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COMPARATIVE STUDY OF TRADITIONAL FORMALDEHYDE VERSUS FORMALDEHYDE FREE- SATURATED SALT AS EMBALMING SOLUTIONS FOR GROSS ANATOMY AND SURGICAL TRAINING OF STUDENTS

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Abstract:

The aim of this study was to compare the chemical and microbiological effects of two preservatives used for the embalming of cadavers for teaching of veterinary students in anatomy laboratory. Twelve cats recommended for euthanasia were included in this study. All the cats (n=12) were divided into two treatment groups (n=6 each). In Group A, 10 % formaldehyde was used with phenol, glycerin and water. In Group B Saturated Salt was used with phenol, glycerin and water. Microbiological examination was performed and evaluated by pour plate technique. The range of motion of the joints and freshness was evaluated by Goniometer and Konica Minolta C-400 Chroma Meter respectively. Data thus obtained were compared between groups using paired T-test. Differences at (P<0.05) were considered significant. Cats administered with saturated salt solution showed reduction in bacterial and mold growth, joints flexibility, retained their natural consistency and color than traditional formaldehyde treated group (P>0.05). The range of motion of joints at day 20th, 40th and 60th were significantly higher (P <0.05) in group B as compared to group A. The muscle consistency and color was graded as L, a*, b*, C* and h* and their values were (65.21±0.50, 6.17±0.20, 12.79±0.31, 11.03±0.25 and 30.71±0.35) and (58.74±0.35, 8.22±0.20, 9.59±0.27, 13.08±0.20 and 25.36±0.22) in formaldehyde and SSS groups respectively. The responses of DVM students were positive for irritation of eyes, sore throat, coughing and bad smell in formaldehyde group. In conclusion, the SSS method is simple and cheap with a low risk of infection as compared to formaldehyde.*

Keywords: *Embalming; Veterinary Gross anatomy and surgery; Saturated salt solution (SSS); formaldehyde 10%.*

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INTRODUCTION:

In the current scenario of veterinary education, anatomist use animal cadaver models to teach veterinary students (1). Cadaver dissection in anatomy teaching laboratory results in exposure of the chemicals used for embalming procedure to students, faculty and laboratory staff. The intensity and duration of exposure varies from person to person and ranges from short to long period of time (2). The embalming chemical is most suitable requirement for cadaver preservation for education purpose. The appropriate preservatives keep the cadaver safe from harm destruction or decomposition (3). A large number of studies have used fresh frozen cadavers. The alternative to the fresh cadaver is the embalmed cadavers (4). From ancient time, the main purpose of embalming was preservation of human body; important motive was religious belief (5). In China deceased people were also embalmed (6) but methods of embalming and solutions were unknown. Embalming became widespread practice to Europe and during Middle Ages included evisceration and immersion of body in alcohol (7). Bodies used for dissection purposes and preservation required more refined embalming techniques (8). With the progress made in embalming by arterial injection, research for new preserving fluids opened (9). The main preserving agents used were alcoholic solutions of arsenic/ alumina salts before introduction of carbolic acid, phenol and formaldehyde. The investigations were made to improve embalming fluid formulation bases on alcohol-glycerine-phenol-formaldehyde. Recently, Hammer et al. 2012 described a formaldehyde free system comprising of ethanol-glycerine. In spite of the chemical properties of embalming solution, they should deliver a long term organ and tissue preservation along with the prevention of toughening and retention of color of tissues and organs. They should also avoid bacterial and fungal growth (10). In human and veterinary medicine, the formaldehyde is extensively used as embalming chemical. It causes protein denaturation, a good preservative, antioxidant and exhibits antimicrobial property (11). However, formaldehyde carries possible negative effects on fetal development and significant health risks such as impending tumor development and allergic reaction (12). In the past, table salt has long been in meat conservation and recently tested for preservation of meat (12). Embalming of human carcasses with a saturated salt solution has been commonly tested and established for medical training especially for surgical skills training as well as plastic surgery (13). After many years' exposure to the formaldehyde, sensitization increases as it causes irritation.

