Journal of Chemical Health Risks

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JCHR (2025) 15(6), 211-224 | ISSN:2251-6727

Comparative Evaluation of Effectiveness of Glutaraldehyde and Partial Formaldehyde Replacement With 2% Phenoxyethanol, Vinegar as an Alternate Non-Formaldehyde Embalming Agents in Wistar Rats: A Morphological and Histological Assessment.

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Conflict of interest : The authors certify that there is no any conflict.

Author's contributions : Dr.Padmini & Dr Mini Mol both listed authors have participated equally in drafting the manuscript , both authors read and approved the final version of manuscrit.

(Received: 27 September 2025 Revised: 05 October 2025 Accepted: 01 November 2025)

KEYWORDS

Embalming, Formaldehyde, Glutaraldehyde, Phenoxyethanol, Vinegar.

ABSTRACT:

Background: Formaldehyde is the most commonly used preservative in embalming process of cadavers which are main tool for teaching & learning anatomy. With advantages of low cost & easy availability, Formaldehyde suffers from many disadvantages due to its known health and environmental hazardous effects. Need of hour is to explore the alternatives or partial replacement of Formaldehyde to create a balance between good tissue preservation and safe working environment.

Objectives: The study was designed to evaluate the effectiveness of embalming solutions by using partial formaldehyde replacement with 2% Phenoxyethanol, Vinegar & Glutaraldehyde on Wistar rats by analysing their odour characteristics, morphological and histological assessment.

Methods: The study was conducted on 72 Wistar rats. They were divided into 4 groups on basis of embalming fluids used. The embalming process started with injection of agents to the common carotid artery followed by their immersion in embalming fluids. At the end of 1st week, 8th & 16th odour characteristics, morphological and histological assessment were observed, evaluated & statically analysed.

Results: Embalmed rats with Glutaraldehyde group retained life like appearance of preserved organs, greatest degree of range of motion & close to normal appearance of histological features of superficial and deep organs as compared to rats embalmed with solution with partial replacement of formaldehyde vinegar & 2 % phenoxyethanol groups.

Conclusion: Further research works with larger sample sizes and different concentrations to investigate the effectiveness of non-formaldehyde-based embalming agents in tissue preservation.

INTRODUCTION

Embalming is a method that involves chemically treating a deceased body to slow down its decay, minimize the presence and growth of microorganisms, and hence assist in restoring an acceptable physical appearance. One of the primary applications of the embalming process is its role in medical education, in addition to restoring bodies for

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JCHR (2025) 15(6), 211-224 | ISSN:2251-6727

transport and preserving them for public viewing.[1] In medical schools, the teaching tools of human anatomy subject relies heavily on cadaver dissection, complemented by museum specimens, bones, and histological slides. Typically, the preservation of cadavers is achieved through the embalming process, which involves immersing them in embalming fluid. [2]

In today's context, Formaldehyde stands as the most prevalent chemical utilized as a preservative for embalming since it was discovered by the British chemist August Wilhelm von Hofmann in 1867. It is available commercially as formalin, which consists of 37% by weight or 40% by volume of formaldehyde gas dissolved in water. Despite its benefits, such as being entirely biodegradable, cost-effective, and possessing germicidal properties against a broad spectrum of microorganisms, formaldehyde continues to be the most widely used preservative in embalming fluids. However, formaldehyde has several drawbacks, including an unpleasant smell, the hardening and discoloration of preserved tissues, and its classification as a potent carcinogen. [1,3] Recent research has revealed negative implications of formaldehyde exposure on medical students. Studies conducted in recent years have shown that medical students experienced adverse effects from formaldehyde exposure. Common symptoms among study participants included odor, excessive lacrimation, eye and nose burning, dry mouth, throat irritation, hand itching, fatigue/dizziness, headache, sleep disturbance, and dermatological symptoms. [2,4–8] In addition to these immediate symptoms, prolonged exposure to formaldehyde can result in or worsen pre-existing malignancies, immunological hypersensitivity, skin conditions, and cardiopulmonary disorders.[9–12] Furthermore, Formaldehyde posed a risk to the environment because its disposal may pollute the air, soil, and water, which would hurt ecosystems. [13] Given the aforementioned acute and chronic health problems and environment risks associated with formaldehyde, the researchers are looking for an alternative for embalming cadavers that is not only an effective preservative but also has a low health and environmental risk. As a result, a thorough investigation into alternative preservatives in embalming agents in the domains of veterinary and human anatomy is required, with the goal of lowering potential harm to teaching staff, students, and other handlers. Despite occasional attempts in past studies on specific natural and synthetic substances, there is a lack of comprehensiveness in assessing the efficacy of many alternative embalming agents at both macroscopic and microscopic scales. [14-17]

To lessen the toxicity and irritation of formaldehyde, a small number of chemicals have been researched for years that can either fully or partially replace it. Glutaraldehyde and phenoxyethanol are two of these that are commonly used as preservatives in research projects. Glutaraldehyde, is considered to be better than formaldehyde at crosslinking proteins. [18] In comparison to formaldehyde, phenoxyethanol was claimed to a good performer in maintaining the histology of samples. [19] Due to its acidity, vinegar has been utilized as a preservative for antiquity. It has been demonstrated to be effective in preserving human tissues, although further research is necessary. [20]

The aim of this study was conducted to compare the effectiveness of three embalming solutions which included partially replacement of formaldehyde with 2% phenoxyethanol, white vinegar & Glutaraldehyde (completely replacing formaldehyde) with formaldehyde in the preservation of Wistar rats for 16 weeks. A comprehensive macroscopic observation (Odour, physical appearance and range of motion of joints) & histological analysis of various organs of rats carried out after 1^{st} , 8^{th} & 16^{th} week of embalming.

MATERIALS & METHODS:

The present study was conducted after the approval from Institutional Animal Ethical committee approval no (N-2023/10/02). A total number of 72 male and female Wistar rats, aged between 8 to 9 weeks were included in the study. These Wistar rats were randomly assigned into four groups: A (Formaldehyde group), B (Phenoxyethanol group), C (Glutaraldehyde group), and D (Vinegar group), with 18 rats in each group on the basis of type of embalming solution used. The composition of the embalming solutions is presented in Table1.

