the authors conclude that concentrations less than saturated NaCl (26% (w/w)) are inappropriate for slice preservation of the human liver and brain. In the human brain, the authors discovered no statistically significant difference between NaCl salt solution and formaldehyde, when the white matter areas were quantitatively measured for discoloration. However, as Figure 3 (A and B) shows, the difference in color of the grey matter areas of brain tissues preserved in formaldehyde were markedly paler, compared to the control sample.

In the human liver, formaldehyde altered tissue color compared to the control subject and the 26% NaCl salt solution. Therefore, 26% NaCl salt solution is an alternative preservation agent, which leads to less discoloration of the human brain and liver slices than formaldehyde.

Even though NaCl salt solution has many benefits including delaying or stopping microbial growth, lower cost, less tissue discoloration and non-carcinogenic effects, there is a drawback of using this preservation technique, as fungi can grow on the parts of organs which are exposed to the air.

There are vast opportunities for further research into this subject. For example, the application of 26% NaCl salt solution to preserve other organ slices, intact organs or other selected body regions, and the preservation of parenchymatous tissue, blood vessels and nerves.

Moreover, further research might attempt to preserve organ slices for a longer period than this study, as samples which are to be exhibited in forensic museums have to be displayed for a long period of time. All this proposed research might lead to a reduction in formaldehyde used for the benefit of health science practitioners who are exposed to carcinogenic substances.

Conclusions

This study shows the effectiveness of applying 26% NaCl salt solution to preserve human brain and liver slices, which results in similar anatomical features to original tissues. The procedure yields less discoloration; is non-carcinogenic, and has lower costs and is convenient for preparation. However, further research is essential before applying this technique for the preservation of other organ slices.

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Conflicts of interest: The authors, hereby, declare that the article is free from conflict of interest, plagiarism, fabrication or falsification.

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