In addition, European association of veterinary education (EAEVE) recommends replacing this fixation method such as formaldehyde by a nontoxic alternative suitable method for preserving anatomical specimen (14). As our dissection facility has no devices to reduce or eliminate formaldehyde fumes, students and teachers of anatomy are subjected to hazardous doses of formaldehyde. We therefore conducted a study to compare 10% formaldehyde solution with saturated salt solution for embalming to avoid the use of formaldehyde in animal specimen for gross anatomy practical session. We tested both solutions in our practical sessions by students with the help of questionnaire distributed among 35 students in the practical class. The aim of our study was to highlight the risk associated with formaldehyde and to establish formaldehyde-free SSS alternative embalming solution in best interest of students, teachers and laboratory staff.

MATERIALS AND METHODS:

This study was conducted on twelve cats breed bangle, mixed male and female and age ranged 3-5 years having 4-6 kg body weight and were recommended for euthanasia at Pet center, University of Veterinary and Animal Sciences, Lahore due to any reason. All the experimental animals were divided in two groups, n=6 in each group and kept in separate cages. The experiment was performed at Anatomy Laboratory, University of Veterinary and Animal Sciences Lahore. The preservation experiment with embalming solutions was carried under general anesthesia in both groups. Vital signs i.e. temperature, pulse and respiration of each animal were recorded prior to the experiment. All the animals were pre medicated with atropine sulphat at the dose rate of (dose must be in mg/kg body weight). Induction of anesthesia was performed with xylazine (mention the company and product name) (dose must be in mg/kg body weight) and ketamine (same as above) cocktail. At the same time, heparin 1ml/100ml normal saline (mention the w/w or w/v) was also injected after induction of anesthesia as anticoagulant agent (Murray et al. 1938). Large vessels of neck common carotid arteries & external jugular veins) were dissected and cannulated with guage 22 cannula and fixed carefully to prevent the displacement (Hayashi et al. 2014) (Fig. No.1).

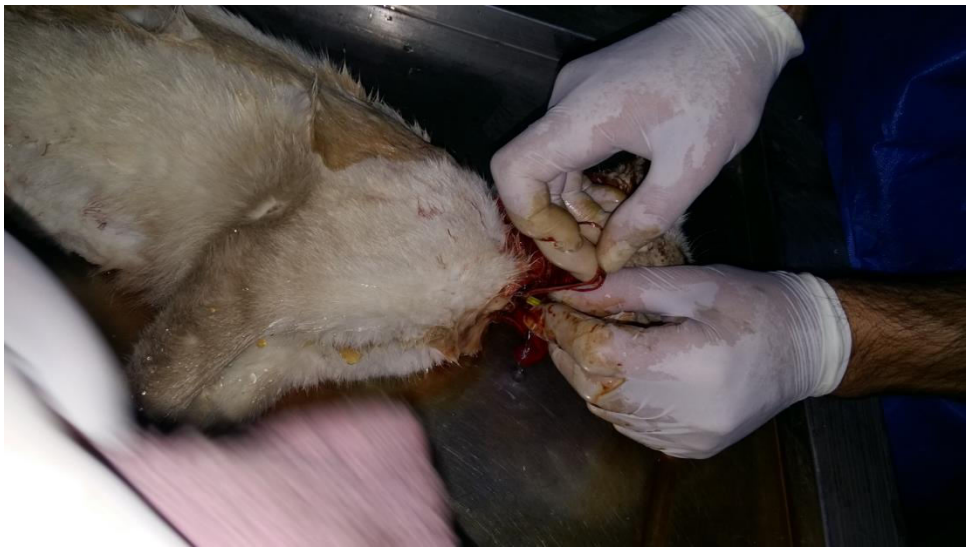

Figure 1: Cannula (22 Guage) fixed in common carotid artery for perfusion of embalming solutions

Group A was administered with 10 % formaldehyde, phenol, glycerin, water and group B was administered with Saturated Salt, phenol, glycerin and water as embalming solutions (also mention the concentration and quantity of each chemical in solution), respectively. Complete perfusion of embalming solution was assessed by monitoring the oral cavity and nostrils. After successful embalming, the cadaveric bodies were kept in freezer at 40C for three days. (Table.1 & 2).

