ORIGINAL RESEARCH

Animal Tissue Preservation Techniques Using Ethanol and Sodium Chloride: An Experimental Study

EAST Edirisinghe[1], KM Chandimal[2], EAAM Wickramarathna[2], KN Kumarasinghe[1], LC Weerabhahu[1], PH Dissanayake[1], SG Yasawardane[1]

[1]Department of Anatomy, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka
[2]Department of Anatomy, Faculty of Medicine, Wayamba University of Sri Lanka

Abstract

Objective: Cadaveric dissection is the primary mode of anatomical teaching in Sri Lanka. Cadaveric preservation is carried and out using formaldehyde phenoxyethanol, which are costly and hazardous. This study aimed to find an alternative preservative medium.

Methods: 15 samples of fresh chicken drumsticks were kept in a preservative mixture of 95% ethanol, tap water, and glycerine with varied Sodium Chloride (NaCl) concentrations for six months to identify the best mixture. Only specimens in formaldehyde, 95% ethanol, NaCl, and Phenoxyethanol mixture were controlled. All were assessed by an independent expert anatomist for long-term macroscopic and microscopic changes in Hematoxylin and Eosin stains at time intervals of, one-week, one-month, and six-months.

Results: Thirteen specimens were well preserved till the end of six months. The two specimens preserved only in pure NaCl solution putrefied in one week. Tissues preserved using the experimental preservative showed well-preserved tissues without a putrefying odor. Histological characteristics of muscle fiber were found to be similar to specimens preserved in formaldehyde and phenoxyethanol.

Conclusion: Ethanol and NaCl has resulted in a better skeletal tissue preservation method compared to formaldehyde. It is easily accessible, cost-effective, safer1, and suited for tropical environments. Experimental studies on other tissues need to be conducted for the wider use of this method.

Key-words: cadaver preservation; animal tissue preservative medium; sodium chloride; formaldehyde

DOI: https://doi.org/10.4038/slaj.v10i2.313

Sri Lanka Anatomy Journal 2026; 10(II): 41–47

Corresponding author

EAST Edirisinghe

email: edirisinghe@sjp.ac.lk

https://orcid.org/0000-0002-8360-5157

CC BY 4.0

This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 license (unless stated otherwise) which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited. Copyright is retained by the author(s).

Received 26/02/2026 *Revised version accepted 15/06/2026* *Published 11/11/2026*

Introduction

Human bodies are irreplaceable in anatomical education (1, 2). Old World has used mummification (3, 4) arsenic, and formaldehyde (5) for cadaveric preservations. In the past, meat was preserved using condiments, bee honey, and sodium chloride (6). The Buddhist culture in Sri Lanka encourages cadaver donation (7). Cadavers are considered as first patients of medical students (8). These cadavers are preserved using various solutions. Medical schools use formaldehyde, which is occupationally hazardous (10) proven carcinogenic element (3, 5, 9–14). Research for less toxic alternatives which are more realistic and safer (4, 14) is needed.

In this study, the researchers explore novel, cost-effective, non-formaldehyde solutions suitable for tropical climates, to overcome the hazards and high costs.

Methods

Sample size was decided based on a similar study which was done utilizing 12 mouse cadavers (15). This experimental study used 15 commercially available fresh chicken drumsticks (Gallus gallus domestica) which on sale for general public consumption. The experiment was carried out for 6 months in 2022 and 2023, in the Department of Anatomy, FMS, USJ due to above said practice at the department. During the experiment as the preservative medium mixture, the team used 95% Ethanol up to 60% of the total volume, tap water up to 25% of the total volume, and glycerine up to 15% of the total volume. The Sodium Chloride (NaCl) concentrations were changed as the variable parameter to find out the best concentration. During the experiment laboratory-grade, Sodium Chloride and commercially available normal table salts were used to assess any difference in tissue preservation method. The fresh chicken drumsticks were used to understand how the skeletal muscles behave in different tissue preservative solutions (Table 1) at room temperature. Sample 01 (Only Formaldehyde), Sample 02 (Only 95% ethanol), Sample 03 and 04 (Only NaCl), and Sample 15 (Phenoxyethanol mixture) were considered as controls/ reference samples. Samples 05 to 14 were the test samples for the novel method.

Currently, the Department of Anatomy, FMS, USJ uses phenoxyethanol-based cadaver preservation techniques. To compare the histological changes in tissues in the novel method and the phenoxyethanol-based preservation method, sample 15 was preserved according to the phenoxyethanol-based technique. For this new experiment, the major component of the solution was 95% ethanol which is locally made as a byproduct of the sugar industry.

