International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 24s, 2025
https://theaspd.com/index.php

Comparative Evaluation Of Formaldehyde, Modified Thiel, And Saturated Salt Solution (SSS) For Embalming In Wistar Rats: A Morphological, Microbial And Histological Study

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Abstract

Background & objectives: *Embalming is the process of artificial preservation of dead body with the help of different chemicals. Formaldehyde is the traditional embalming chemical that works effectively, but it raises health and environmental concerns. Other alternatives, such as the Thiel and Saturated Salt Solution (SSS), show potential benefits with enhanced safety and preservation of tissue. Nonetheless, comparative research is scarce, especially involving animal studies. This study aims to evaluate and compare the embalming effectiveness of formaldehyde, Modified Thiel, and SSS in Wistar rats over a 16-week duration, concentrating on morphological preservation, resistance to microorganisms, and maintaining the histological integrity.*

Methods*: A total of 54 Wistar rats were divided randomly into three groups (n=18 each), with Group A treated with formaldehyde, Group B with Modified Thiel solution, and Group C treated with Saturated salt solution(SSS). The embalming process involved carotid perfusion followed by immersion in the designated embalming fluid. Evaluations of morphological features, microbial resistance, and histological analysis of superficial and internal organs were conducted at weeks 1, 8, and 16. Grading was conducted by six evaluators who were blinded to the groups, and statistical analysis was performed using the Kruskal-Wallis test and Fleiss' Kappa.*

Results*: In terms of morphology, the SSS group maintained a color and tonicity that was close to natural at all time points, followed by the Thiel method, while formaldehyde performed the least with tissue hardening and discoloration. The joint range of motion was highest in the SSS group and lowest in the group embalmed with formaldehyde. No microbial growth was detected in any of the embalming solutions. In the histological assessments, at the end of the 16 weeks, SSS showed superior preservation across the majority of organs, while formaldehyde exhibited the most degeneration.*

Conclusion*: SSS emerged as the most effective embalming method of the three, providing superior preservation of physical characteristics, joint mobility, and histological integrity over the 16 weeks. Modified Thiel exhibited moderate efficacy, while formaldehyde, despite its antimicrobial properties, proved to be the least effective over time. These results advocate for the use of safer and more functional embalming alternatives, such as SSS, in anatomical education and research.*

Keywords*: Embalming, cadaver, Formaldehyde, Thiel, saturated salt solution*

INTRODUCTION

Embalming is a vital process for preserving anatomical specimens, enabling their long-term storage for both educational and research purposes, as well as forensic uses. Especially in medical teaching, there is an increased demand for surgical workshops that include hands-on training over the embalmed cadavers to improve surgical and clinical skills, as well as for the practice procedures. Formaldehyde has been the most preferred embalming agent due to its advantages of being a strong fixative and preservative, as well as its low cost and consistent results. However, its common use has raised considerable health and environmental concerns, such as toxicity, respiratory issues, and possible carcinogenic effects. These drawbacks have led researchers to investigate alternative embalming techniques that can preserve tissue quality while minimizing health hazards.(1) In addition, formaldehyde-embalmed cadavers are less realistic in appearance, so there is a need for an effective soft embalming agent that can be used in a surgical workshop or training session. Among the alternatives to formaldehyde, the Thiel embalming solution and saturated salt solution (SSS) have emerged as promising options.(2) In 1922, the Thiel method, first developed by Walter Thiel, which was noted for its ability to maintain tissue color, texture, and flexibility, making it ideal for surgical education and realistic anatomical dissections.(3) Likewise, SSS , introduced by Coleman and Kogan, presents a straightforward, cost-effective, and less toxic method of preservation, especially advantageous in settings with limited resources.(4) Despite the increasing interest

in these alternatives, comparative data are scarce regarding their effectiveness in relation to formaldehyde, especially in small animal studies. Wistar rats are a suitable and consistent model for assessing the quality of preservation and the physiological effects of embalming agents due to their manageable size and well-defined anatomy.

This research aimed to evaluate the effectiveness of partial replacement of formaldehyde with Thiel and SSS as embalming methods compared to the traditional formaldehyde technique using Wistar rats. The evaluation concentrated on morphological, microbial, and histological variables, such as color retention, joint flexibility, physical appearance, microorganism growth, and histopathology of various organs at different time periods of assessment (1st, 8th & 16th week of embalming) to explore safer and more efficient alternatives for anatomical preservation and training.

MATERIALS & METHODS:

This animal study received approval from the Institutional Animal Ethical Committee under approval number (N-2023/10/02). The study involved a total of 54 male and female Wistar rats, aged between 8 and 9 weeks. These Wistar rats were randomly divided into three groups: A, B, and C, with each group consisting 18 rats. The allocation to groups was determined by the type of embalming solution administered (Group A: Formaldehyde, Group B: Theil embalming solution, and Group C: Saturated Salt Solution). The composition of these solutions is shown in Table 1. The rats were euthanized through an intraperitoneal injection of sodium phenobarbitone at a dosage of 150 mg/kg, followed by the injection of the specified embalming solution into the carcasses via the common carotid artery until the fluid was seen flowing from natural orifices, ensuring thorough perfusion. The carcasses (n=6) were then submerged in tanks containing 6 liters of fluid for different time periods (the composition of which is provided in Table 1). The carcasses from each tank were assessed for physical appearance, joint range of motion, microbial growth, and histological evaluation. After the 1st, 8th, and 16th weeks of embalming, the gross and histological changes were evaluated by six independent and blinded examiners (comprising three anatomists and three pathologists). Their findings were scored using a Likert scale, and the most frequently occurring score (mode) among the three assessors were utilized; if no single mode was present, the median score was used.