Table 1: Composition of different embalming solutions

Formaldehyde embalming solutions (FAS)		**Saturated salt solution (SSS)**	
Ingredients	**Volume (liters)**	**Ingredients**	**Volume (liters)**
10% formaldehyde	0.8	Saturated salt	4 kg
Phenol	0.08	Phenol	0.04
Glycerin	0.2	Glycerin	0.1
Water	2.92	Water	3.86
Total	**4**	**Total**	**4**

Table 2: Composition of nutrient agar oxoid and sabouraud dextrose agar

Nutrient agar oxoid	**Gram/Liter**	**Sabouraud dextrose agar**	**Gram/Liter**
'Lab-Lemco' powder	1.0	Mycological peptone	10.0
Yeast extract	2.0	Glucose (dextrose)	40.0
Peptone	5.0	Agar	15.0
Sodium chloride	5.0		
Agar	15.0		

Range of motion (ROM) of joint

After three days of freezing, the range of motion of elbow and stifle joint was observed before disarticulation of pectoral and pelvic limbs of Cats Cadavers The flexion and extension of both right and left sides of elbow and stifle joint of each cadaver were assessed by using Goniometer at day 4th, 20th, 40th and 60th to evaluate the rigor mortis (Fig No. 2).

Figure 2: Extension of range of motion (ROM) of elbow joint using Goniometer

Microbiological examination

Afterwards disarticulation of joints of pelvic and pectoral limb, the skin was incised to obtain meat sample from thigh regions using sterile tubes for bacterial and fungal growth examination in both groups at 4th, 20th, 40th and 60th days.

Meat/muscles color examination

The freshness of meat/muscles and organs was determined and graded by change in color at day 4th, 20th, 40th and 60th of experiment. Konica Minolta CR-400 Chroma Meter was used (Fig No. 3) for grading of color change of embalmed organs and meat/muscles in both groups (Table No. 3).

Table 3: Grading of color change

L*	**Lightness**
a*	**Redness**
b*	**Yellowness**
C*	**Chroma**
h*	**Hue**

Figure 3: Meat/muscles color observation using Konica Minolta CR-400 Chroma Meter

Responses on questionnaire

The questionnaire for DVM students was prepared to test the authenticity of two embalming solutions about their experience with dissection during their practical class, where cadavers were preserved with conventional formaldehyde versus saturated salt solution (SSS). After ninety minutes of class time, a Performa was distributed to get a response from students. Similar observation of the embalmed cadavers in saturated salt solution (SSS) was performed on next day.

RESULTS:

Evaluation of range of motion

In our study, we attempted a less invasive approach, through cannulation of common carotid arteries for the perfusion of two different embalming solutions that enabled us to get good results of musculoskeletal preservation allowing wide range of motion of the joints, similar to a living animal especially in saturated salt solution perfused cadavers. The results showed that joints were stiffed in formaldehyde embalmed cadavers. There was significant difference in range of motion of joints between formaldehyde and saturated salt solution ($P<0.05$) as shown in (Fig No. 4). There was significant difference of degree of extension and flexion of elbow and stifle joints at day 20, 40 and 60 in both groups.

