JOURNAL OF APPLIED ANIMAL BIOSCIENCES (JAAB)

Open Access • Peer Reviewed • Biannual
https://jaab.in

Volume 1 • Issue 1 • July 2026 pp 4-9 | DOI: https://doi.org/10.5281/zenodo.21584229 | eISSN: in process

A Practical Approach for Preservation of Animals through Fluid Injection Technique

Satish K. Pathak*[1] and Archana Mahapatra[1]

[11]Department of Veterinary Anatomy, Faculty of Veterinary & Animal Sciences, Institute of Agricultural Sciences, RGSC, Banaras Hindu University, Mirzapur, India, 231307

*Corresponding author: satishpathak@bhu.ac.in | ORCID: 0000-0003-2778-2885

Received	Revised	Accepted	Published
22/07/2026	24/07/2026	25/07/2026	25 /07/2026

Abstract

Preservation of animal specimens is an essential component of veterinary, zoological, and biological education, allowing repeated demonstration of anatomical structures while minimizing dependence on freshly euthanized or deceased animals. Conventional preservation techniques rely primarily on immersing specimens in formalin-based solutions, which require large volumes of preservative and expose students and laboratory personnel to prolonged formaldehyde vapors. The present study describes a practical fluid injection technique for preserving whole animal specimens through localized infusion of a preservative solution rather than complete immersion. The preservative consisted of 10% formalin, glycerine, and thymol crystals. The solution was injected systematically into different anatomical regions, including the head, neck, thoracic cavity, abdominal cavity, pelvic cavity, vertebral region, and limbs, after proper cleaning and positioning of the specimen. Glycerine maintained tissue pliability and reduced dehydration, whereas thymol prevented fungal growth during storage. Preserved specimens retained their external morphology, flexibility, and anatomical details for extended periods while exhibiting a marked reduction in formalin odor after several days. The technique minimizes chemical consumption, reduces occupational exposure to formaldehyde, simplifies storage and transportation, and provides durable teaching specimens suitable for museums and anatomy laboratories. This preservation approach represents an economical and practical alternative to conventional immersion methods, particularly for educational institutions.

Keywords

Animal preservation, Anatomy Museum, Fluid injection, Formalin, Veterinary education

Introduction

Preservation of biological specimens is a fundamental component of veterinary anatomy, zoology, wildlife biology, comparative anatomy, and medical education. Well-preserved specimens provide indispensable teaching resources for demonstrating anatomical structures, facilitating repeated practical instruction, supporting museum collections, and serving as valuable reference materials for research and biodiversity conservation. The long-term preservation of animal specimens is therefore essential for maintaining high-quality educational and scientific collections while minimizing the need for repeated specimen acquisition (Alvites et al., 2026; Nachman et al., 2023).

Conventionally, whole animal specimens are preserved by complete immersion in aqueous formaldehyde (formalin) solutions. Immersion fixation effectively stabilizes tissue architecture by cross-linking proteins, thereby preventing autolysis and microbial decomposition. Despite its widespread use and proven effectiveness, this method requires large volumes of preservative solution, substantial storage space, and continuous maintenance of fluid levels. Moreover, specimens stored in open or partially sealed containers continuously emit formaldehyde vapors, creating an unpleasant laboratory environment and increasing occupational exposure among students, instructors, museum personnel, and technical staff (Azari et al., 2012; Bhat et al., 2019).

Formaldehyde remains the most extensively used biological tissue preservative because of its excellent tissue penetration, antimicrobial activity, affordability, and ability to preserve gross anatomical relationships with minimal distortion. However, prolonged or repeated exposure to formaldehyde has well-documented adverse health effects, including irritation of the eyes, skin, and respiratory tract, allergic sensitization, and adverse effects on pulmonary function (Khoshakhlagh et al., 2023; Mathur & Rastogi, 2007). More importantly, formaldehyde has been classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC), with sufficient evidence linking occupational exposure to nasopharyngeal cancer and leukemia (Kang et al., 2021; Protano et al., 2021). Consequently, reducing formaldehyde exposure has become a major concern in anatomy laboratories, museums, and veterinary teaching institutions worldwide.

To overcome these limitations, numerous alternative preservation techniques have been developed, including ethanol-based preservation, glycerination, plastination, Thiel embalming, and modified embalming solutions (Hammer et al., 2015; Toy & Secgin, 2022). Although these methods can reduce formaldehyde exposure or improve specimen handling, many require specialized equipment, expensive chemicals, prolonged processing times, or sophisticated technical expertise. Furthermore, several techniques are better suited for surgical training or soft-tissue flexibility rather than the routine preservation of museum specimens intended for repeated educational use (Rakuša & Kocbek Šaherl, 2023; Srivastava & Nagwani, 2019) .

