Indian Journal of Clinical Anatomy and Physiology 2023;10(2):66–73

Content available at: https://www.ipinnovative.com/open-access-journals

Indian Journal of Clinical Anatomy and Physiology

Journal homepage: https://www.ijcap.org/

Review Article

Alternative natural and chemical substances to traditional formalin-based embalming fluid for cadaveric dissection: A review

Bikash Chandra Satapathy[1,*], **Biswa Bandita Kar**[2]

[1]*Dept. of Anatomy, All India Institute of Medical Sciences, Mangalagiri, Andhra Pradesh, India*
[2]*School of Applied Sciences, KIIT (Deemed to be University), Bhubaneswar, Odisha, India*

ARTICLE INFO

Article history:
Received 17-04-2023
Accepted 13-06-2013
Available online 08-07-2023

Keywords:
Embalming
Formalin
Thiel's solution
Larssen solution
Genelyn
Essential oils
Honey
Cadaver preservation
Dissection

ABSTRACT

Formalin, a widely used embalming fluid in the preservation of cadavers, poses significant health risks to anatomists, medical students, and the environment. This review article evaluates alternative natural and chemical substances for embalming fluid in cadaveric dissection. We conducted a comprehensive literature search on PubMed, Scopus, and Web of Science, focusing on alternative embalming fluids in the past 20 years. The main outcome of interest was the effectiveness of alternative fluids in preserving cadavers for dissection. The review identified several alternatives, including Thiel's solution, modified Larssen solution, Genelyn, and natural alternatives such as honey and essential oils. Thiel's solution has emerged as a popular alternative, providing excellent preservation and flexibility of cadavers. However, the high costs and the need for specialized equipment limit its widespread adoption. Essential oils and honey have also shown potential as effective, environmentally friendly alternatives to formalin. Future research should optimise the composition and application of these alternatives to improve their cost-effectiveness, feasibility, and environmental impact.

1. Introduction

Formalin-based embalming fluid has long been the standard for cadaveric preservation in anatomical education and research, dating back to the early 20th century. The effectiveness of formalin as a fixative and preservative can be attributed to its ability to cross-link proteins, thereby halting cellular processes and preventing decomposition.[1] This preservation process ensures that cadavers retain their natural appearance, making them an invaluable resource for teaching and learning anatomy.

Despite its widespread use and effectiveness, formalin presents several drawbacks. Its pungent odour is unpleasant and can distract students and faculty during dissection. Furthermore, formalin exposure has been associated with various health risks, which vary depending on the level and duration of exposure. Acute exposure can cause respiratory and dermal irritation and sensitisation, leading to allergic reactions. Chronic exposure to formalin has been linked to more severe health consequences, including neurological disorders and cancer. Formaldehyde, the main component of formalin, is a known human carcinogen. Studies indicate increased risks for nasopharyngeal and Sino nasal cancers and leukaemia among those exposed to high levels of formaldehyde.[2,3]

In addition to its health risks, using formalin raises environmental concerns. Disposing of formalin-contaminated waste can lead to soil and water pollution, impacting ecosystems and posing potential threats to human health.[4] The growing awareness of the adverse effects of formalin on human health and the environment has led to increased demand for alternative embalming

* *Corresponding author*.
E-mail address: bikash.satapathy@gmail.com (B. C. Satapathy).

https://doi.org/10.18231/j.ijcap.2023.016

fluids that offer comparable preservation quality without the associated risks.

Although formalin has been indispensable in anatomical education, exploring and developing safer and more environmentally friendly alternatives for cadaveric preservation is essential. The identification and adoption of such alternatives would not only protect the health of students and faculty and minimise the negative environmental impact of cadaveric preservation practices.

2. Methodology

PubMed/Medline and Scopus databases were searched with key words, Embalming, Formalin and preservation without limitation of year or language. MeSH words were used to narrow down the searches in PubMed. Both the authors searched the databases independently. 70 articles were collected on the basis of searches. The inclusion criteria included any article with description of alternative to traditional formalin based embalming. The exclusion criteria included articles those formulations which were reported ineffective. A collective assessment revealed 20 articles that had discussed about alternative embalming fluids. These were included for detailed review.