These rats were euthanised by an intraperitoneal injection of sodium phenobarbitone (150mg/kg) followed by injection of the designated embalming solution into the carcasses of common carotid artery until the fluid was observed exiting through natural orifices, ensuring complete perfusion.(21) Finally these carcasses were submerged into tanks containing 10 liters of

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respective embalming solutions. All carcasses were examined for morphological and histological assessment immediately after 1, 8 and 16 weeks of submerged periods by three blinded examiners (Comprising of Anatomist and Pathologists). Their characteristics were graded by using Likert scale as follows:

A. Morphological Assessment:

1. Assessment of Odor were scored I to III i.e. Grade I: strongly irritant, Grade II: less irritant than I, Grade III: Non- irritant.

2. Assessment of Physical appearance (Colour, texture and tonicity) of Skin, Fascia, Skeletal muscle and visceral organs were scored I to V i.e. Grade I : Very poor, totally distorted

,Grade II: Poor, moderately distorted, Grade III: Fair, mild distorted, Grade IV: Good not distorted, but less intact Grade V: Very good, totally intact. (Figure-1)

3. Range of motion of bilateral elbow & knee joint was recorded (0 to180^{o}) in flexion and extension position by stainless steel Goniometer.

B. Histological assessment:

Tissue sample of skeletal, cardiac muscle, tendon, liver, kidney and cerebrum from embalmed rats were taken after 1st, 8th & 16th weeks of embalming. Sample were sectioned to a thickness of 4 micron, immersed in 10% formaldehyde for 48 hours sample were processed by using paraffin embedding method, and stained with Haematoxylin and Eosin stain. To assess histology of Tendon, a special stain Masson's trichome was used and examined under light microscope and photomicrograph were taken by using Evos microscope under 10X and 40X magnification. The quality of stained sections was assessed on the basis of general microscopic architecture and cell morphology. HistopathologyGrading was performed on a scale I to III as Grade I: High degree of cell distortion, Grade II: Moderately good sections, Grade III: Sections are very close to normal.

Table1. Group wise composition of 3 litres of embalming fluid

Group A	**Group B**	**Group C**	**Group D**
Formaldehyde group	Phenoxyethanol group (Partially replacement of Formaldehyde with Phenoxyethanol)	Glutaraldehyde group	Vinegar group (Partially replacement of Formaldehyde with Vinegar)
Formalin (37%) 30ml Water 637ml Methanol 2000ml Normal saline 300ml Sodium citrate 15ml Glycerine15ml Phenol3ml Thymol 1gm	Formalin 10ml Methanol 1000ml 2% Phenoxyethanol 90ml Water 1570 ml Normal saline 300ml Sodium citrate 15ml Glycerine 15ml Phenol 3ml Thymol 1 gm	2%Glutaraldehyde 1300ml Methyl alcohol 200ml Water 1300ml Centrimide 165ml Glycerine 15ml Eosin 15ml Oil of eucalypts 5ml Thymol 1gm	Vinegar 1000ml Formalin 30ml Methanol 100ml Table Salt 100gms + 637ml water Normal saline 300ml Sodium citrate 15ml Glycerine 15ml Phenol 3 ml Thymol 1gm
6 liter Submerged solution			
37% formaldehyde	Thiel solution	2% glutaraldehyde	White vinegar

STATISTICS:

Statistical methods were employed to compare the effects of embalming agents in preserving macroscopic and microscopic details of the samples. Shapiro–Wilk test was used to test normality of the data set. Interrater agreement was carried out for each morphological and histological parameter by Fleiss' Kappa statistical method. According to the normality distribution data, one way-Anova and Kruskal Willis test was used for comparison among multiple variables of groups followed

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by the post hoc analysis or multiple comparison analysis by SPSS version 29.

RESULTS:

Interobserver agreement (Fleiss' Kappa) regarding the odour and physical appearance of organs indicated moderate consensus among the observers concerning the physical appearance of the pancreas, κ=.429(95% CI, .343 to .515), p = .000, while a strong agreement was noted for all other organs, with κ values between 0.633 and 0.808, p = .000. The level of agreement on Odour was classified as Very good, with κ=1 (95% CI, .897 to 1.013), p =.000. For the histological evaluation, the observers exhibited good agreement on the Liver, κ=.643(95% CI, .518 to .768), p = .000, while Very Good agreement was recorded for all other organs, with κ values ranging from 0.841 to 1, p = .000.

The observations (odour, physical appearance, range of motion of joints and histological assessment) of the present study are as follows at the end of 1st , 8th and 16th week of embalming period. After 8th week, the carcasses emersed in Phenoxyethanol was discarded as the severe degree of disintegration of tissue was observed making it difficult to handle.

a. **Odour:** At the end of 1st week, when the odour grading of carcases immersed in four groups of embalming solutions was compared, a Kruskal-Wallis test indicated that there was a significant difference among these groups, χ^2 ([3], N = [24]) = [23.000], p = [< .001]. With highest median rank (21.5), Phenoxyethanol was found to be non-irritant followed by Glutaraldehyde and Vinegar group. The Formaldehyde found to be the most irritant of all groups with median rank of 3.5. A similar result was observed at the end of 8th week of embalming. At end of 16th week, as Phenoxyethanol was removed as group, Glutaraldehyde and Vinegar group emerged as less irritant with mean rank of 12.5 as Formaldehyde was observed to be a highly irritant embalming solution with mean rank of 3.5.

b. **Physical Appearance of organs:** Upon evaluating the effectiveness of the four embalming agents in preserving the physical characteristics (color and texture) of organs, it was observed that formaldehyde and glutaraldehyde achieved higher scores in comparison to the other fluids (p < .001) across all three time points from the 1st to the 16th week. In Formaldehyde group, skin colour of rats turned yellowish while maintaining its tonicity till 16th week. Muscles & other visceral organs were brownish in colour & integrity was maintained up to 8 weeks and then turn into blacked. Mild distortion began in every organ after 8 weeks of embalming which was maintained till 16th week, except pancreas which was moderately distorted at 1 week. Cerebrum was well preserved up to 8 weeks of embalming then showed mildly distortion till 16 weeks of embalming. When it came to maintaining the physical attributes of practically every organ at 16th week, glutaraldehyde outperformed others. The skin colour was slight yellowish. Other organs maintained their texture till 16th week including organs like cerebrum, tendon, blood vessels and nerves except pancreas which showed moderate distortion after 8th week. In case of Phenoxyethanol, the fluid converted into sticky fluid after 1 week of embalming which turned skin yellowish in colour, loss of tonicity in 1st week itself, which made the specimens very difficult to handle. Colour of muscles & other visceral organs turned brownish in colour and showed moderately distortion after 8 weeks. In this case, pancreas presented moderately distortion even at 1st week. Because of the difficult handling the attached organs which showed considerable tissue degeneration, phenoxyethanol was removed from the study after 8th week. In Vinegar, Skin appeared bleached but maintained its tonicity up to 8 weeks of embalming. At the end of 1st week, muscles & other visceral organs are brownish in colour & intact then colour turned whitish with loss of tonicity. Pancreas showed early and moderate distortion at 1st week. Surprisingly, cerebrum was well preserved up to 8 weeks, showed mild to moderate distortion after 16 weeks.