Table 1: Composition of embalming fluid and submerged solution

Composition of 3 liters of embalming fluid		
Group A	Group B	Group C
Formalin (37%) 30ml Water 637ml Methanol 2000ml Normal saline 300ml Sodium citrate 15ml Glycerine15ml Phenol3ml Thymol 1gm	Hot tap water 1307ml. Boric acid 60 gm Ammonium nitrate 168ml Pottasium nitrate 84gm Sodium sulphate 140gm Propylene glycol 500ml Stock Cholocerol solution 100ml Formaldehyde 420ml Morpholine 30ml Ethanol 200ml	Formalin (37%) 50ml Isopropyl alcohol 200ml, 2717ml water +600gm Nacl (SSS) Glycerine 15ml Phenol 3ml Sodium citrate 15ml Thymol 1 gm
Composition of Submerged solution in 6 litres of tank (Tank Fluid)		
10% formaldehyde	Thiel solution	1lit formaldehyde + 5 liter water + 1.2 kg Nacl

A. **Morphological Assessment:**

1. The physical appearance (color, texture, and tonicity) of the skin, fascia, skeletal muscle, and visceral organs was scored from I to V, where Grade I indicates very poor and totally distorted, Grade II indicates poor and moderately distorted, Grade III indicates fair and mildly distorted, Grade IV indicates good but less intact, and Grade V indicates very good and completely intact. (**Figure-1**)
2. The range of motion of both elbow and knee joints was measured (0 to 180 degrees) for both flexion and extension using a stainless-steel goniometer. (**Figure-2**)

B. **Microbial Assessment:**

Procedure

Sample Collection and Preparation: Aseptic collection of the embalming solution samples was done from a preserved rat carcass (post 16 weeks). Sterile test tubes were labelled appropriately for the In-Use Method, indicating the solution name. All tests were performed in duplicate to ensure reproducibility.

A. In-Use Method

- Dilution Preparation: 1:10 dilution was prepared by transferring 1 ml of the embalming solution into 9 ml of sterile nutrient broth.
- Plating: 0.2 ml of the diluted sample was aseptically spread onto:
  - Nutrient Agar (NA) – for bacterial detection
  - Sabouraud Dextrose Agar (SDA) – for fungal detection
2. Incubation: One set of plates at room temperature incubated. The second set was incubated at 37°C in an incubator.
3. Observation: Plates were monitored for microbial growth at 24, 48, and 72 hours.

B. Tube Dilution Method

- Tube Labelling: Sterile test tubes were labelled with the sample name and dilution factors: 1:2, 1:4, 1:8, 1:16, and 1:32.
- Preparation of Dilutions**:**2 ml of sterile peptone water was added to each labelled test tube. Then 2 ml of the embalming solution was transferred into the first tube (1:2 dilution) and mixed thoroughly. Two-fold serial dilutions were continued to be prepared by transferring 2 ml from the previous tube into the next, mixing after each transfer. 2 ml was discarded from the final (1:32) tube to maintain equal volume across all tubes.
- Incubation**:** All dilution tubes were incubated at 37°C for 24–72 hours.
- Observation**:** Each tube was observed for any signs of microbial growth, such as turbidity or sediment, at 24, 48, and 72 hours.(**Figure-3**)

C. Histological Assessment:

Tissue samples from skeletal muscle, cardiac muscle, tendon, liver, kidney, and cerebrum of the embalmed rats were collected after the 1st, 8th, and 16th weeks of embalming. The samples were cut to a thickness of 4 microns, immersed in 10% formaldehyde for 48 hours, and processed using the paraffin embedding technique. They were then stained with Hematoxylin and Eosin. For the evaluation of tendon histology, a specific stain called Masson's trichrome was employed and examined under a light microscope, with photomicrographs taken using an Evos microscope at 10X and 40X magnification. The quality of the stained samples was assessed based on overall microscopic architecture and cellular morphology. Histopathology grading was conducted on a scale from I to III, where Grade I denotes a high degree of cellular distortion, Grade II denotes moderately good sections, and Grade III indicates sections that closely resemble normal tissue. The histological evaluation was compared with that of non-embalmed rats. (**Figure-4 to 9**)

Statistics:

Statistical methods were employed to evaluate the effects of embalming agents on preserving both macroscopic and microscopic details of the samples. The Shapiro–Wilk test was utilized to determine the normality of the data set. Interrater agreement for each morphological and histological parameter was assessed using Fleiss' Kappa statistical method. Based on the normal distribution of the data, one-way ANOVA and the Kruskal-Wallis test were applied to compare multiple group variables, followed by post hoc analysis or multiple comparison analysis using SPSS version 29.

RESULT:

Interobserver agreement (Fleiss' Kappa) showed strong consensus among the observers for all organs while assessing physical appearance of organs, with κ values between 0.640 and 0.828, p = .000. For the histological evaluation, the observers exhibited Very Good agreement was recorded for all organs, with κ values ranging from 0.852 to 1, p = .000.

The Kolmogorov-Smirnov Normality test revealed that the grade scores for physical appearance and histologic assessment were not normally distributed. As a result, the Kruskal-Wallis test, a non-parametric alternative to one-way ANOVA, was used to compare grading scores across groups

A. Morphological Assessment:

Overall odour of Formaldehyde solution was highly irritant till 16 weeks of embalming. In case of modified Thiel embalming, it had non – irritant pungent smell while Saturated salt solution was most

non -irritant in order up to 16 weeks of embalming.