2.1. Thiel's solution

Thiel's solution, first introduced by Walter Thiel in 1992, has gained popularity as an alternative to formalin for cadaveric preservation due to its numerous advantages.[5] The solution is a mixture of salts, ethanol, glycerol, and a reduced amount of formaldehyde, which provides a more flexible and life-like preservation of human tissue.

The technique has a injection solution and an immersion solution followed by storage in an airtight sealed bag.

Table 1:

Solution A		**Solution B**	
Chemical	**Amount**	**Chemical**	**Amount**
Boric acid	3gm	Ethylene glycol	10ml
Ethylene glycol	30ml	4-chloro-3-methyl-phenol	1gm
Ammonium nitrate	20gm		
Potassium nitrate	5gm		
Hot water	100ml		

For making embalming solution for injecting 1 cadaver 15.8 literes of fluid are required, which is made as follows.

Table 2:

Solution A	14300ml
Solution B	500ml
Formaldehyde	300ml
Sodium Sulfate	700gm
	15800ml

Table 3:

Components	**Percentage**
Ethylene glycol	10%
Formaldehyde	2%
Solution B of Thiel	2%
Boric acid	3%
Ammonium nitrate	10%
Potassium nitrate	5%
Sodium sulphate	7%
Water	Rest

After injection of the reconstituted fluid into the cadaver, it is completely submerged in a tank containing the Immersion Solution that has the following for atleast 30 days.

This unique combination of ingredients results in a softer, more pliable preservation that allows for a more realistic dissection experience than traditional formalin-fixed cadavers. Thiel-embalmed cadavers retain tissues' natural colour and texture, making it easier for students to identify anatomical structures and distinguish between different tissue types.[6]

One of the main advantages of Thiel's solution is its reduced formaldehyde concentration, which has the potential to decrease the health risks associated with formalin exposure. Thiel's solution contributes to a healthier working environment for students and faculty engaged in dissection activities by using lower levels of formaldehyde. This benefit is especially important considering the increased awareness of the health hazards associated with formaldehyde exposure.

Another advantage of Thiel's solution is its suitability for various applications, including surgical training and research. Thiel-embalmed cadavers can be used for anatomical education and practicing surgical techniques, as their flexibility and realistic texture closely mimic living tissue. This versatility has led to the adoption of Thiel's method in various surgical disciplines, including orthopaedics, neurosurgery, and plastic surgery.[6,7]

Despite its many advantages, Thiel's method has some limitations. One of the main drawbacks is the high cost associated with the solution and the embalming process. The ingredients used in Thiel's solution, can be expensive and the which may limit its adoption by institutions with limited resources. Furthermore, the Thiel embalming process is more time-consuming than formalin-based methods, requiring a longer fixation period to achieve optimal tissue preservation.[8]

2.2. Modified larssen solution

The modified Larssen solution is another alternative to formalin-based embalming fluids that has gained attention

recently. This solution consists of phenol, glycerol, ethanol, and sodium chloride, which offer a different approach to cadaveric preservation.[9] The modified Larssen solution has been reported to provide satisfactory tissue preservation while using lower concentrations of formaldehyde than traditional formalin-based methods. This reduction in formaldehyde concentration can help to mitigate the health risks associated with formaldehyde exposure, making the modified Larssen solution a safer alternative for students and faculty involved in dissection activities.[10]

One of the key advantages of the modified Larssen solution is its cost-effectiveness. Compared to Thiel's solution, the modified Larssen solution is less expensive to produce and implement, making it a more accessible option for institutions with limited financial resources. This cost-effectiveness can be attributed to the use of more readily available and affordable ingredients and the reduced need for specialized equipment and facilities for the embalming process. The modified Larssen solution's lower cost can help promote its adoption in a wider range of educational settings, ultimately benefiting more students and faculty.[11]

Additionally, the modified Larssen solution has been reported to provide adequate preservation of cadaveric tissue, making it a viable alternative for anatomical education and research purposes. However, it is important to note that the preservation quality achieved with the modified Larssen solution may not be as superior as that achieved with Thiel's solution, particularly regarding tissue flexibility and colour retention. Further studies are needed to optimise the modified Larssen solution's preservation quality and compare its long-term effects on cadaveric tissue to those of other embalming fluids.[10]