All 15 samples were prepared and fixed in December 2022. Each chicken drumstick was subjected to a few longitudinal cuts to increase the contact surface area of the experimental solution. Each sample was dipped in 160ml of prepared solution and 40ml was injected intramuscularly. All the labeled samples were kept at room temperature in sealed clear thick polyethylene bags (Figure 1).



Figure 1: Tissue samples preserved in different solutions containing polyethylene bags.

The specimens were observed and photographed for their external appearance in one week, one month, and six months from the start. The reason for observing the tissues for 06 months is to observe any long-term changes in the tissues by the novel method. During each observation session, physical appearance and characters were recorded, 1 cm^3 size samples were obtained and the rest were dipped in the same solution. The obtained samples were subjected to paraffin-based tissue fixation and routine hematoxylin and eosin stain. Histological assessment was done by an independent expert anatomist.

Results

All 15 samples were kept under observation at room temperature. Sample numbers 3 and 4 were excluded from the study by 1st week, as they were putrefied, indicating that isolated pure NaCl solution is not suitable preservative medium. The rest of the samples were proceeded with the study. Macroscopically, the tissues preserved using the novel method showed hardened tissues with a color conversion from pink to yellow (Figure 2) and there was no putrefying odor (Table 2).

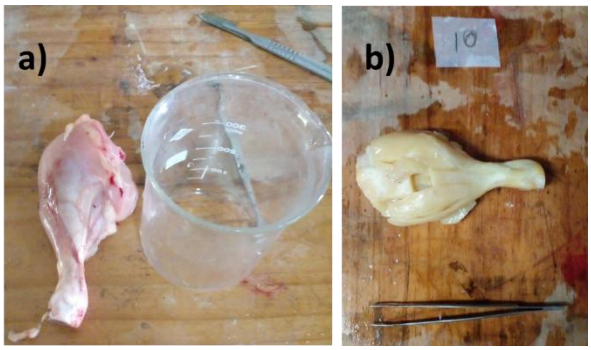


Figure 2: Tissue sample - number 10 a) before the experiment b) after 06 months of experiment

The samples preserved using the novel method were well preserved for histological examination. The samples were hard enough to obtain sections, not brittle or broken easily which are usually seen in samples that are not properly fixed. Even during the histology, tissue preparation samples were well maintained their integrity and well responded during microtome sectioning. The team observed well-preserved muscle fibres and typical characteristics of skeletal muscles on the processed slides (Figures 3 and 4). No significant tissue architectural changes were observed in samples 05 to 12.

Table 1: Different tissue preservative solutions used on the samples

Sample number	Description
01	20% Formaldehyde
02	95% Ethanol
03	100% Laboratory grade Sodium Chloride in tap water - NaCl
04	100% Table salt in tap water
05	Mixture* + Laboratory grade Sodium Chloride 5g
06	Mixture* + Table salt 5g
07	Mixture* + Laboratory grade Sodium Chloride 10g
08	Mixture* + Table salt 10g
09	Mixture* + Laboratory grade Sodium Chloride 15g
10	Mixture* + Table salt 15g
11	Mixture* + Laboratory grade Sodium Chloride 2.5g
12	Mixture* + Table salt 2.5g
13	Mixture* + Laboratory grade Sodium Chloride 1g
14	Mixture* + Table salt 1g
15	95% ethanol 60% + Phenoxyethanl 8%+ Glycerine 15% + Formaldehyde 2% + Tap water 15% o

Mixture - 95% Ethanol up to 60% of the total volume, tap water up to 25% of the total volume, and glycerine up to 15% of the total volume.*

During the microscopic examination, the tissues preserved under the novel method did not show any difference compared to tissues preserved with formaldehyde only and Phenoxyethanol mixture (Figure 05).