- **Physical appearance of visceral organs: (Table-2)**

At the end of 1st week : It was observed that wister rats embalmed with formaldehyde solution show a yellowish colour of skin and a bleached colour of fascia, but maintained its tonicity & elasticity, which was comparable with Theil embalmed rats (reddish colour with life like and more pliable), while SSS embalmed rats showed natural look with reddish brown colour of organs while maintaining their tonicity. These findings were statistically not significant. While only one organ, i.e., the pancreas, showed deviation from the above finding. Pancreas of Formaldehyde embalmed rats showed more distortion with the lowest grading scores as compared with Thiel and SSS (highest grading score), H(2) = 15. 811p = 0.000.

At the end of the 8th week: In the Formaldehyde group, the colour of rat skin remained similar to the 1st week, while internal organs changed into dark brownish colour. In the case of Thiel, muscles were pinkish red, thoracic organs showed reddish colour while abdominal organs looked pinkish in colour. In rats of the SSS group, the natural appearance of the skin & fascia was maintained, and all visceral organs showed a reddish-brown in color. With respect to maintaining tonicity and elasticity of organs, the Thiel group performed less than the formaldehyde group (statistically nonsignificant), while the SSS group was highest in ranking, even in maintaining the morphology of the pancreas and blood vessels. (P=.002 and 0.049 respectively)

At the end of 16th week: The colour of all visceral organs in the Formaldehyde group was tuned black, while the Thiel and SSS groups exhibited retention of their natural appearance. All organs show mild to moderate degree of distortion. While comparing the tonicity and pliability of organs, the performance of formaldehyde was the lowest, followed by the Thiel group, and the highest ranking in grade score was observed in the SSS group.

Organs	**Embalming Agents**	AT 1ST WEEK				AT 8TH WEEK				AT 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 & Fascia	Formalin	8.50	2.000	2	.368	7.50	3.662	2	.160	4.50	12.364	2	**.002**
	Thiel	10.00				9.00				9.50			
		10.00				12.00				14.50			
Lungs & Heart	Formalin	8.50	2.000	2	.368	8.50	2.429	2	.297	4.25	12.183	2	**.002**
	Thiel	10.00				8.50				10.25			
	SSS	10.00				11.50				14.00			
Liver, stomach, Intestines spleen	Formalin	8.50	2.000	2	.368	8.00	2.267	2	.322	4.25	12.183	2	**.002**
	Thiel	10.00				9.50				10.25			
	SSS	10.00				11.00				14.00			
Pancrea s	Formalin	4.00	15.811	2	**.000**	6.50	12.750	2	**.002**	5.00	14.733	2	**.001**
	Thiel	9.00				6.50				8.00			
	SSS	15.50				15.50				15.50			
Kidney	Formalin	8.50	2.000	2	.368	10.08	4.406	2	.110	4.25	12.183	2	**.002**
	Thiel	10.00				6.92				10.25			
	SSS	10.00				11.50				14.00			
Muscle	Formalin	9.50	.000	2	1.000	10.00	4.250	2	.119	5.50	10.092	2	**.006**
	Thiel	9.50				7.00				8.50			

	SSS	9.50				11.50				14.50			
Blood vessels	Formalin	9.50	.000	2	1.000	9.00	6.025	2	**.049**	6.50	8.972	2	**.011**
	Thiel	9.50				6.50				7.50			
	SSS	9.50				13.00				14.50			
Cerebrum	Formalin	10.00	2.000	2	.368	8.25	4.192	2	.123	6.00	8.880	2	**.012**
	Thiel	8.50				7.75				8.33			
	SSS	10.00				12.50				14.17			

Table 2 Comparative analysis of physical appearances of different organs embedded in Wistar rats at 1st, 8th, and 16th week

Figure-1

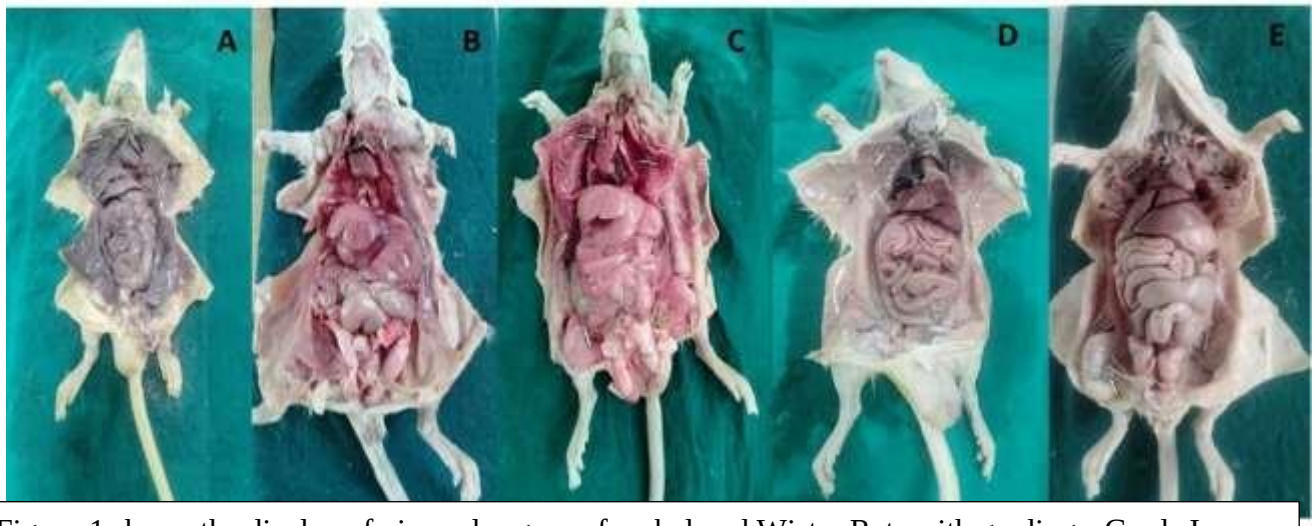

Figure-1 shows the display of visceral organs of embalmed Wistar Rats with gradings. Grade I : Very poor totally distorted (A) ,Grade II: Poor, moderately distorted (B), Grade III: Fair, mild