2.3. Genelyn

Genelyn is a non-formaldehyde-based embalming fluid that has emerged as another alternative for cadaveric preservation. Composed of glutaraldehyde, quaternary ammonium compounds, and a surfactant, Genelyn offers a distinct approach to preserving human tissue, providing a viable alternative for institutions seeking to move away from formalin-based methods.[12]

One of the key advantages of Genelyn is its ability to preserve soft tissue and organs with minimal tissue distortion. This preservation quality is particularly important in anatomical education and research, as it allows students and faculty to examine the intricate structures of the human body with greater accuracy and clarity. The absence of formaldehyde in Genelyn also contributes to a safer working environment, reducing the health risks associated with formaldehyde exposure.[13] This benefit is crucial, given the growing awareness of the hazards of formaldehyde and the increasing demand for safer embalming fluids.

Genelyn's unique composition also offers additional benefits for anatomical education, including improved tissue colour retention and reduced odour compared to formalin-based methods. These attributes can enhance the overall dissection experience for students and faculty, making learning more enjoyable and effective.

Despite its numerous advantages, Genelyn's limitations may hinder its widespread adoption. One such limitation is its high cost, which can be a barrier for institutions with limited budgets.[12] The production and implementation of Genelyn may require significant financial investment, making it less accessible than more affordable alternatives like the modified Larssen solution. Additionally, the limited availability of Genelyn may further restrict its adoption in certain regions or countries where access to the product may be challenging.

2.4. Essential oils

Essential oils, derived from various plants, have been gaining attention as potential alternatives to formalin-based embalming fluids due to their natural antimicrobial and preservative properties. Eucalyptus, thyme, and lavender are among the essential oils explored for their potential in cadaveric preservation.[14] The use of essential oils in embalming offers a more natural and environmentally friendly approach compared to conventional chemical-based methods.

One of the primary advantages of using essential oils for cadaveric preservation is the reduced health risks compared to formalin-based methods. As essential oils are natural substances, they generally pose fewer hazards to human health, making them a safer option for students and faculty involved in dissection activities. Furthermore, essential oils often have pleasant, natural fragrances, which can help to create a more enjoyable learning environment.

Another notable benefit of essential oils is their environmental impact. Essential oils are biodegradable and generally have lower ecological footprints compared to chemical-based embalming fluids, such as formalin or glutaraldehyde. As awareness of the environmental impact of embalming practices grows, the adoption of more sustainable alternatives, such as essential oils, becomes increasingly important.[15]

Despite these advantages, there are still challenges to overcome in using essential oils for cadaveric preservation. One such challenge is determining the optimal concentration and combination of essential oils to provide effective long-term preservation. Further research is needed to establish the most suitable formulations for preserving human tissue while maintaining its natural appearance and texture.

Moreover, the cost-effectiveness of essential oils as embalming fluids remains to be determined. While essential oils are natural and renewable, they can also be expensive,

particularly when used in large quantities. The cost of essential oils may be a limiting factor for some institutions, especially when compared to more affordable alternatives like the modified Larssen solution.

2.5. Honey

Honey, a natural substance produced by bees, has been recognised for its antimicrobial and preservative properties for centuries. In recent years, there has been growing interest in exploring the potential of honey as an alternative to formalin-based embalming fluids for cadaveric preservation. Honey is primarily composed of sugars, which can draw out moisture from tissues and inhibit microbial growth, thus contributing to the preservation of cadaveric tissue.

One of the main advantages of using honey for cadaveric preservation is its natural origin, which is associated with fewer health risks compared to formalin-based methods. Honey poses minimal hazards to human health, making it a safer option for students and faculty involved in dissection activities.[16] Furthermore, honey has a pleasant aroma, contributing to a more enjoyable learning environment and potentially enhancing the overall dissection experience.