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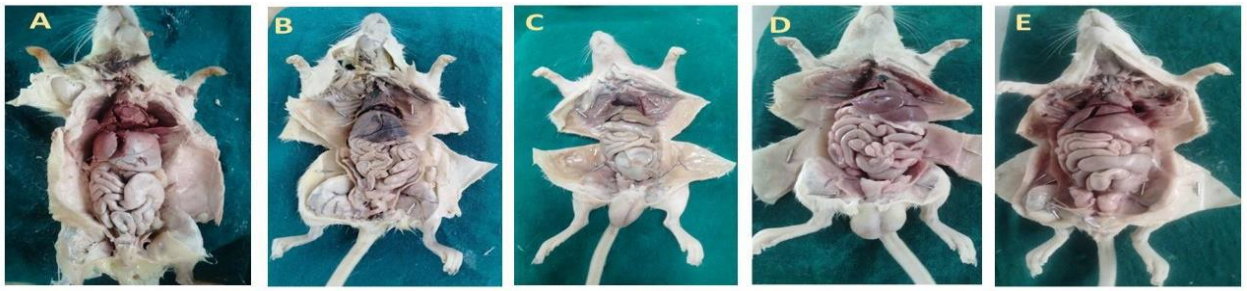

Figure-1 shows the display of visceral organs of embalmed Wistar Rats with gradings. Grade I : Very poor totally distorted (A) ,Grade II: Poor, moderately distorted (B), Grade III: Fair, mild distorted (C), Grade IV: Good not distorted, but less intact (D), Grade V: Very good, totally intact (E)

Range of motion:

Table 4 displays the findings of the 1-way ANOVA comparing the bilateral elbow and knee joint ranges of motion among the four treatment groups. Throughout the investigation, the glutaraldehyde group showed the greatest range of motion in their elbows and knees, followed by the formaldehyde group and the vinegar group (in both groups, decreased mobility observed after 8th week) and the phenoxyethanol group (the least as loss of mobility observed in 1st week).

d. Histological analysis:

Histological microscopic images of slides stained with Hematoxylin–Eosin for skeletal muscle,cardiac muscle, liver, kidney, and cerebrum tissues & Masson's Trichome for tendon are used which are shown in Figure-2 to Figure -7. The histological assessment of these tissues is based on their architectural structure and cellular characteristics such as structural integrity, necrosis, cellular/nuclear degeneration, vacuolization of cells, deposition of extracellular matrix, inflammation, fibrosis, changes in vascular structure, and calcification. In all examined tissues, the groups treated with formaldehyde and Glutaraldehyde exhibited a greater degree of restoration of tissue and cellular integrity up to the 16th week of embalming, although an increase in microscopic distortion was noted in both groups at the 16th week, with the most significant distortion occurring in the cerebrum. The Phenoxyethanol group exhibited the greatest degree of tissue and cellular degeneration across all tissues during both the 1st and 8th weeks of embalming. This group was subsequently eliminated due to the tissue distortion that complicated the handling of specimens. Regarding the cerebrum, vinegar demonstrated a positive effect on the preservation of microscopic details of the tissue until the 8th week, after which moderate distortion appeared, comparable to that observed with formaldehyde and Glutaraldehyde at the 16th week.

Figure-2 Histology images of Skeletal muscle

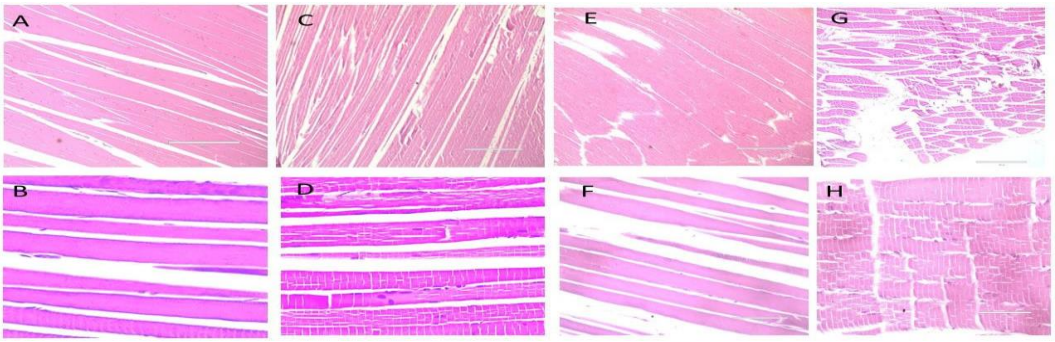

Figure 2 shows histological assessment of skeletal muscle stained with H&E displaying the tissue architecture and cellular details (10x and 40x view: upper row and lower row respectively) of normal sections (A,B), Sections are very close to normal (C,D), Moderately good sections (E, F), section with high degree of cell distortion (G,H

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Figure-3 Histology images of Tendon