Injection preservation represents a practical and economical alternative in which preservative fluid is delivered directly into the vascular system or body cavities, allowing the fixative to diffuse throughout the tissues without the need for complete immersion (Brenner, 2014). Because only a relatively small volume of preservative is required, this approach substantially reduces chemical consumption, storage requirements, and environmental contamination. Following adequate fixation, the residual odor of formaldehyde gradually diminishes, making preserved specimens safer and more comfortable to handle during practical demonstrations. In addition, injection preservation better maintains the natural external appearance of specimens and facilitates long-term storage with minimal maintenance, making it particularly suitable for educational museums and anatomy teaching laboratories.

The present study describes a simple, economical, and effective fluid injection technique for preserving whole animal specimens using a preservative formulation consisting of formalin, glycerine, and thymol. Formalin serves as the primary fixative, glycerine acts as a humectant to maintain tissue flexibility and reduce desiccation, and thymol functions as an antifungal preservative to inhibit microbial growth during long-term storage. The proposed method aims to produce durable, odor-reduced museum specimens while substantially decreasing the quantity of formaldehyde required for preservation. This technique offers a practical solution for veterinary anatomy departments, zoological museums, and educational institutions seeking an affordable, safe, and effective alternative to conventional immersion preservation.

Materials and Methods

Study Design

The study was conducted to standardize a practical fluid injection technique for preserving whole animal specimens intended for anatomical teaching and museum display. Freshly 6 dead animal specimens (4 squirrels, 1 monitor lizard and 1 mongoose) free from advanced decomposition were selected for preservation.

Materials Required

- 10% Formalin
- Glycerine
- Thymol crystals
- Disposable syringes (20–50 mL)
- Needles (18–20 gauge)
- Disinfectant solution
- Gloves and protective equipment

Pathak S.K. and Mahapatra A., 2026, Volume 1 • Issue 1 • July 2026 pp 4-9. Journal of Applied Animal Biosciences | DOI: 10.5281/zenodo.21584229

- Glass jars or preservation platform

Preparation of Preservative Solution

A preservative mixture was prepared consisting of:

- **10% Formalin** – primary fixative
- **Glycerine** – to maintain tissue softness and prevent excessive hardening
- **Thymol crystals** – antifungal agent to inhibit fungal contamination during storage

The ingredients were mixed thoroughly until a homogeneous preservative solution was obtained.

Preservation Procedure

The preservation process consisted of four sequential steps.

Step 1. Cleaning of the Animal

Freshly dead animals were washed thoroughly using disinfectant water to remove dirt, blood, mucus, and other contaminants from the body surface. Cleaning minimized microbial contamination and improved preservation efficiency.

Step 2. Preparation of the Preservative

The preservative solution containing formalin, glycerine, and thymol was freshly prepared before use.

Each component served a specific purpose:

- Formalin fixed tissues by cross-linking proteins.
- Glycerine prevented dehydration and maintained flexibility.
- Thymol inhibited fungal growth during long-term storage.

Step 3. Orientation of the Animal

The animal was positioned carefully in the desired anatomical posture before injection.

Proper orientation ensured:

- Natural appearance
- Correct limb positioning
- Easy demonstration of anatomical structures
- Attractive museum display

The specimen was placed either on a flat preservation platform or inside a glass display jar.

Fig.1 The squirrel was positioned carefully in the desired anatomical posture before injection

Step 4. Infusion of the Preservative

The preservative solution was injected systematically into different regions of the body.

Injection sites included:

- Head
- Around the eyes
- Base of the ears
- Neck muscles
- Forelimbs
- Hind limbs
- Thoracic cavity
- Abdominal cavity
- Vertebral region
- Pelvic cavity

Special attention was given to the abdominal and pelvic cavities because these regions undergo rapid postmortem decomposition. A relatively larger quantity of preservative was infused into these cavities to prevent tissue maceration.

The preservative was injected slowly to allow uniform distribution throughout the tissues while avoiding excessive leakage.

Pathak S.K. and Mahapatra A., 2026, Volume 1 • Issue 1 • July 2026 pp 4-9. Journal of Applied Animal Biosciences | DOI: 10.5281/zenodo.21584229

Fig.2 The preservative solution was injected systematically into different regions of the body of squirrel

Results

The fluid injection technique successfully preserved the external morphology of whole animal specimens without requiring complete immersion in preservative solution.