Another notable benefit of honey is its positive environmental impact. As a natural, biodegradable substance, honey has a lower ecological footprint than chemical-based embalming fluids, such as formalin or glutaraldehyde. Adopting honey as an embalming fluid could contribute to more sustainable anatomical education and research practices.

Despite its potential benefits, there are challenges associated with using honey for cadaveric preservation. One key challenge is determining the optimal concentration and formulation of honey to achieve effective long-term preservation while maintaining human tissue's natural appearance and texture. Further research is needed to establish the most suitable methods for using honey as an embalming fluid.

Moreover, the cost-effectiveness of honey as an embalming fluid remains to be determined. While honey is a natural and renewable substance, it can be expensive, especially when used in large quantities. The cost of honey may limit its adoption by some institutions, particularly when compared to more affordable alternatives like the modified Larssen solution.

2.6. Phenol-based solutions

Phenol-based solutions have been used as embalming fluids, and recent research has revisited their potential as alternatives to formalin-based methods. Phenol, also known as carbolic acid, is an aromatic organic compound with antimicrobial and preservative properties. It has been used in various industries, including the medical field, for its disinfecting and preserving capabilities.[17] Using phenol-based solutions for cadaveric preservation could offer a potential alternative to formalin while maintaining satisfactory preservation quality.

One of the advantages of phenol-based solutions is their relatively low cost compared to some other alternative embalming fluids, such as Thiel's solution or Genelyn. This cost-effectiveness could make phenol-based solutions more accessible for institutions with limited budgets, encouraging their adoption in a wider range of educational settings.

Phenol-based solutions have also been reported to provide adequate tissue preservation, making them suitable for anatomical education and research.[18] However, it is important to note that the preservation quality achieved with phenol-based solutions may not be as superior as that achieved with Thiel's solution or Genelyn, particularly regarding tissue flexibility and colour retention. Further research is needed to optimise phenol-based solutions' preservation quality and compare their long-term effects on cadaveric tissue to those of other embalming fluids.

Despite these potential advantages, there are concerns regarding phenol-based solutions' safety and environmental impact. Phenol is a toxic substance and can pose health risks if not handled with care, including skin irritation, respiratory problems, and systemic toxicity.[19] Moreover, the disposal of phenol-contaminated waste can contribute to soil and water pollution, posing potential threats to ecosystems and human health.[19] Therefore, it is crucial to implement proper safety measures and waste disposal protocols when using phenol-based solutions for cadaveric preservation.

2.7. Alcohol-based solutions

Alcohol-based solutions, primarily utilizing ethanol or isopropanol, have been considered as potential alternatives to formalin-based embalming fluids in cadaveric preservation. These solutions are known for their disinfecting, antimicrobial, and tissue-preserving properties, making them suitable candidates for use in anatomical education and research.[20] The use of alcohol-based solutions for cadaveric preservation could offer a safer and more environmentally friendly alternative to formalin, while still maintaining satisfactory preservation quality.

One of the advantages of alcohol-based solutions is their relatively low toxicity compared to formalin, which is associated with several health risks, including respiratory issues and carcinogenic potential. Alcohol-based solutions can help reduce the health risks for students and faculty involved in dissection activities, creating a safer learning environment. Alcohols such as ethanol and isopropanol are less pungent than formalin, which can contribute to a more pleasant dissection experience.

Alcohol-based solutions have also been reported to provide adequate tissue preservation, making them suitable

for anatomical education and research. However, it is important to note that the preservation quality achieved with alcohol-based solutions may not be as superior as that achieved with Thiel's solution or Genelyn, particularly regarding tissue flexibility and colour retention. Further research is needed to optimize the preservation quality of alcohol-based solutions and to compare their long-term effects on cadaveric tissue to those of other embalming fluids.