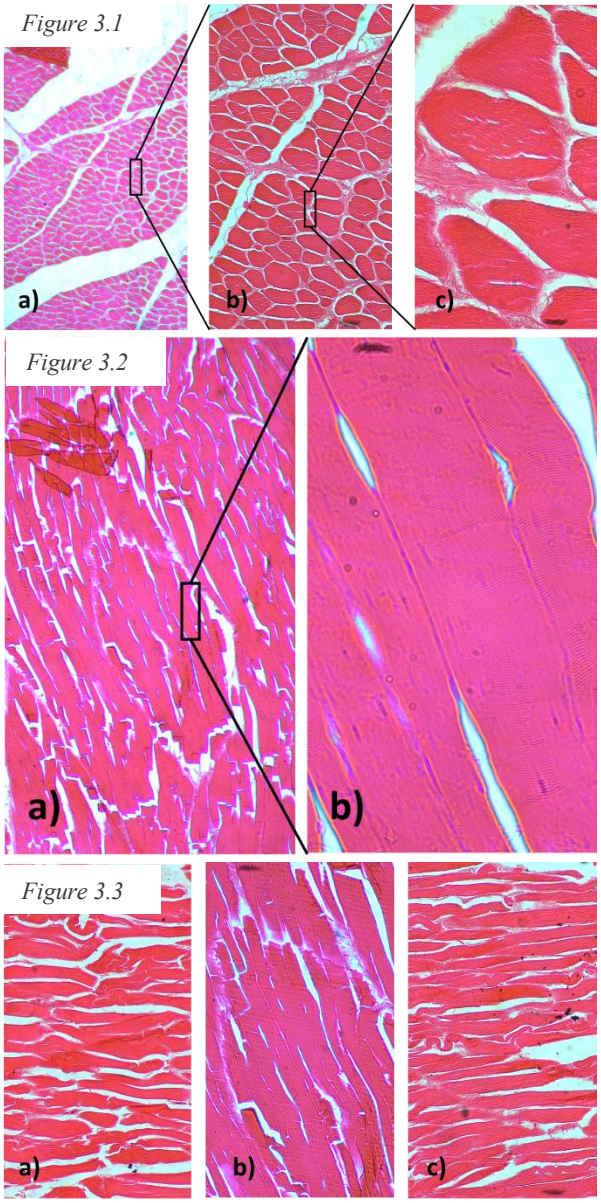


Figure 3: 3.1 - Microscopic appearance of the transverse sections of the tissues preserved according to the novel methods (sample number 10), a) low power, b) middle power, and c) high power. 3.2 - Macroscopic appearance of the longitudinal section of the tissues preserved according to the novel methods (sample number 10) after 6 months a) low power and b) high power. 3.3 - Microscopic appearance of the test samples under middle power, a) Preserved using Formaldehyde only, b) novel Ethanol, tap water Glycerin, and NaCl method, and c) Phenoxyethanol mixture

Table 2: Comparison of macroscopic appearance of the samples after 6 months

	Formaldehyde only	**Novel methods: Ethanol, Tap water, Glycerin, and NaCl mixture**	**Phenoxyethanol mixture**
	(Sample no 01)	(Sample no 05 – 14)	(Sample no 15)
Muscles	Hardened strongly gray-yellow colour	Hardened slightly Bleached and converted to a yellow colour	Bleached and converted to a yellow colour
Appearance	Hard and shrunken	Shrunken and stiffened	Shrunken and stiffened but soft
Odor	Typical irritating smell of the formaldehyde	Ethanol smell	Fruity and ethanol smell

Discussion

Since human cadavers have been preserved over generations (16) as the primary mode of anatomical medical education (17). Although formaldehyde has been commonly used for cadaver preservation in the past, anatomists with concerns about occupational health hazards attempt to use alternative solutions to formaldehyde (4) considering its carcinogenicity (13, 18).

Additionally, formaldehyde hardens the tissues strongly and makes them gray-yellow with an irritating smell making it chemically hazardous (4) (Table 2). Through our experiment, we were able to find a novel

method (Table 1) that resulted in less hardened, yellowish, but not greyish in colour, tissues with a non-irritating smell (Table 2). Additionally, the usage of NaCl solutions (Table 1) is less occupationally hazardous as it has a low risk of infection spread.

Different chemicals have been used as alternative preservatives for formaldehyde which preserve human cadavers identical to live tissue and give a more natural appearance (14, 16, 19, 20). However, most of them are costlier than formaldehyde, namely, Thiel, glutaraldehyde, and ethanol-glycerin tissue preservations. Therefore, the current trend is to use preservatives that are most cost-efficient, less health-hazardous, and effective in the preservation of tissue architecture and structure (16). Our novel method is the least costly of all, as its chemicals are locally manufactured and easily available within Sri Lanka.