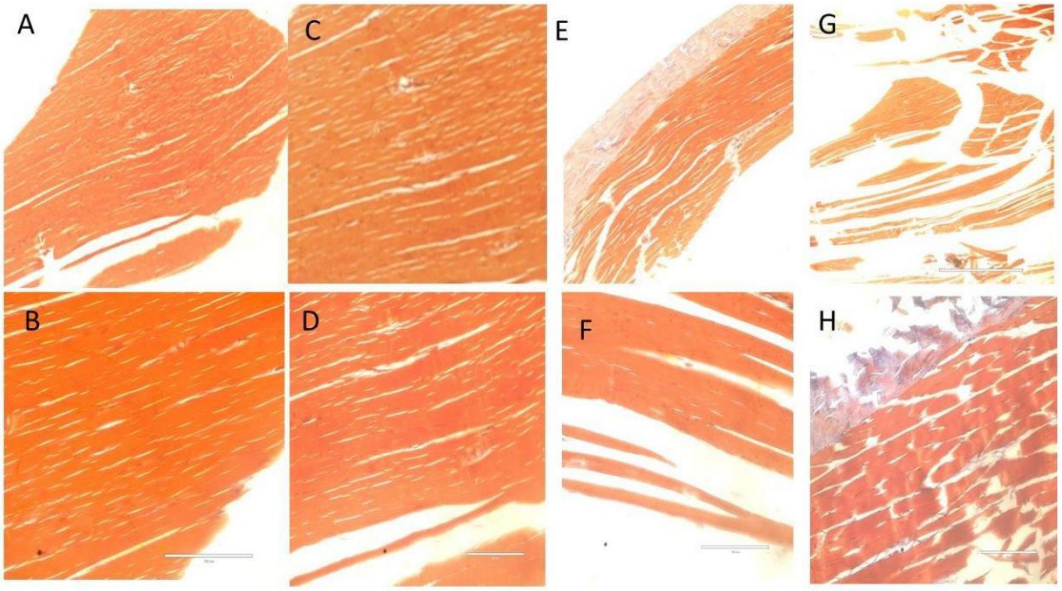

Figure 3 shows histological assessment of Tendon stained with Masson's Trichrome stain displaying the tissue architecture and cellular details (10x and 40x view: upper row and lower row respectively) of normal sections (A,B), Sections are very close to normal (C,D), Moderately good sections (E, F), section with high degree of cell distortion (G,H).

Figure-4 Histology images of Cardiac muscle

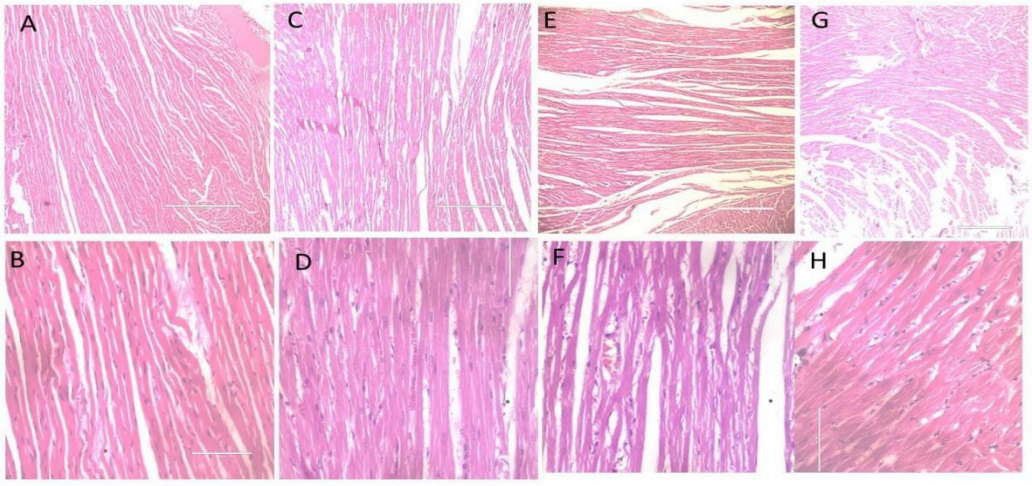

Figure-4 shows histological assessment of cardiac muscle stained with H&E displaying the tissue architecture and cellular details (10x and 40x view: upper row and lower row respectively) of normal sections (A,B), Sections are very close to normal (C,D), Moderately good sections (E, F), section with high degree of cell distortion (G,H).

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Figure-5 Histology images of Liver

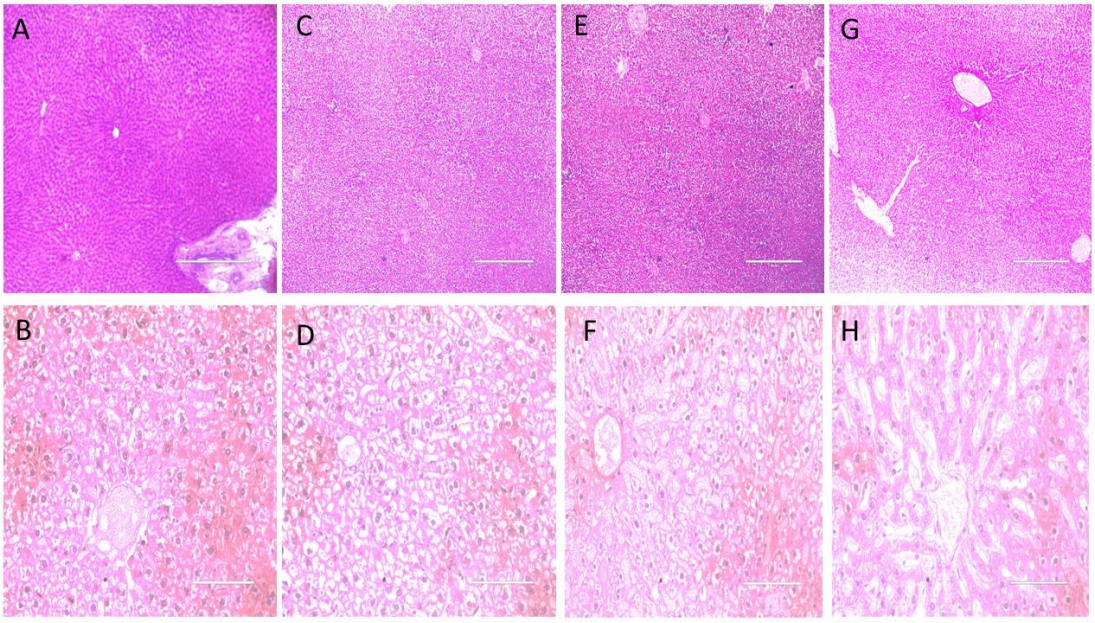

Figure-6 shows histological assessment of Liver stained with H&E displaying the tissue architecture and cellular details (10x and 40x view: upper row and lower row respectively) of normal sections (A,B), Sections are very close to normal (C,D), Moderately good sections (E, F), section with high degree of cell distortion (G,H)