Despite these potential advantages, there are some concerns regarding the flammability and volatility of alcohol-based solutions. Both ethanol and isopropanol are flammable, which can pose safety risks if not handled and stored properly.[21] Moreover, alcohol-based solutions can evaporate relatively quickly, which may affect the preservation quality of the cadaveric tissue if not managed carefully. Therefore, it is crucial to implement proper safety measures and storage protocols when using alcohol-based solutions for cadaveric preservation.[22]

2.8. Gas-based preservation methods

Gas-based preservation methods have been investigated as potential alternatives to formalin-based embalming fluids for cadaveric preservation. These methods employ gases, such as nitrogen or carbon dioxide, to create an environment that inhibits microbial growth and decomposition, thus preserving the cadaveric tissue. Gas-based preservation methods offer a unique approach to cadaveric preservation that could provide a safer and more environmentally friendly alternative to traditional embalming fluids.[23]

One of the main advantages of gas-based preservation methods is their potential to provide effective tissue preservation without using chemicals, such as formaldehyde or other aldehyde-based compounds. This eliminates the health risks associated with chemical exposure, creating a safer working environment for students and faculty involved in dissection activities. Gas-based preservation methods do not produce pungent odours or hazardous waste, contributing to a more pleasant dissection experience and reduced ecological footprint.

In some cases, gas-based preservation methods have been reported to provide satisfactory tissue preservation, making them suitable for anatomical education and research purposes. However, it is important to note that the preservation quality achieved with gas-based methods may not be as superior as that achieved with some alternative embalming fluids, such as Thiel's solution or Genelyn, particularly regarding tissue flexibility and colour retention. Further research is needed to optimise the preservation quality of gas-based methods and to compare their long-term effects on cadaveric tissue to those of other embalming fluids.

Despite their potential benefits, there are challenges associated with using gas-based preservation methods for cadaveric preservation. One key challenge is requiring specialised equipment and infrastructure to create and maintain a controlled gas environment, which may be costly and difficult to implement in some institutions. Additionally, the effectiveness of gas-based preservation methods can be influenced by factors such as temperature and humidity, which may require ongoing monitoring and adjustments to ensure optimal preservation conditions.[24]

2.9. Cryopreservation

Cryopreservation has been explored as an alternative method for cadaveric preservation, using extremely low temperatures to preserve cadaveric tissue by slowing down biological processes and inhibiting microbial growth and decomposition.[25] This technique has been widely used for the preservation of cells, tissues, and organs for transplantation and research purposes. It has the potential to provide a safer and more environmentally friendly alternative to traditional embalming fluids for cadaveric preservation.

One of the main advantages of cryopreservation is its potential to provide effective tissue preservation without using chemicals, such as formaldehyde or other aldehyde-based compounds. This eliminates the health risks associated with chemical exposure, creating a safer working environment for students and faculty involved in dissection activities. Cryopreservation does not produce pungent odours or hazardous waste, contributing to a more pleasant dissection experience and reduced ecological footprint.[25]

Cryopreserved cadavers have been reported to provide satisfactory tissue preservation in some cases, making them suitable for anatomical education and research purposes. However, it is important to note that the preservation quality achieved with cryopreserved cadavers may not be as superior as that achieved with some alternative embalming fluids, such as Thiel's solution or Genelyn, particularly in terms of tissue flexibility and colour retention. Further research is needed to optimize cryopreserved cadavers' preservation quality and compare their long-term effects on cadaveric tissue to those of other embalming fluids.[26]

Despite the potential benefits of cryopreservation, there are challenges associated with using this method for cadaveric preservation. One key challenge is the requirement for specialized equipment and infrastructure to maintain extremely low temperatures, which may be costly and difficult to implement in some institutions.[26] Additionally, the process of thawing cryopreserved cadavers can be time-consuming, and improper thawing techniques may result in tissue damage or reduced preservation quality.

2.10. Saturated salt solution

Saturated salt solutions have been investigated as potential alternatives to formalin-based embalming fluids for cadaveric preservation. These solutions utilize high concentrations of salts, such as sodium chloride, to create an osmotic environment that inhibits microbial growth and decomposition, thus preserving the cadaveric tissue.[27] Saturated salt solutions offer a unique approach to cadaveric preservation that could provide a safer and more environmentally friendly alternative to traditional embalming fluids.

One of the main advantages of saturated salt solutions is their potential to provide effective tissue preservation without using chemicals, such as formaldehyde or other aldehyde-based compounds. This eliminates the health risks associated with chemical exposure, creating a safer working environment for students and faculty involved in dissection activities.[28] Additionally, saturated salt solutions do not produce pungent odours or hazardous waste, contributing to a more pleasant dissection experience and reduced ecological footprint.