Range of Motion of Joints: At the end of the 1st, 8th, and 16th weeks, Formaldehyde showed a lesser degree of range of motion in the bilateral elbow and knee joints, followed by the Thiel group. The highest range of motion of all joints was observed in the SSS group. This observation was statistically significant. (**Table-3**)

Figure-2:

Figure-2 Range of motion of knee joint was recorded (0 to180^{o}) in flexion and extension position by stainless steel Goniometer

Table-3: Comparative analysis of the Range of motion of joints of embedded Wistar rats at 1st, 8th, and 16th weeks.

Joints	Embalming Agents	AT 1ST WEEK				AT 8TH WEEK				AT 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.**
Right Elbow Joint	ormalin	3.50	12.986	2	**.002**	3.50	12.425	2	**.002**	3.50	13.406	2	**.001**
	hiel	11.00				11.17				10.92			
		14.00				13.83				14.08			
Left Elbow Joint	ormalin	3.50	12.491	2	**.002**	3.50	12.591	2	**.002**	3.50	13.041	2	**.001**
	hiel	11.17				12.00				11.58			
	SSS	13.83				13.00				13.42			
Right Knee Joint	Formalin	3.50	13.470	2	**.001**	3.50	12.790	2	**.002**	3.50	12.110	2	**.002**
	Thiel	10.50				11.17				11.58			
	SSS	14.50				13.83				13.42			
Left Knee Joint	Formalin	3.50	13.377	2	**.001**	3.50	12.776	2	**.002**	3.50	12.284	2	**.002**

B. Microbial assessment:

At the end of the 16th week, no microbial growth was detected in any group of embalming solution, indicating their sterility and antimicrobial efficacy.

Figure-3:

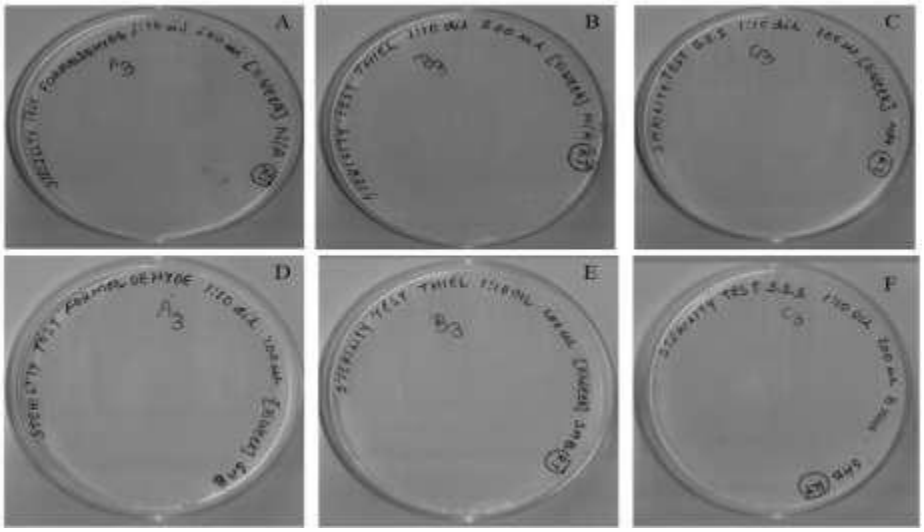

Figure-3 : Bacterial and fungal growth assessment respectively at the end of 16 weeks: Formaldehyde(A&D), Thiel (B&E) and SSS (C&F)

Histological assessment:

Histological microscopic images of slides stained with Haematoxylin–Eosin for skeletal muscle, cardiac muscle, liver & cerebrum while tendons stained with Masson's trichome The histological assessment of these tissues is based on their structural 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.

At the end of 1st week of embalming, skeletal muscle, tendon, and liver all showed well-preserved architecture and cellular details without any signs of distortion in all three groups. In the case of the Kidney and cerebrum, Group B rats showed better tissue histology preservation as compared to the other two groups, which performed at a similar level. In restoring the cardiac muscle, Formaldehyde performed better than Thiel and SSS.