Major observations included:

- Excellent preservation of body shape.
- Retention of natural posture.
- Soft and pliable tissues due to glycerine.
- Absence of fungal growth during storage.
- Significant reduction in formalin odor after several days.
- Reduced quantity of preservative required compared with immersion methods.
- Easy handling during practical anatomy classes.
- Suitable specimens for museum display.

The specimens remained suitable for repeated educational use while requiring minimal maintenance

Fig.3 The final preserved specimen.

Discussion

The present preservation technique demonstrates that whole animal specimens can be effectively preserved through localized injection of a formalin-based preservative rather than conventional immersion. The approach substantially reduces the volume of preservative required while maintaining excellent anatomical integrity.

Formalin remains one of the most effective tissue fixatives because it rapidly penetrates tissues and stabilizes cellular proteins. However, continuous exposure to formaldehyde vapors is associated with irritation of the eyes and respiratory tract and has long-term occupational health implications (Tesfaye et al., 2021). By confining most of the preservative within body tissues rather than storing specimens in open formalin containers, the present method markedly reduces environmental formaldehyde exposure.

The incorporation of glycerine improves tissue quality by preventing excessive dehydration and hardening, problems frequently encountered with prolonged formalin fixation (Harris et al., 2025; Setayesh et al., 2013). The resulting specimens remain flexible and more closely resemble fresh anatomical material, thereby enhancing teaching effectiveness. Thymol serves as an effective antifungal agent and prevents fungal colonization during prolonged storage, especially under humid environmental conditions (Awlqadr et al., 2026; Escobar et al., 2020).

Injection into multiple anatomical regions ensures uniform diffusion of the preservative throughout the body. Greater volumes administered into the abdominal and pelvic cavities are particularly important because these regions contain digestive organs that undergo rapid autolysis and microbial decomposition following death.

Compared with immersion preservation, the injection technique offers several practical advantages. It requires considerably less formalin, reduces storage costs,

Pathak S.K. and Mahapatra A., 2026, Volume 1 • Issue 1 • July 2026 pp 4-9. Journal of Applied Animal Biosciences | DOI: 10.5281/zenodo.21584229

eliminates the need for large preservation tanks, facilitates specimen transportation, and minimizes occupational exposure to harmful vapors. Furthermore, preserved specimens can be displayed openly in anatomy museums without being submerged continuously in preservative solution, thereby improving their educational value.

Although the present method is highly suitable for gross anatomical teaching and museum display, periodic inspection of specimens is recommended to detect any localized drying or fungal contamination. Supplemental injection may be required after prolonged storage in exceptionally dry environments.

Conclusion

This technique represents a simple, economical, and effective preservation method that can be readily adopted in veterinary colleges, medical institutions, zoological museums, and biological teaching.

Future Scope

Future studies should compare this fluid injection technique with conventional immersion preservation and alternative fixatives by evaluating tissue integrity, color retention, flexibility, microbial resistance, and long-term durability. Quantitative assessment of airborne formaldehyde concentrations before and after preservation would further validate the occupational safety benefits of this method. Optimization of preservative composition using lower formaldehyde concentrations or less toxic alternatives may further enhance the technique for widespread use in veterinary, medical, and zoological institutions.

Funding Statement

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Approval

Formal ethical approval was not required for this study because only dead animals were used.

Data Availability Statement

No new data generated in this study.

References

Alvites, R., Sá, S., Lei, M. C., Quinteira, S., & Miranda, C. (2026). Biosafety implications of cadaver preservation methods in veterinary anatomy education. *Veterinary Research Communications*, *50*(5), 422. https://doi.org/10.1007/s11259-026-11378-y

Awlqadr, F. H., Saeed, M. N., Altemimi, A. B., Qadir, S. A., Faraj, A. M., Mohammed, O. A., Salih, T. H., Tlay, R. H., & Abedelmaksoud, T. G. (2026). Sustainable Applications of Thymol: Advances in Formulation Technologies, Drug Delivery Systems, and Food Preservation Strategies. *Food Science & Nutrition*, *14*(5), e71779. https://doi.org/10.1002/fsn3.71779

Azari, M. R., Asadi, P., Jafari, M. J., Soori, H., & Hosseini, V. (2012). Occupational exposure of a medical school staff to formaldehyde in tehran. *Tanaffos*, *11*(3), 36–41.