Saturated salt solutions have been reported to provide satisfactory tissue preservation in some cases, making them suitable for anatomical education and research purposes.[29] However, it is important to note that the preservation quality achieved with saturated salt solutions may not be as superior as that achieved with some alternative embalming fluids, such as Thiel's solution or Genelyn, particularly regarding tissue flexibility and colour retention. Further research is needed to optimise the preservation quality of saturated salt solutions and to compare their long-term effects on cadaveric tissue to those of other embalming fluids.

Despite their potential benefits, there are challenges associated with using saturated salt solutions for cadaveric preservation. One key challenge is the requirement for specialized storage conditions to prevent desiccation and maintain the osmotic environment necessary for preservation.[30] Additionally, the use of saturated salt solutions may result in tissue dehydration, leading to potential alterations in tissue appearance and texture. Further research is needed to address these challenges and optimize the use of saturated salt solutions for effective cadaveric preservation.

3. Discussion

Thiel's solution comes close to a perfect alternative low formaldehyde solution than the traditional 4-10% formalin used in many set ups. Many institutions are teaching surgical trainees through Thiel's embalmed cadavers in workshops. However, the ingredients are of high cost and most of its components needs to be freshly prepared before mixing. For the complete fixation earlier six months of immersion was required. Then, at least one month of immersion was found needed for full fixation. Many researchers have modified the original Thiel's recipe differently in search of making the method cost-effective and decreasing immersion duration.

The modified Larssen solution offers a cost-effective and potentially safer alternative to formalin-based embalming fluids for cadaveric preservation.[11] While its preservation quality may not be as exceptional as Thiel's solution, its lower cost and reduced formaldehyde concentration make it an attractive option for institutions seeking a more accessible alternative. Future research should focus on optimising the preservation quality of the modified Larssen solution and comparing its long-term effects on cadaveric tissue to those of other embalming fluids.

Genelyn potentially provides excellent tissue preservation and reducing health risks associated with formaldehyde exposure. However, the high cost and limited availability of Genelyn may constrain its widespread use in anatomical education. Due to proprietary nature and higher cost the use is still limited.[12]

Essential oils present an environmentally friendly alternative to formalin-based embalming fluids. However other than anecdotal usage and history of usage in mummification, they have not been used widely in modern embalming techniques.[31]

Honey offers a natural and environmentally friendly alternative to formalin-based embalming fluids. The purity of honey, its long term efficacy has been questioned, but due to its organic origin widespread use has not been seen. But as it is the best natural substance that preserves even the histological features it holds potential for research in this field.

Phenol-based solutions provide adequate tissue preservation at a lower cost. Many researchers have complained of the health hazards of phenol. It is still part of many traditional formalin-based embalming fluids as it is used as an effective and cheap antiseptic. In autopsied bodies, many centres use direct phenol injection as a preservative.[18]

Alcohol-based solutions offer a potential alternative to formalin-based embalming fluids, providing adequate tissue preservation and reducing health risks. Alcohol smell is non pungent hence though it is an astringent, but is more tolerable as a chemical to work on. However, flammability and volatility safety concerns warrant caution and further investigation. However, preservation for histological usage is limited as alcohol dries the tissues.[20]

Saturated salt solutions can be a great alternative. Many centers use them for soft embalming as there is no pungent smell. Bodies are comparatively soft to dissect. But long term preservation is not there and dehydration is always a challenge.[32]

4. Conclusion

This review has discussed various alternative natural and chemical substances to formalin-based embalming fluid for cadaveric dissection, including Thiel's, modified Larssen, Genelyn, essential oils, honey, and phenol-based, alcohol-based solutions, aldehyde-free embalming fluids, gas-based preservation methods, cryopreservation, and saturated salt solutions. Each alternative method presents its unique advantages and challenges in terms of preservation quality, safety, environmental impact, and feasibility of implementation.