Fig 6 -Histology images of Kidney

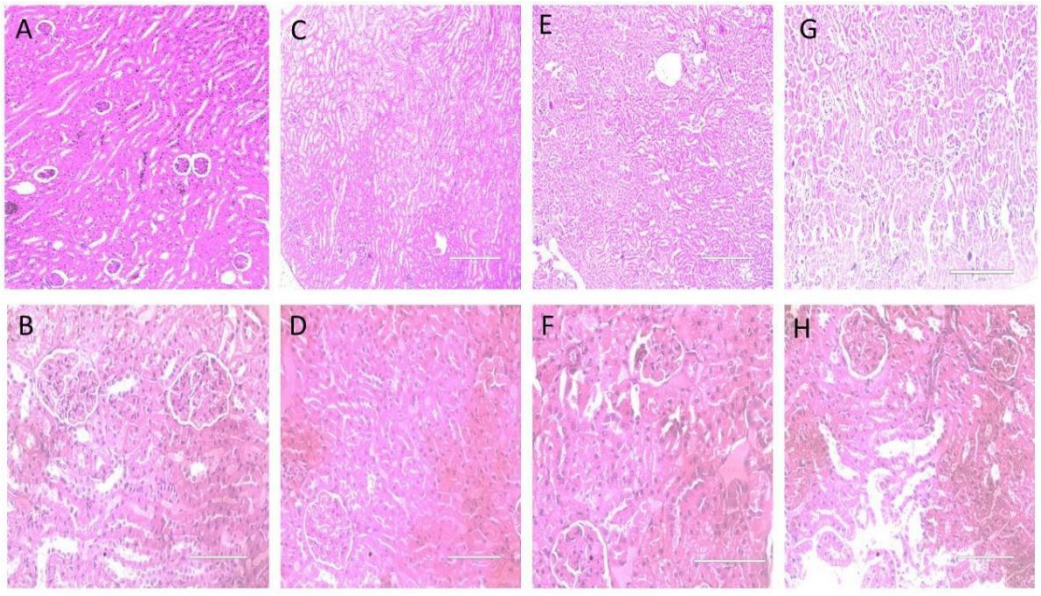

Figure-6 shows histological assessment of Kidney stained with H&E displaying the tissue architecture and cellular details (10x and 40x view: upper row and lower row respectively) of normal sections (A,B), Sections are very close to normal (C,D), Moderately good sections (E, F), section with high degree of cell distortion (G,H)

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Figure-7 Histology images of cerebrum

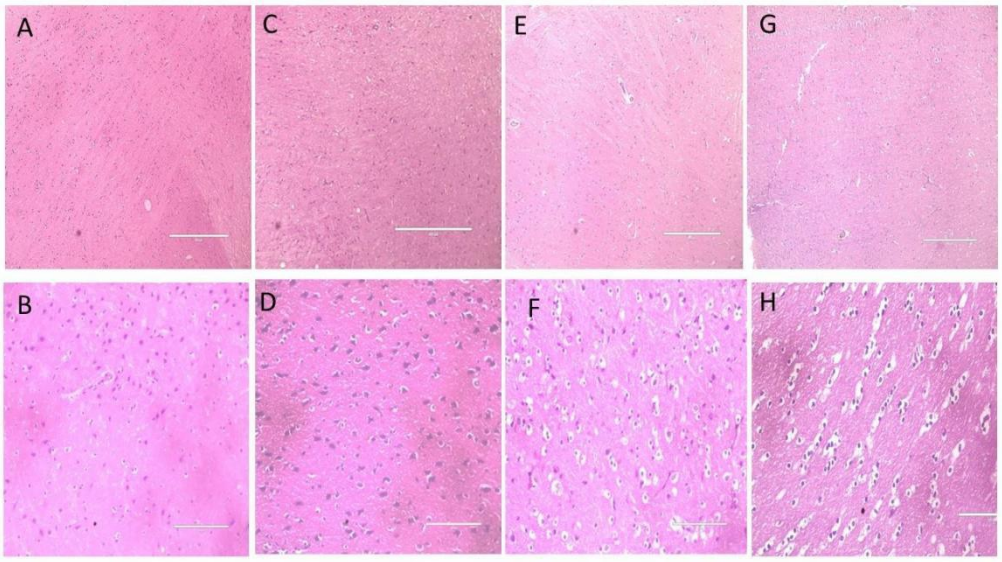

Figure-7 shows histological assessment of Cerebrum stained with H&E displaying the tissue architecture and cellular details (10x and 40x view: upper row and lower row respectively) of normal sections (A,B), Sections are very close to normal (C,D), Moderately good sections (E, F), section with high degree of cell distortion (G,H).

DISCUSSION:

The current study focused on both morphological (odour, gross anatomical) and microscopic/histological assessments of rats embalmed using different groups of embalming agents. The aim was to assess the efficacy of these agents in preserving external anatomy and internal tissue structure, as well as to compare their effectiveness, toxicity thus to explore potential applications in education or research

Odour Characteristics:

Odour is a significant yet frequently neglected element of embalming process especially in academic settings where extended exposure may affect the health and comfort of its handlers. The current study investigated the odour properties of rats embalmed using various preservation agents, emphasizing both the strength and acceptability of the resulting smells. In this study, formalin was associated with a strong and highly irritating odour throughout the study observation period. Though there is no evidence of previous animal studies on odour characteristics, still clinical studies consistently reported the unpleasant odour is the most common complaint in handlers of formaldehyde embedded cadavers. [2, 22] Phenoxyethanol was emerged as a non-irritant chemical among all embalming agents, which is in accordance to observations of research study conducted by Chhabra et al who compared the effectiveness of Phenoxyethanol with formaldehyde. [23] Natekar et al conducted a study on Glutaraldehyde as embalming chemical agent which advocated its use as they found it less irritant and environmentally safe for users. [24] In case of vinegar, rarity of published work noticed except the research work by Said et al. According to them, Vinegar is a traditional safe substance to use as embalming agent as compared to formaldehyde when the authors verified the suitability of vinegar to be used as preservative in gelatin-based material for the development of realistic human head phantom. [20]

Morphological assessment:

Assessing the morphology of organs after embalming is crucial for understanding the structural integrity of specimens, aiding in pathological diagnosis, teaching anatomy, and conducting forensic investigations. Color and texture are two of the most visibly affected features, and evaluating them offers insights into how effective the embalming process was, how well the tissues are preserved, and the potential for further histological