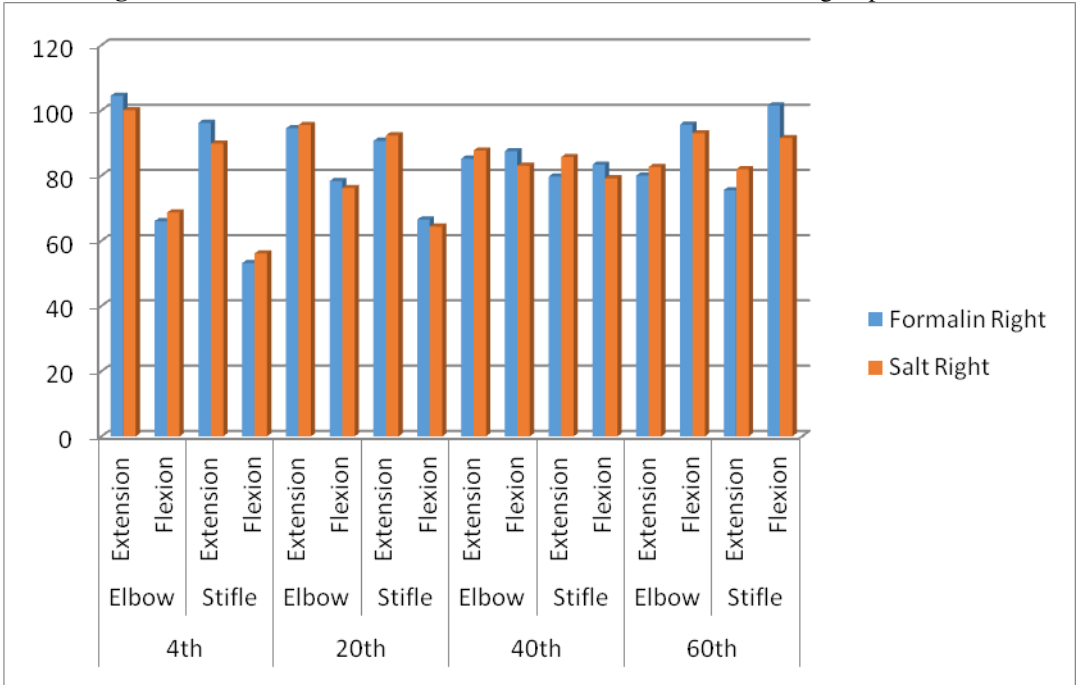

Figure 4: Comparison of ROM of joints by SAS and SSS solutions at different days

Assessment of infectious risk

Our results showed that both embalming solutions possessed bactericidal property. The bacterial colonies were counted from meat/muscle samples of each cat and bacterial growth was determined. There was no significant difference at day 20th, 40th and 60th. The results indicated that none of the solution promoted bacterial colonies. Similarly, we also observed meat samples of each group on day 4th, 20th, 40th and 60th for mold growth but negative growth was examined till the last day of our trial. It showed that both solutions prevented bacterial growth as well as fungal in (Fig No. 2).

Testing of freshness of meat

The freshness of meat sample was also evaluated on basis of discoloration and unnatural texture. Samples showed lightness in color but lightness was less in saturated salt solution group as compared to formaldehyde embalmed group that showed more lightness in color. There was significant difference between lightness of two groups ($P<0.05$). The color of saturated salt solution sample was more close to the normal meat color. The results showed that lightness increased with passage of time at day 20th, 40th and 60th in both groups but to lesser extent in group B as show in (Table No. 4) and it was observed that both groups showed decreased in redness from original color but redness of group B was near to original sample, there was less decreased in redness of group B as compared to group A. ($P<0.05$). Similarly, there was significant difference between two groups for yellowness, chroma and hue ($P<0.05$). The degree of hardening, discoloration, deterioration was higher with unpleasant odor in formaldehyde

treated group (Table.4).