Bhat, D., Chittoor, H., Murugesh, P., Basavanna, P. N., & Doddaiah, S. (2019). Estimation of occupational formaldehyde exposure in cadaver dissection laboratory and its implications. *Anatomy & Cell Biology*, *52*(4), 419. https://doi.org/10.5115/acb.19.105

Brenner, E. (2014). Human body preservation – old and new techniques. *Journal of Anatomy*, *224*(3), 316–344. https://doi.org/10.1111/joa.12160

Escobar, A., Pérez, M., Romanelli, G., & Blustein, G. (2020). Thymol bioactivity: A review focusing on practical applications. *Arabian Journal of Chemistry*, *13*(12), 9243–9269. https://doi.org/10.1016/j.arabjc.2020.11.009

Hammer, N., Löffler, S., Bechmann, I., Steinke, H., Hädrich, C., & Feja, C. (2015). Comparison of modified thiel embalming and ethanol-glycerin fixation in an anatomy environment: Potentials and limitations of two complementary techniques. *Anatomical Sciences Education*, *8*(1), 74–85. https://doi.org/10.1002/ase.1450

Harris, G. G., Jones, J. M., & McNary, T. G. (2025). Analysis of the Elnady Technique on Cadaveric Tissues: A Low Barrier-to-Entry to Increase Tissue Handling Comfort, Longevity, and Safety. *Cureus*. https://doi.org/10.7759/cureus.99483

Kang, D. S., Kim, H. S., Jung, J.-H., Lee, C. M., Ahn, Y.-S., & Seo, Y. R. (2021). Formaldehyde exposure and leukemia risk: A comprehensive review and network-based toxicogenomic approach. *Genes and Environment*, *43*(1), 13. https://doi.org/10.1186/s41021-021-00183-5

Khoshakhlagh, A. H., Mohammadzadeh, M., Manafi, S. S., Yousefian, F., & Gruszecka-Kosowska, A. (2023).

Inhalational exposure to formaldehyde, carcinogenic, and non-carcinogenic risk assessment: A systematic review. *Environmental Pollution*, *331*, 121854. https://doi.org/10.1016/j.envpol.2023.121854

Mathur, N., & Rastogi, S. (2007). Respiratory effects due to occupational exposure to formaldehyde: Systematic review with meta-analysis. *Indian Journal of Occupational and Environmental Medicine*, *11*(1), 26. https://doi.org/10.4103/0019-5278.32461

Nachman, M. W., Beckman, E. J., Bowie, R. C., Cicero, C., Conroy, C. J., Dudley, R., Hayes, T. B., Koo, M. S., Lacey, E. A., Martin, C. H., McGuire, J. A., Patton, J. L., Spencer, C. L., Tarvin, R. D., Wake, M. H., Wang, I. J., Achmadi, A., Álvarez-Castañeda, S. T., Andersen, M. J., … Zink, R. M. (2023). Specimen collection is essential for modern science. *PLOS Biology*, *21*(11), e3002318. https://doi.org/10.1371/journal.pbio.3002318

Protano, C., Buomprisco, G., Cammalleri, V., Pocino, R. N., Marotta, D., Simonazzi, S., Cardoni, F., Petyx, M., Iavicoli, S., & Vitali, M. (2021). The Carcinogenic Effects of Formaldehyde Occupational Exposure: A Systematic Review. *Cancers*, *14*(1), 165. https://doi.org/10.3390/cancers14010165

Rakuša, M., & Kocbek Šaherl, L. (2023). Thiel embalming method used for anatomy dissection as an educational tool in teaching human anatomy, in research, and in training in comparison of different methods for long term preservation. *Folia Morphologica*, *82*(3), 449–456. https://doi.org/10.5603/FM.a2022.0055

Setayesh, M., EbrahimEsfandiari, Rabiei, A., Hanaei, M., & Rashidi, B. (2013). Comparing two methods of plastination and glycerin preservation to study skeletal system after Alizarin red-Alcian blue double staining. *Advanced Biomedical Research*, *2*(1), 19. https://doi.org/10.4103/2277-9175.108003

Srivastava, G., & Nagwani, M. (2019). EMBALMING OF HUMAN CADAVERS FROM EGYPTIAN ERA TO THE MOST MODERN TECHNIQUES - A REVIEW ON PRESERVATION OF HUMAN CADAVERS. *Era's Journal of Medical Research*, *6*(2), 94–97. https://doi.org/10.24041/ejmr2019.137

Tesfaye, S., Hamba, N., Gerbi, A., & Negeri, Z. (2021). Occupational formaldehyde exposure linked to increased systemic health impairments and counteracting beneficial effects of selected antioxidants. *Alexandria Journal of Medicine*, *57*(1), 157–167. https://doi.org/10.1080/20905068.2021.1926172

Toy, S., & Secgin, Y. (2022). Cadaver embalming and fixing solutions from past to present. *Journal of Clinical Medicine of Kazakhstan*, *19*(5), 9–11. https://doi.org/10.23950/jcmk/12551