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studies. In the present study, Glutaraldehyde embedded rats exhibited maximum pliability and life like elasticity without any noticeable hardness and stiffness in organs throughout the embalming period of 16 weeks, even higher than formaldehyde though the difference was not significant. This observation was in accordance to results Natekar et al who reported a satisfying result on flexibility and cosmetic effect on skin of cadaver embedded in glutaraldehyde. [24] In contrast to formaldehyde, glutaraldehyde produces a fast and irreversible cross-linkage with proteins with slow perfusion process. Hence, it provides more permanent end point fixation, whereas in case of formaldehyde, quick tissue perfusion produces irreversible fixation. [25] In case of phenoxyethanol, few studies reported a better outcome in preservation tissue integrity as compared to formaldehyde. In this context research work by Crosado et al also indicated the slight difference between scores were not statistically significant. In contrast, the tissue integrity was severely affected in phenoxyethanol group in the present study. The contrasting observation could be explained by the large variation of the concentration of phenoxyethanol used in previous studies as it ranges from 1% to 7% volume. [19, 26] In present study, a combination of 2% of phenoxyethanol and formaldehyde used for 8 weeks. Vinegar was also not effective in maintaining an optimal physical appearance of tissue embedded in it. As there is no detailed comparable study on effect of vinegar on tissue preservation, there is a need of further research work to validate our observation.

Similarly, regarding the range of joint movements (bilateral elbows and knees) in rats, the Glutaraldehyde group showed the greatest mobility compared to the other groups, which aligns with the findings reported in human cadavers by Natekar et al. [24] The lack of comparative studies on these embalming agents highlights the need for further research to confirm the results of this study.

Histological assessment:

In our research, we analyzed various tissues from both superficial and deep organs, including skin, tendons, cardiac muscle, kidneys, liver, skeletal muscle, and the cerebrum. All samples demonstrated similar levels of preservation, as evidenced by the quality of the sections, which suggests good perfusion, especially in the formaldehyde and glutardehyde groups. These aldehyde-based embalming agents are well-known fixatives. Glutaraldehyde has an advantage over formaldehyde in better preserving tissue ultrastructure, likely due to its more effective protein cross-linking and enzyme activity inhibition. [27] However, its slower penetration rate compared to formaldehyde might pose a challenge for larger specimens. Studies by Frolich et al. and Nicholson et al. reported good histological section quality with phenoxyethanol, which contrasts with our findings. [26,28] Frolich et al. used a different embalming method for longer preservation, initially fixing cadavers in formaldehyde before transferring them to 1% phenoxyethanol. Nicholson et al. conducted a comparative study using an alcohol-based phenoxyethanol mixture containing 7% phenoxyethanol for cadaver preservation, though the duration of embalming was not specified. Frolich et al. noted that phenoxyethanol cannot prevent autolysis, leading to rapid tissue decomposition, making it unsuitable for initial fixation. In our study, we observed similar results with the phenoxyethanol group, where tissue structure and cellular details deteriorated progressively from the first week to the eighth week. Although the effectiveness of vinegar has not been extensively studied, we observed that it could preserve cerebrum histology, aligning with findings by Said et al., who recommended vinegar as a good preservative for developing human head phantoms due to its acidity, which inhibits microbial growth. [20] Likewise, Gotur et al. reported good preservation efficacy using Shirka (sugarcane vinegar) in maintaining cellular details when compared with formaldehyde. [29] Keeping these results in mind, we would like suggest that there is a need for more research work on non-formaldehyde based embalming agents like Vinegar to explore the effectiveness in tissue preservation.

CONCLUSION:

The current study attempted to observe the effectiveness of embalming chemicals in tissue preservation in wistar rats by analysing morphological and microscopic criteria with the goal of finding an alternative to formaldehyde. Despite its unpleasant odor, glutaraldehyde emerged as an excellent embalming agent with promising outcomes when compared to others in terms of color, texture, and histopathologic features. Further structured research on the comparative evaluation of embalming agents with larger sample sizes and different concentrations is

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required to validate our findings and investigate the effectiveness of non-formaldehyde-based embalming agents in balancing tissue preservation and creating a safe environment for their handlers.

Table-2: Comparison of physical appearance (mean rank) of various organs treated with embalming agents at the end of 1st ,8th and 16th week

Organs	Embalming agents	N	1st week				8th week				16th week *			
			Mean Rank	Kruskal-Wallis H	df	Asymp. Sig.	Mean Rank	Kruskal-Wallis H	df	Asymp. Sig.	Mean Rank	Kruskal-Wallis H	df	Asymp. Sig.
Skin and Fascia	Formalin	6	17.42	18.462	3	**.000**	17.00	19.951	3	**.000**	9.50	12.621	2	**.002**
	Phenoxyethanol	6	7.33				5.50				-			
	Glutaraldehyde	6	19.00				20.00				14.75			
	Vinegar	6	6.25				7.50				4.25			
Lungs and Heart	Formalin	6	17.42	18.462	3	**.000**	17.50	20.024	3	**.000**	11.75	7.650	2	**.022**
	Phenoxyethanol	6	7.33				5.50				-			
	Glutaraldehyde	6	19.00				19.50				11.75			
	Vinegar	6	6.25				7.50				5.00			
Liver, stomach, Intestines and spleen	Formalin	6	17.42	18.462	3	**.000**	17.50	20.024	3	**.000**	11.75	7.650	2	**.022**
	Phenoxyethanol	6	7.33				5.50				-			
	Glutaraldehyde	6	19.00				19.50				11.75			
	Vinegar	6	6.25				7.50				5.00			
Pancreas	Formalin	6	15.50	21.930	3	**.000**	14.75	16.921	3	**.000**	10.50	15.057	2	**.001**
	Phenoxyethanol	6	7.50				5.75				-			
	Glutaraldehyde	6	21.50				20.00				14.50			
	Vinegar	6	5.50				9.50				3.50			
Kidney	Formalin	6	17.17	19.111	3	**.000**	17.75	20.399	3	**.000**	12.25	10.788	2	**.005**
	Phenoxyethanol	6	8.00				5.00				-			
	Glutaraldehyde	6	19.00				19.00				12.25			
	Vinegar	6	5.83				8.25				4.00			
	Formalin	6	18.50	21.686	3	**.000**	17.75	20.399	3	**.000**	10.50	12.194	2	**.002**
	Phenoxyethanol	6	8.00				5.00				-			