At the end of the 8th week, in the case of liver, kidney, and cerebrum, formaldehyde had a lower ranking in grading score compared with Thiel and SSS. Group C, while SSS embalmed rats, displayed better histology of cardiac muscle than groups A and

At the end of the 16th week, Group C rats showed better preservation of architectural and cellular details of the majority of organs, like tendon, cardiac muscle, liver, kidney, and cerebrum whereas Group A rats scored least ranking in the same. In the case of skeletal muscle, better preservation of fiber arrangement, cellular and nuclear morphology in Group A and C rats was better than in Group B. However, all the above correlations were not statistically significant. (**Table-4**)

Figure-4: Histological assessment of skeletal muscle

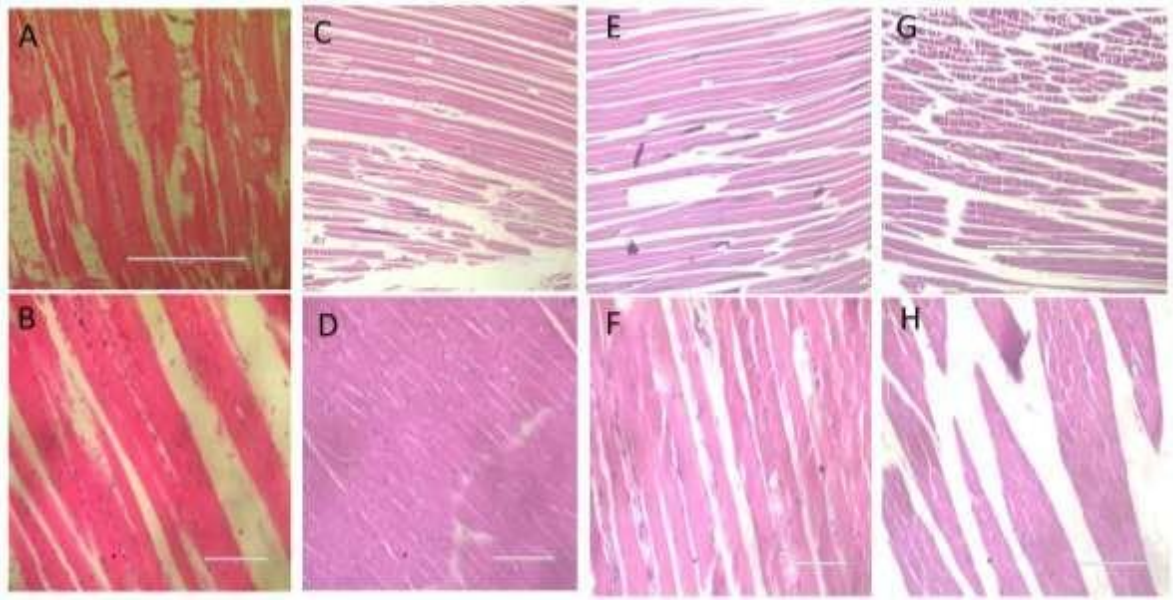

Figure-4 : shows histological assessment of skeletal muscle stained with H&E displaying the tissue architecture and cellular details (10x and 40x view: upper row and lower row respectively) of normal sections of non-embalmed (A,B), Sections are very close to normal (C,D), Moderately good sections (E,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

Figure-5: Histological assessment of Tendons

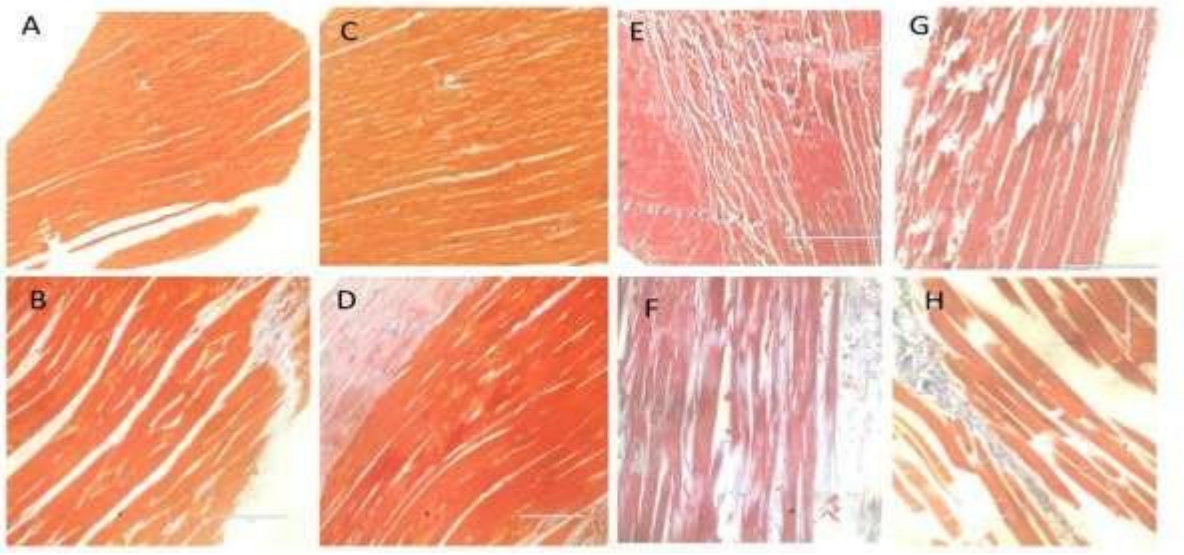

Figure-5: shows histological assessment Tendon stained with Masson's Trichrome stain displaying the tissue architecture and cellular details (10x and 40x view: upper row and lower row respectively) of sections of non-embalmed (A,B), Sections are very close to normal (C,D), Moderately good sections (E, F), section with high degree of cell distortion (G,H).