While some of the discussed alternatives, such as Thiel's solution and Genelyn, have demonstrated superior preservation quality, others, like essential oils and saturated salt solutions, may require further research and optimisation to achieve comparable results. Moreover, many of these alternatives are more environmentally friendly and pose fewer health risks than formalin-based embalming fluids, making them more suitable for modern anatomical education and research.

When selecting an appropriate alternative embalming method, it is essential to consider individual institutions' specific needs and constraints. Factors such as cost, availability, infrastructure requirements, and the desired preservation quality must be considered to ensure the chosen method is suitable for educational and research purposes.

Further research is necessary to optimise using these alternative embalming fluids, better understand their long-term effects on cadaveric tissue preservation, and assess their feasibility and cost-effectiveness compared to traditional formalin-based embalming fluids. As anatomical education and research continue to evolve, developing and implementing safer and more sustainable cadaveric preservation methods will be crucial in advancing our understanding of human anatomy and improving the quality of education and training for future healthcare professionals.

5. Source of Funding

None.

6. Conflict of Interest

None.

References

1. Rezoana R, Akter L, Islam R, Bhakta S, Ayman U, Karim MR, et al. The hazardous effects of formalin and alcoholic fixative in mice: A public health perspective study. *Saudi J Biol Sci*. 2022;29(5):3366–71.
2. Kwon SC, Kim I, Song J, Park J. History of the hazards of formalin and the birth of formalin substitutes. *Ann Occup Environ Med*. 2018;30(1).
3. Rana I, Rieswijk L, Steinmaus C, Zhang L. Formaldehyde and Brain Disorders: A Meta-Analysis and Bioinformatics Approach. *Neurotox Res*. 2021;39(3):924–48.
4. Bashir I, Lone FA, Bhat RA, Mir SA, Dar ZA, Dar SA. Concerns and Threats of Contamination on Aquatic Ecosystems. *Bioremed Biotechnol*. 2020;p. 1–26. doi:10.1007/978-3-030-35691-0_1.
5. Thiel W. The preservation of the whole corpse with natural color. *Ann Anat*. 1992;174(3):185–95.
6. Yiasemidou M, Roberts D, Glassman D, Tomlinson J, Biyani S, Miskovic D. A Multispecialty Evaluation of Thiel Cadavers for Surgical Training. *World J Surg*. 2017;41(5):1201.
7. Eisma R, Lamb C, Soames RW. From formalin to Thiel embalming: What changes? One anatomy department's experiences. *Clin Anat*. 2013;26(5):564–71.
8. Liao P, Wang Z. Thiel-embalming technique: investigation of possible modification in embalming tissue as evaluation model for radiofrequency ablation. *J Biomed Res*. 2019;33(4):280–8.
9. Celik S, Bilge O, Ozdemir M, Dionigi G, Anuwong A, Makay O. Modified Larssen solution (MLS)-fixed cadaver model for transoral endoscopic thyroidectomy vestibular approach (TOETVA) education: a feasibility study. *Surg Endosc*. 2022;36(7):5518–30.
10. Kaliappan A, Motwani R, Gupta T, Chandrupatla M. Innovative Cadaver Preservation Techniques: a Systematic Review. *Maedica (Bucur)*. 2023;18(1):127–35.
11. Bilge O, Celik S. Cadaver embalming fluid for surgical training courses: modified Larssen solution. *Surg Radiol Anat*. 2017;39(11):1263–72.
12. Rajasekhar S, Kumar VD, Raveendranath V, Kalayarasan R, Gnanasekaran S. An improved embalming method for long-term preservation of the cadaver for anatomical study. *J Minim Access Surg*. 2021;17(4):495–501.
13. Balta JY, Cronin M, Cryan JF, O'Mahony SM. Human preservation techniques in anatomy: A 21st century medical education perspective. *Clin Anat*. 2015;28(6):725–34.
14. Wińska K, Mączka W, Łyczko J, Grabarczyk M, Czubaszek A, Szumny A. Essential oils as antimicrobial agents—myth or real alternative? *Molecules*. 2019;24(11):2130.
15. Dagli N, Dagli R, Mahmoud R, Baroudi K. ssential oils, their therapeutic properties, and implication in dentistry: A review. *J Int Soc Prev Community Dent*. 2015;5(5):335.
16. Bhattacharyya A, Gupta B, Singh A, Sah K, Gupta V. Probing natural substitute for formalin: Comparing honey, sugar, and jaggery syrup as fixatives. *Natl J Maxillofac Surg*. 2018;9(1):14.
17. McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. *Clin Microbiol Rev*. 1999;12(1):147–79.
18. 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. *Anat Sci Educ*. 2020;13(6):778–93.
19. Brancato DJ. Recognizing potential toxicity of phenol. *Vet Hum Toxicol*. 1982;24(1):29–30.
20. Essen HFV, Verdaasdonk M, Elshof SM, De Weger R, Diest PJV. Alcohol based tissue fixation as an alternative for formaldehyde: influence on immunohistochemistry. *J Clin Pathol*. 2010;36(12):1090–4.
21. Saha T, Khadka P, Das SC. Alcohol-based hand sanitizer – composition, proper use and precautions. *Germs*. 2021;11(3):408–17.
22. Lachenmeier DW. Safety evaluation of topical applications of ethanol on the skin and inside the oral cavity. *J Occup Med Toxicol*. 2008;3(1):26.
23. Kobayashi E, Sano M. Organ preservation solution containing dissolved hydrogen gas from a hydrogen-absorbing alloy canister improves function of transplanted ischemic kidneys in miniature pigs. *PLoS One*. 2019;14(10):e0222863.
24. Rodriguez MBR, Junior C, Carneiro CS, Franco RM, Mano SB. The effect of carbon dioxide on the shelf life of ready-to-eat shredded chicken breast stored under refrigeration. *Poult Sci*. 2014;93(1):194–9.
25. Jaiswal AN, Vagga A. Cryopreservation: A Review Article. *Cureus*. 2022;14(11):e31564.
26. Zarb F, McNulty J, Gatt A, Formosa C, Chockalingam N, Evanoff MG, et al. Comparison of in vivo vs. frozen vs. Thiel cadaver specimens in visualisation of anatomical structures of the ankle on proton density Magnetic Resonance Imaging (MRI) through a visual grading analysis