The Phenoxyethanol technique is considered an effective alternative to standard human tissue preservation methods. It produces tissues that are aesthetic, pliable, durable, and odourless, with less bleaching compared to traditional methods using ethanol or formaldehyde (4). However, our experiment found that tissues preserved using Phenoxyethanol solution were stiff yet soft, yellowish in colour, and had an ethanol smell (table 2). It is important to note that Phenoxyethanol is more expensive as it needs to be imported, and there is evidence suggesting it may cause allergic contact dermatitis, headache, and depression (16). In contrast, our novel method using locally manufactured NaCl for tissue preservation is simple and cost-effective, making it a preferable option to Phenoxyethanol.

Thiel preservation also has been shown to provide better results with in terms of tissue efficacy, quality, and elasticity compared to formaldehyde preservation (19). But its limited tissue dissection time is a disadvantage (14). Our experimental method demonstrated improved tissue preservation compared to formaldehyde, with less muscle hardening and overall appearance of shrined and stiffed tissues (Table 2), Unlike Thiel preservation, our method is not technically complicated (14) and was cost-effective.

Glutaraldehyde is reported to be best in preserving cell structure with DNA, compared to other preservatives (20). Genelyn solution also has better tissue identification but the preservation period between death and embalming has to be less than 6 hours without cold storage making it practically problematic (14). Another non-carcinogenic preservative; N-Vinyl-2-pyrrolidone reported to provide no tissue stiffness, but there is limited evidence available regarding that (14). Compared to those preservatives, our experimented preservative did not have those time limitations and temperature adjustment issues as the solutions were prepared and preserved under room temperature for 6 months.

However, since NaCl causes corrosion of metals, the embalmed human bodies, cannot be stored in metal containers (14). Therefore, to introduce the novel method for medical faculties, the traditional metal containers used for storing human cadavers need to be replaced with non-corrosive alternatives.

Histologically, phenoxyethanol is a solution that gives good results, similar to formaldehyde preservations (4). One of the key advantages of our method is that the preserved tissues were well-maintained for histological slide preparation. The samples were hard enough to obtain sections, and they were easily breaking during the preparation process. This is important because it allows for

a more precise assessment of tissue and cellular morphology. The histological appearances and skeletal muscle characteristics (figure 3.1, 3.2, 3.3b) of the specimens preserved in our method were similar to those preserved either in formaldehyde (Figure 3.3a) or phenoxyethanol (Figure 3.3c).

Conclusion

In conclusion, following this experimental study, we were able to preserve the commercially available chicken (Gallus gallus domestica) samples for six months, using the above-described novel method, based on 95% ethanol and NaCl. The method is easily accessible, affordable, and less hazardous. The next experimental step of the study is hoped to be carried out on a fresh human cadaver that will be donated to the medical faculty for research and anatomical teaching purposes.

References

1. Longrigg J. At Last, At Last - Heinrich von Staden (ed., tr.): Herophilus: The Art of Medicine in Early Alexandria (Edition, Translation and Essays). Pp. xliii + 666. Cambridge University Press, 1989. £75. The Class Rev [Internet]. 1990 [cited 2025 Nov 20]; 40(2):238–40. Available from: https://www.cambridge.org/core/product/identifier/S0009840X00253444/type/journal_article
2. Brenner E, Maurer H, Moriggl B, Pomaroli A. General educational objectives matched by the educational method of a dissection lab. 2003.
3. Mekereş F, Buhaş C, Rahotă D, Moga I, Voiţă F, Mekereş GM. A new approach to exploring human anatomy. Romanian Journal of Functional & Clinical, Macro- & Microscopical Anatomy & of Anthropology/Revista Română de Anatomie funcţională şi clinică, macro- şi microscopică şi de Antropologie. 2017; 16(3):213–4.
4. Crosado B, Löffler S, Ondruschka B, Zhang M, Zwirner J, Hammer N. Phenoxyethanol-Based Embalming for Anatomy Teaching: An 18 Years' Experience with Crosado Embalming at the University of Otago in New Zealand. Anatomical Sciences Ed [Internet]. 2020 [cited 2025 Nov 20]; 13(6):778–93. Available from: https://anatomypubs.onlinelibrary.wiley.com/doi/10.1002/ase.1933
5. Souchon E. Embalming of bodies for teaching purposes. Anat Rec [Internet]. 1908 [cited 2025 Nov 20]; 2(6):244–7. Available from: https://onlinelibrary.wiley.com/doi/10.1002/ar.1090020605
6. Shephard S. Pickled, Potted, and Canned: How the Art and Science of Food Preserving Changed the World. Simon and Schuster; 2006.
7. Edirisinghe TS, Dissanayake H, Yasawardene S. Paying Respect to the Silent Mentors: A Sri Lankan Experience. SE Asian Jnl Med Educ [Internet]. 2023 [cited 2025 Nov 20]; 17(2):33–41. Available from: https://account.seajme.sljol.info/index.php/sljo-j-seajme/article/view/557
8. Ferguson KJ, Iverson W, Pizzimenti M. Constructing Stories of Past Lives: Cadaver as First Patient: "Clinical Summary of Dissection" Writing Assignment for Medical Students. TPJ [Internet]. 2008 [cited 2025 Nov 20]; 12(2):89–92. Available from: http://www.thepermanentejournal.org/doi/10.7812/TPP/07-145
9. Aziz MA, Mckenzie JC, Wilson JS, Cowie RJ, Ayeni SA, Dunn BK. The human cadaver in the age of biomedical informatics. Anat Rec [Internet]. 2002 [cited 2025 Nov 20]; 269(1):20–32.