Figure-6 Histological assessment of Cardiac muscle

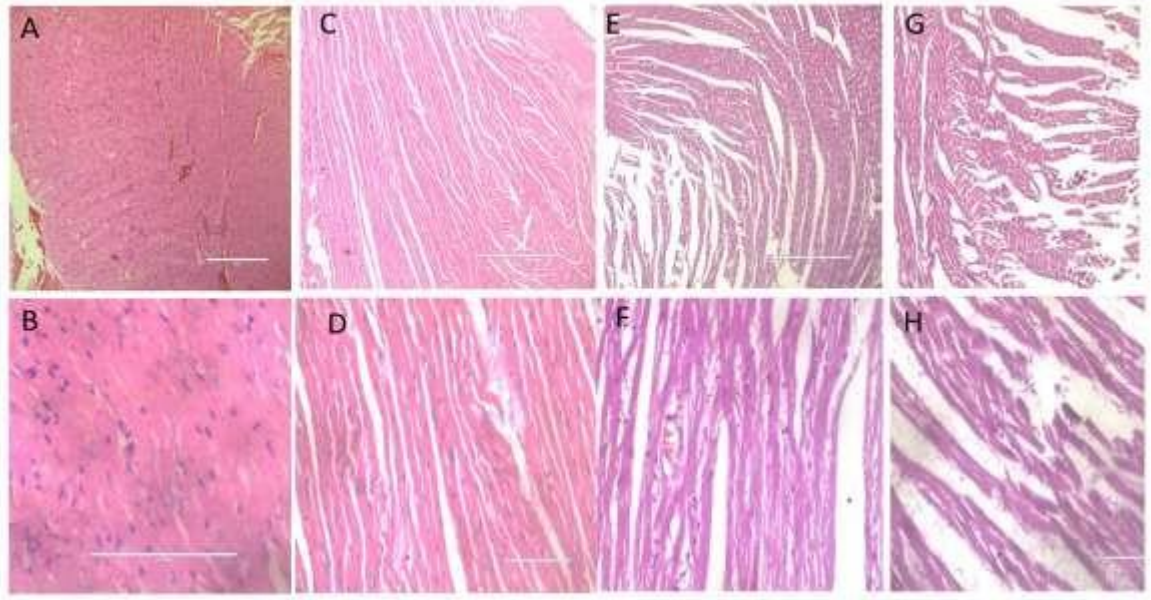

Figure-6: shows histological assessment of cardiac muscle stained with H&E displaying the tissue architecture and cellular details (10x and 40x view: upper row and lower row respectively) of sections of non-embalmed (A,B), Sections are very close to normal (C,D), Moderately good sections (E, F), section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

Figure-7: Histological assessment of Liver

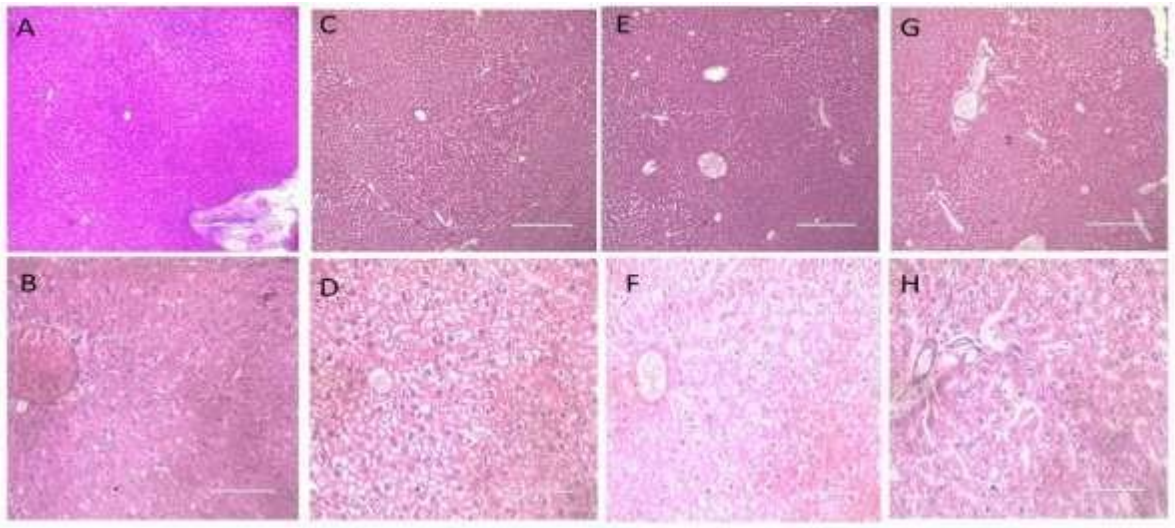

Figure-7: shows histological assessment of Liver stained with H&E displaying the tissue architecture and cellular details (10x and 40x view: upper row and lower row respectively) of sections of non-embalmed (A,B), Sections are very close to normal (C,D), Moderately good sections (E, F), section with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