(VGA) study. *Radiography (Lond)*. 2017;23(2):117–24.
27. Burns DM, Bell I, Katchky R, Dwyer T, Toor J, Whyne CM, et al. Comparison of salt solution and formalin as a preservative for cadavers: An experimental study. *J Bone Joint Surg Am*. 2018;100(15):e104.
28. Hayashi S, Naito M, Kawata S, Qu N, Hatayama N, Hirai S, et al. History and future of human cadaver preservation for surgical training: from formalin to saturated salt solution method. *Anat Sci Int*. 2016;91(1):1–7.
29. Watanabe M, Yoneyama Y, Hamada H, Kohno M, Hasegawa O, Takahashi H, et al. The Usefulness of Saturated Salt Solution Embalming Method for Oral Surgical Skills Training: A New Cadaveric Training Model for Bone Harvesting. *Anat Sci Educ*. 2020;13(5):628–35.
30. Homma H, Oda J, Sano H, Kawai K, Koizumi N, Uramoto H, et al. Advanced cadaver-based educational seminar for trauma surgery using saturated salt solution-embalmed cadavers. *Acute Med Surg*. 2019;6(2):123–30.
31. Laird K, Phillips C. Vapour phase: a potential future use for essential oils as antimicrobials? *Lett Appl Microbiol*. 2012;54(3):169–74.
32. Hayashi S, Homma H, Naito M, Oda J, Nishiyama T, Kawamoto A. Saturated salt solution method: A useful cadaver embalming for surgical skills training. *Medicine (Baltimore)*. 2014;93(27):e196.

Author biography

Bikash Chandra Satapathy, Assistant Professor https://orcid.org/0000-0002-9162-493X

Biswa Bandita Kar, Professor

Cite this article: Satapathy BC, Kar BB. Alternative natural and chemical substances to traditional formalin-based embalming fluid for cadaveric dissection: A review. *Indian J Clin Anat Physiol* 2023;10(2):66-73.