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Skeletal Muscle	Glutaraldehyde	6	18.50				19.00				13.75			
	Vinegar	6	5.00				8.25				4.25			
Blood vessels	Formalin	6	18.50	23.000	3	**.000**	15.25	16.204	3	**.000**	12.75	10.767	2	**.005**
	Phenoxyethanol	6	9.50				8.00				-			
	Glutaraldehyde	6	18.50				20.00				11.67			
	Vinegar	6	3.50				6.75				4.08			
Cerebrum	Formalin	6	18.50	21.481	3	**.000**	17.00	20.579	3	**.000**	11.00	14.733	2	**.001**
	Phenoxyethanol	6	8.50				5.00				-			
	Glutaraldehyde	6	18.50				20.00				14.00			
	Vinegar	6	4.50				8.00				3.50			

Foot note : The significance level is at 0.05Table-3 : Range of motion (in degrees) of joints treated with various embalming agents at 1st , 8th and 16th week

			1st week							8th week							16th week						
						95% Confidence Interval for Mean							95% Confidence Interval for Mean							95% Confidence Interval for Mean			
		N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	F	Sig.	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	F	Sig.	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	F	Sig.
Right Elbow Joint	Formaldehyde	6	113.33	10.328	4.216	102.49	124.17	51.865	**.000**	100.00	10.954	4.472	88.50	111.50	96.786	**.000**	100.00	10.954	4.472	88.50	111.50	73.098	**.000**
	Phenoxyethanol	6	86.67	8.165	3.333	78.10	95.24			51.67	9.832	4.014	41.35	61.98			-	-	-	-	-		
	Glutaraldehyde	6	136.67	5.164	2.108	131.25	142.09			135.00	5.477	2.236	129.25	140.75			135.00	5.477	2.236	129.25	140.75		
	Vinegar	6	95.83	4.916	2.007	90.67	100.99			90.83	6.646	2.713	83.86	97.81			70.83	10.206	4.167	60.12	81.54		
Left Elbow	Formaldehyde	6	115.00	11.832	4.830	102.58	127.42	28.183	**.000**	104.17	8.010	3.270	95.76	112.57	102.52	**.000**	104.17	8.010	3.270	95.76	112.57	94.332	**.000**
	Phenoxyethanol	6	85.83	9.174	3.745	76.21	95.46			47.50	12.550	5.123	34.33	60.67			-	-	-	-	-		

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Joint	Glutaraldehyde	6	129.17	4.916	2.007	124.01	134.33			129.17	4.916	2.007	124.01	134.33			129.17	4.916	2.007	124.01	134.33		
	Vinegar	6	96.67	8.165	3.333	88.10	105.24			92.50	5.244	2.141	87.00	98.00			70.00	8.944	3.651	60.61	79.39		
Right Knee Joint	Formaldehyde	6	121.67	7.528	3.073	113.77	129.57	32.394	**.000**	108.33	9.832	4.014	98.02	118.65	71.944	**.000**	108.33	9.832	4.014	98.02	118.65	81.774	**.000**
	Phenoxyethanol	6	84.17	13.571	5.540	69.92	98.41			52.50	11.726	4.787	40.19	64.81			-	-	-	-	-		
	Glutaraldehyde	6	130.00	6.325	2.582	123.36	136.64			125.00	5.477	2.236	119.25	130.75			125.00	5.477	2.236	119.25	130.75		
	Vinegar	6	100.00	6.325	2.582	93.36	106.64			96.67	7.528	3.073	88.77	104.57			69.17	7.360	3.005	61.44	76.89		
Left Knee Joint	Formaldehyde	6	120.00	8.944	3.651	110.61	129.39	20.735	**.000**	113.33	8.165	3.333	104.76	121.90	54.912	**.000**	113.33	8.165	3.333	104.76	121.90	51.500	**.000**
	Phenoxyethanol	6	90.83	10.206	4.167	80.12	101.54			46.67	15.055	6.146	30.87	62.47			-	-	-	-	-		
	Glutaraldehyde	6	130.00	10.954	4.472	118.50	141.50			123.33	12.111	4.944	110.62	136.04			123.33	12.111	4.944	110.62	136.04		
	Vinegar	6	101.67	7.528	3.073	93.77	109.57			96.67	8.165	3.333	88.10	105.24			68.33	9.309	3.801	58.56	78.10		

Table-4: Comparison of histology (mean rank) of various organs treated with embalming agents at the end of 1st ,8th and 16th week

Organs	Embalming agents	N	1st week				8th week				16th week *			
			Mean Rank	Kruskal-Wallis H	df	Asymp. Sig.	Mean Rank	Kruskal-Wallis H	df	Asymp. Sig.	Mean Rank	Kruskal-Wallis H	df	Asymp. Sig.
Skeletal Muscle	Formalin	6	15.50	7.667	3	.053	17.00	18.239	3	**.000**	12.50	16.320	2	**.000**
	Phenoxyethanol	6	9.50				4.00				-			
	Glutaraldehyde	6	15.50				17.00				12.50			
	Vinegar	6	9.50				12.00				3.50			
Tendon	Formalin	6	15.50	7.667	3	.053	16.17	12.267	3	**.007**	11.67	11.472	2	**.003**
	Phenoxyethanol	6	9.50				5.17				-			
	Glutaraldehyde	6	15.50				16.17				12.83			
	Vinegar	6	9.50				12.50				4.00			

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Cardiac Muscle	Formalin	6	16.00	9.857	3	**.020**	15.00	10.615	3	**.014**	12.17	6.549	2	**.038**
	Phenoxyethanol	6	8.00				5.00				-			
	Glutaraldehyde	6	16.00				15.00				10.75			
	Vinegar	6	10.00				15.00				5.58			
Liver	Formalin	6	17.00	16.867	3	**.001**	14.50	7.299	3	.063	12.33	7.484	2	**.024**
	Phenoxyethanol	6	5.00				6.50				-			
	Glutaraldehyde	6	17.00				14.50				11.00			
	Vinegar	6	11.00				14.50				5.17			
Kidney	Formalin	6	17.00	16.867	3	**.001**	14.50	7.299	3	.063	9.50	2.833	2	.243
	Phenoxyethanol	6	5.00				6.50				-			
	Glutaraldehyde	6	17.00				14.50				11.83			
	Vinegar	6	11.00				14.50				7.17			
Cerebrum	Formalin	6	17.00	17.706	3	**.001**	14.25	7.820	3	**.050**	9.75	.097	2	.953
	Phenoxyethanol	6	4.25				6.00				-			
	Glutaraldehyde	6	17.00				14.25				9.75			
	Vinegar	6	11.75				15.50				9.00			

Foot note : The significance level is at 0.05

REFERENCE:

1. Batra APS., Embalming and Other Methods of dead body preservation. Int J Med Toxicol Leg Med [Internet], 2010.12(3),15–8. Available from: https://www.researchgate.net/publication/261438780
2. Saikia R., Baruah P., Biswas K.K., Sinha T., Saikia R., A study on the adverse effects of embalming fluid on medical students. Int J Heal Clin Res, 2022.5(3),482–4.
3. Pal A., Bhanarkar U., Ray B., Embalming with Formalin – Benefits and Pitfalls. Sch Int J Anat Physiol, 2022.5(3),70–7.
4. Tiruneh C., Acute adverse effects of formaldehyde treated cadaver on new innovative medical students and anatomy staff members in the dissection hall at Wollo University, Northeast Ethiopia. Adv Med Educ Pract, 2021.12,41–7.
5. Kumar Dhar D., Chaudhuri S., Kumar Singh S., Bhattacharya S., Effect of exposure to formaldehyde on the skin of first-year medical students during Anatomy dissection: A longitudinal study. J Community Heal Manag, 2018.5(4),202–4.
6. Chia S.E., Ong C.N., Foo S.C., Lee H.P.. Medical students' exposure to formaldehyde in a gross anatomy dissection laboratory. J Am Coll Health Assoc, 1992.41(3),115–9.
7. Fernandes R., Anturlikar V., Patil D., A Study of Toxic Effects of Formalin on Health of First Year Medical and Dental Students. Int J Anat Radiol Surg, 2022.11(1),48–50.
8. Yadav S., Chaudhary S., Examining the harmful effects of formaldehyde vapours on Indian medical students while they are in the dissection hall. J Surv Fish Sci , 2023.10(1),2023.
9. Xu J., Shi Y., He G., Guo Y., Ruan Y., Hu J., Effects of Long-Term Exposure to Ambient Formaldehyde on Hypertension and Angina Pectoris Symptoms: Evidence From the WHO SAGE Cohort Study. J Am Heart Assoc, 2024.13(19), e035341.
10. Sun X., Yang C., Zhang W., Zheng J., Ou J., Ou S., Toxicity of formaldehyde, and its role in the formation of harmful and aromatic compounds during food processing. Food Chem X [Internet], 2025.25(November 2024), 102225. Available from: https://doi.org/10.1016/j.fochx.2025.102225
11. Khoshakhlagh A., Mohammadzadeh M., Manafi S.S., Yousefian F., Gruszecka-Kosowska A., Inhalational exposure to formaldehyde, carcinogenic and non-carcinogenic risk assessment: A systematic review.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 211-224 | ISSN:2251-6727

Environ Pollut, 2023.331(1),121854.

12. Mazurkiewicz W., Sak J., Nowiński M., Fus-Mazurkiewicz L.A., Environmental exposure to formaldehyde and effects on human health. Environ Med, 2024.27(2),41–5.
13. Bashir I., Lone L., Bhat R., Mir S., Dar Z., Dar S., Concerns and Threats of Xenobiotics on Aquatic Ecosystems. In: Bioremediation and Biotechnology, Vol 3: Persistent and Recalcitrant Toxic Substances. 2020. p. 15–23.
14. Chhabra N., Long Term Preservation of Cadavers: Substitution of Formalin With Phenoxyethanol: Need of the Hour. Int J Anat Res, 2020.8(2),7557–63.
15. Janczyk P., Weigner J., Luebke-Becker A., Kaessmeyer S., Plendl J., Nitrite pickling salt as an alternative to formaldehyde for embalming in veterinary anatomy-A study based on histo- and microbiological analyses. Ann Anat [Internet], 2011.193(1),71–5. Available from: http://dx.doi.org/10.1016/j.aanat.2010.08.003
16. Goodarzi N., Akbari G., Tehrani P.R., Zinc Chloride, A New Material for Embalming and Preservation of the Anatomical Specimens. Anat Sci J, 2018.15(1),25–30.
17. Jochebed S. R., S. T.M., Phenoxyethanol as a substitute for formaldehyde in the preservation of anatomical specimens. Int J Res Trends Innov, 2020.5(1),140–3.
18. Natekar P., DeSouza F., Glutaraldehyde:A New Embalming Chemical Composition For Preservation of Cadavers for Innovative Surgical Procedures. Indian J Anat, 2014.3(1),39–44.
19. 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.
20. Said M.S., Seman N., Investigation on Vinegar as presevative in materials for brain phantom. In: Proceeding - 2013 IEEE SInternational RF and Microwave Conference(RFM2013). 2013. p. 391–4.
21. Laferriere C.A., Pang D.S., Review of intraperitoneal injection of sodium pentobarbital as a method of euthanasia in laboratory rodents. J Am Assoc Lab Anim Sci, 2020.59(3),254–63.
22. Elshaer N.S.M., Mahmoud M.A.E., Toxic effects of formalin-treated cadaver on medical students, staff members, and workers in the Alexandria Faculty of Medicine. Alexandria J Med [Internet]. 2017.53(4),337–43. Available from: https://doi.org/10.1016/j.ajme.2016.11.006
23. Chhabra N., Long Term Preservation of Cadavers: Substitution of Formalin With Phenoxyethanol: Need of the Hour. Int J Anat Res, 2020.8(2),7557–63.
24. Natekar P., DeSouza F., Glutaraldehyde:A New Embalming Chemical Composition For Preservation of Cadavers for Innovative Surgical Procedures. Indian J Anat, 2014.3(1),39–44.
25. Kalliappan A., Motwani R., Chandrupatla M., Innovative Cadaver Preservation Techniques: a Systematic Review. Maedica - A J Clin Med, 2023.18(1),127–35.
26. Frølich K.W., Andersen L.M., Knutsen A., Flood P.R., Phenoxyethanol as a nontoxic substitute for formaldehyde in long-term preservation of human anatomical specimens for dissection and demonstration purposes. Anat Rec, 1984.208(2),271–8.
27. Bhat A.H., Hussein S., Fixation and different types of fixatives: Their role and functions: A review. Int J Clin Diagnostic Pathol, 2021.4(4),113–9.
28. Nicholson H.D., Samalia L., Gould M., Hurst P.R., Woodroffe M., A comparison of different embalming fluids on the quality of histological preservation in human cadavers. Eur J Morphol, 2005.42(4–5),178–84.
29. Gotur S.P., Kamarthi N., Wadhwan V., Sirka: An alternative to the formalin fixative. Indian J Pathol Microbiol, 2024.67(1),238–40.