Figure-8: Histological assessment of Kidney

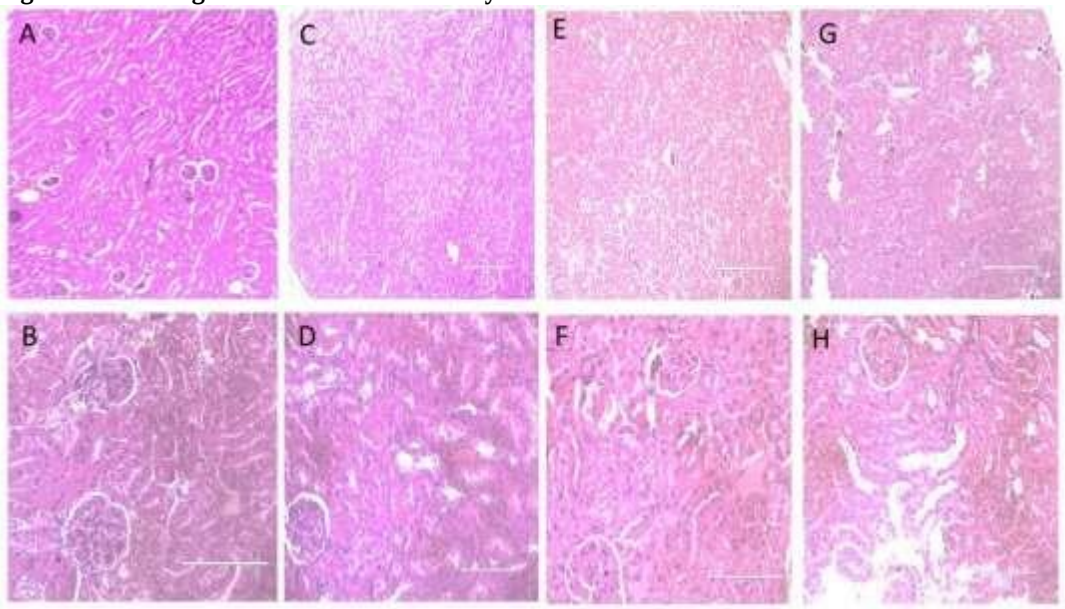

Figure-8: shows histological assessment of Kidney stained with H&E displaying the tissue architecture and cellular details (10x and 40x view: upper row and lower row respectively) of sections of non-embalmed (A,B), Sections are very close to normal (C,D), Moderately good sections (E, F), section with high degree of cell distortion (G,H).

Figure-7: Histological assessment of Cerebrum

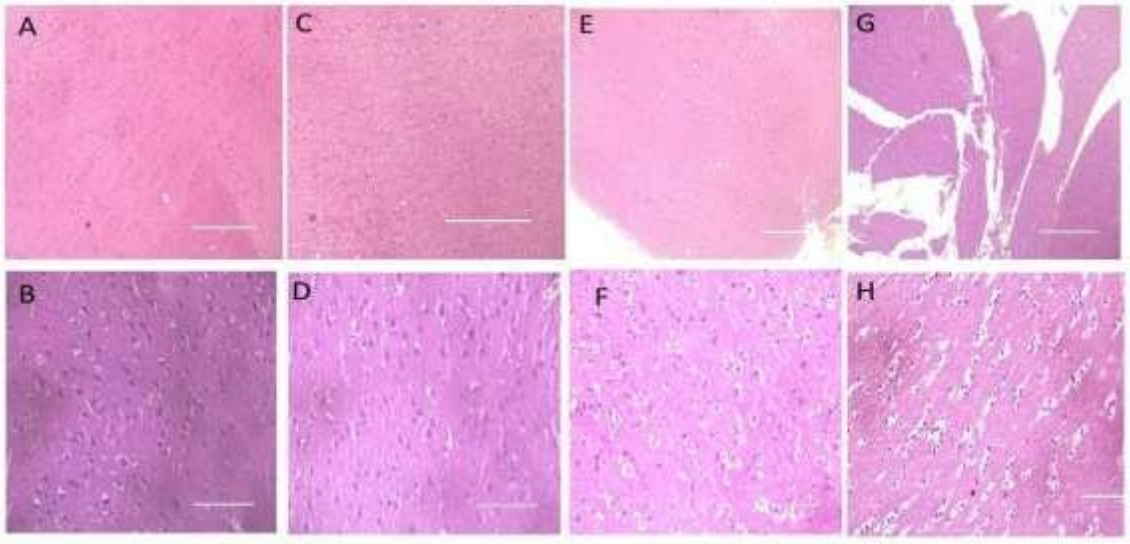

Figure-9: shows histological assessment of Cerebrum stained with H&E displaying the tissue architecture and cellular details (10x and 40x view: upper row and lower row respectively) of sections of non-embalmed (A,B), Sections are very close to normal (C,D), Moderately good sections (E, F), section with high degree of cell distortion (G,H).

Table 4: Histological analysis of different organs embedded in Wistar rats at 1st, 8th, and 16th weeks.

Organs	Embalming Agents	AT 1ST WEEK				AT 8TH WEEK				AT 16TH WEEK			
		Mean rank	Kruskal-	df	Asymp. Sig.	Mean rank	Kruskal-Wallis H	df	Asymp. Sig.	Mean rank	Kruskal-	df	Asymp. Sig.

			Wallis H								**Wallis H**		
Skeletal Muscle	ɒrmalin	9.50	.000	2	1.000	9.50	.000	2	1.000	10.00	2.000	2	.368
	hiel	9.50				9.50				8.50			
		9.50				9.50				10.00			
Tendon	ɒrmalin	9.50	.000	2	1.000	9.50	.000	2	1.000	8.50	2.429	2	.297
	hiel	9.50				9.50				8.50			
	SSS	9.50				9.50				11.50			
Cardiac Muscle	Formalin	10.50	1.063	2	.588	9.00	.523	2	.770	10.00	.442	2	.802
	Thiel	9.00				9.00				10.00			
	SSS	9.00				10.50				8.50			
Liver	Formalin	9.50	.000	2	1.000	7.50	2.092	2	.351	8.17	2.028	2	.363
	Thiel	9.50				10.50				9.33			
	SSS	9.50				10.50				11.00			
Kidney	Formalin	10.00	2.000	2	.368	7.50	2.092	2	.351	6.50	5.440	2	.066
	Thiel	8.50				10.50				9.50			
	SSS	10.00				10.50				12.50			
Cerebrum	Formalin	10.50	4.250	2	.119	8.58	.617	2	.734	6.17	4.533	2	.104
	Thiel	7.50				9.25				10.17			
	SSS	10.50				10.67				12.17			

DISCUSSION

The present study aimed to compare the efficacy of three embalming techniques, i.e., Formaldehyde, Modified Thiel, and SSS, in preserving anatomical integrity, tissue morphology, and microbial resistance in Wistar rats over 16 weeks. Parameters evaluated included physical appearance, joint range of motion (ROM), microbial growth, and histopathological preservation. The findings suggest significant differences in performance across these embalming methods, with SSS emerging as the most effective technique for long-term preservation.