Available from: https://onlinelibrary.wiley.com/doi/10.1002/ar.10046

10. Wilbur SB, Harris MO, McClure PR, Spoo W. Toxicological profile for formaldehyde [Internet]. 1999 [cited 2025 Nov 21]. Available from: https://stacks.cdc.gov/view/cdc/6388/cdc_6388_DS1.pdf
11. Staden H von, editor. Herophilus: The Art of Medicine in Early Alexandria: Edition, Translation and Essays. Cambridge University Press; 1989.
12. Soffritti M, Maltoni C, Maffei F, Biagi R. Formaldehyde: An Experimental Multipotential Carcinogen. Toxicol Ind Health [Internet]. 1989 [cited 2025 Nov 20]; 5(5):699–730. Available from: https://journals.sagepub.com/doi/10.1177/074823378900500510
13. Swenberg JA, Moeller BC, Lu K, Rager JE, Fry RC, Starr TB. Formaldehyde Carcinogenicity Research: 30 Years and Counting for Mode of Action, Epidemiology, and Cancer Risk Assessment. Toxicol Pathol [Internet]. 2013 [cited 2025 Nov 20]; 41(2):181–9. Available from: https://journals.sagepub.com/doi/10.1177/0192623312466459
14. Kaliappan A, Motwani R, Gupta T, Chandrupatla M. Innovative Cadaver Preservation Techniques: a Systematic Review. Maedica (Bucur) [Internet]. 2023 [cited 2025 Nov 25]; 18(1):127–35. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10231151/
15. Sajjacharoenpong K, Chuesiri P. Effectiveness of embalming solutions with partial replacement of formaldehyde and phenol with ethanol and sodium chloride on mice over 18 months. The Thai Journal of Veterinary Medicine [Internet]. 2020 [cited 2026 May 15]; 50(2):233–8. Available from: https://he01.tci-thaijo.org/index.php/tjvm/article/view/244189
16. Aslam F, Kumarasinghe N, Lowe WC. Cadaver Preservation Methods for Human Anatomy Teaching; a Three-Dimensional Approach. 2021; 3:1–004.
17. Durosaro O, Lachman N, Pawlina W. Use of Knowledge-sharing Web-based Portal in Gross and Microscopic Anatomy. Annals of the Academy of Medicine, Singapore. 2009; 37:998–1001.
18. Shian SW, Soenggono A, Pramod SV. Comparison of Tissue Preservation using Formalin and Ethanol as Preservative Formula. Althea Medical Journal [Internet]. 2016 [cited 2025 Nov 21]; 3(3):359–63. Available from: https://journal.fk.unpad.ac.id/index.php/amj/article/view/880
19. Denis-Rodríguez E, Aguirre-Gutiérrez ÁA. Thiel Soft-Fix method for long term preservation. Rev Mex Med Forense [Internet]. 2019 [cited 2025 Nov 21]; 3(2):91–8. Available from: https://www.medigraphic.com/cgi-bin/new/resumenI.cgi?IDREVISTA=340&IDARTICULO=88036&IDPUBLICACION=8407
20. Kiernan JA. Formaldehyde, Formalin, Paraformaldehyde and Glutaraldehyde: What They Are and What They Do. Microscopy Today [Internet]. 2000 [cited 2025 Nov 21]; 8(1):8–13. Available from: https://academic.oup.com/mt/article/8/1/8/6822388