Morphological Assessment

Physical Appearance

Formaldehyde is widely used for tissue preservation due to its protein cross-linking properties; however, it is also known for its irritant vapors and tissue-hardening effects. (5)In this study, rats embalmed with formaldehyde demonstrated a persistently irritating odor up to 16 weeks and exhibited a gradual blackening of visceral organs. Although it initially maintained tonicity and elasticity, its performance deteriorated over time, with visible distortion and loss of tonicity making the tissue harder in consistency, especially by week 16. Conversely, Thiel embalming showed a more lifelike appearance of organs with less odor, as previously documented in various human cadaver studies. (3,6) Villacorta et al conducted a comparative perception study in which the physical appearance of formaldehyde and Thiel preserved cadaver used in human grossing. In the study, ratings of Thiel Method-preserved cadavers were higher compared to the formaldehyde in terms of tolerable odor, presenting color, better skin, muscle blood vessels,l texture, and integrity as observed in the present study.(7) In another 3-year-long Thiel embalming study on human cadavers, clearly stated the advantages of Thiel in embalming regarding its ability to retain skin colour, excellent flexibility of joints and long term preservation without dehydration. (8) The life-like appearance of embalmed cadavers, the pinkish or reddish color of Thiel- embalmed cadavers could be attributed to the presence of nitrate, which can be responsible to plays a vital role in colour preservation. (9)However, it was the SSS method that most effectively preserved the natural appearance and tonicity of tissues throughout the study period. A similar animal study conducted by Lombardero M et al in dogs embalmed with SSS through an innovative approach of recirculation instead of maceration. The results were impressive in terms of non-irritant smell, good preservation of the musculoskeletal system, natural consistency, and better joint mobility as compared to the conventional Formaldehyde.(10)In a comparative human cadaver study, Hayashi et al reported about superiority of SSS over Thiel and formaldehyde embalming in terms of maintaining high tissue quality. (4) Notably, the pancreas in SSS-embalmed rats retained its structure significantly better ($p = 0.000$), suggesting superior long-term preservation capability in delicate tissues.

Range of Motion (ROM) of Joints

Preservation of joint mobility is critical for educational and surgical simulation purposes. In this study, SSS-embalmed specimens consistently showed the highest ROM at the elbow and knee joints at all time points, followed by Thiel and then formaldehyde, with statistical significance ($p < 0.05$). The superior ROM in the SSS group likely reflects its ability to prevent the stiffening commonly induced by formaldehyde-induced protein fixation.(11) These findings support prior literature where SSS has been reported to maintain tissue flexibility better than formaldehyde.(12)

Microbial Assessment

It is noteworthy that microbial resistance plays a pivotal role in embalming efficacy. Formaldehyde is known to possess strong antimicrobial properties. (1) Balta et al observed the highest microbial growth inhibition efficacy of Formaldehyde, which was followed by Thiel when examined for 2 months (13) In the present study, we did not observe any microbial growth (both bacterial and fungal) when embalming fluids were examined for 16 weeks. This deviated result could be attributed to the methodology of sample collection. however, SSS has also demonstrated antimicrobial activity, particularly due to its high osmotic concentration, which inhibits microbial growth.(4) Future studies should provide quantitative microbial data to complement these preliminary observations.

Histopathological Assessment

Histological preservation is a cornerstone of embalming efficacy, especially for research and diagnostic applications. At week 1, all groups showed intact tissue architecture in skeletal muscle, tendon, and liver. However, differences emerged at 8th week and became pronounced by week 16. SSS provided superior preservation across most organs, especially cardiac muscle, kidney, liver, and cerebrum. This may be attributed to reduced tissue dehydration and better maintenance of the extracellular matrix. Interestingly, Thiel fixation offered the least preservation of skeletal muscle fibers and nuclear morphology at 16 weeks, consistent with its known content, such as boric acid, which induces protein degeneration involved in maintaining muscle integrity. (14) Formaldehyde, while initially effective, showed the most degeneration by week 16, particularly in neural and hepatic tissues.

Despite the consistent histological trends favouring SSS and Thiel over formaldehyde, most of these differences did not reach statistical significance. This may be attributed to sample size limitations or inherent biological variability. Nonetheless, the observed patterns suggest a clear trajectory favouring SSS for longer-term anatomical preservation.

CONCLUSION

Based on the physical, functional, and histological parameters assessed, SSS emerged as the most effective embalming technique for long-term preservation in Wistar rats, maintaining near-natural tissue appearance, pliability, joint mobility, and cellular integrity across multiple organ systems. Thiel embalming showed intermediate results, particularly excelling in skeletal muscle preservation. Formaldehyde, while traditionally favoured for its antimicrobial strength and structural fixation, was least effective in maintaining functional and histological quality over time and posed significant safety concerns due to its irritant vapours. These findings have important implications for preclinical education and animal model preservation, especially in scenarios requiring repeated handling or simulation-based training. Further research with larger sample sizes and more detailed microbial assessments is warranted.

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