Table 4: Meat/ muscles color examination on day 4th, 20th, 40th and 60th days

Day 4

Parameters	Day 4		Day 20		Day 40		Day 60	
	FAS	SSS	FAS	SSS	FAS	SSS	FAS	SSS
L*	52.23	51.91	55.98	53.29	59.86	55.90	65.21	58.74
a*	14.26	14.27	12.30	13.04	10.20	11.35	6.171	8.221
B*	3.572	3.643	5.782	4.551	9.032	5.791	12.79	9.590
C*	17.73	17.66	15.46	16.04	13.64	14.67	11.03	13.08
h*	11.01	11.27	19.06	14.67	22.83	19.86	30.71	25.36

Assessment of embalming solution by students

In both visual and tactile assessments, the students felt that saturated salt solution embalmed cadavers were more suitable to study for hands-on training of anatomy teaching classes. They suggested safe in terms of coughing, bad smell, irritation, sore throat and watery eyes and nose. The percentage of positive replies for SSS and negative replies for formaldehyde embalmed cadavers is shown in (Table No. 5).

Table 5: Summary of response of students included in questionnaire

Response category	Total replies	Positive replies as % of total FAS	Positive replies as % of total SSS
Respiratory distress	35	80.01	40.02
Dry or sore throat	35	62.85	31.42
Unusual thirst	35	45.71	37.14
Itching or sore eyes	35	68.57	25.71
Red eyes	35	65.71	28.57
Excessive lacrimation	35	85.71	17.14
Disturbance of sight	35	31.42	22.85
Headache	35	77.14	37.14
Dry or sore nose	35	62.85	40.01
Running or congested nose	35	77.14	57.14

DISCUSSION AND CONCLUSION:

Mummification has been in practice since prehistoric times. Salt has been used in preserving process since Egyptian and earliest Biblical era (15). In this study, we observed that the cadavers embalmed with both 10% formalin and SSS had adequate antibacterial potential. The widespread practice of formalin as a therapeutic and embalming agent is dependent on its outstanding antiseptic property that inhibit the entrance of putrefying organism as well as it also tans tissues without abolishing their delicate structure. Our study is in accordance to Brenner.2014. Our study proves that considering the presence of multiple bacteria and fungi before embalming, SSS-embalmed cadavers are measured to be no less safe than formaldehyde embalmed cadavers. At least it is sure that they have lower infectious risk and are less fragile than fresh frozen cadavers. Our findings are similar to Hayashi, 2014 who reported also that bacterial and fungal tests proved that SSS has bactericidal effect. In current study, our results showed that bacterial examination on day 20th, 40th

and 60th did not vary significantly ($p>0.05$) between formaldehyde (0.500%), (1.16%) and (1.27%) and saturated salt solution (0.111%), (0.888%) and (1.611%). The current findings are supported by the work of other scientist (16) who reported that using low formaldehyde concentrations in combination with ethanol and polyethylene glycol inhibits microbial growth on animal cadavers. Similar results were also showed by (17) who preserved and fixed in nitrite pickling salt and ethanol glycols solutions. Similarly, there were negative mold growth examinations. For the reason of the wellbeing and environmental hazards linked by formaldehyde, severe health precautions are obligatory during handling cadavers pickled with formaldehyde. These precautions are much cost consuming. In the face of present economic cutbacks, universities are searching for all possible cost cutting measures. Moreover, proper waste management of formalin residues transfers with its high financial and environmental cost. We are thus farsighted a renaissance in the search for perfect embalming agent, one that is both safe and inexpensive. Our study confirms the usefulness of SSS as in embalming agent (18). The present study proposes an alternative fixative method for veterinary specimen to be used in gross anatomy teaching.

Their joints remained flexible and their soft tissue freshness in quality was acceptable in saturated salt treated group. Hayashi et al. (2014) used a standard goniometer (19) to test range of motion (ROM) of joints. The range of motion (ROM) of joints were significantly higher ($p<0.05$) in SSS treated group. Softness of tissue and joints in a cadaver is an important factor for SSS. Moderately high levels of formalin harden tissues and have been found to severely affect the quality of cadaveric tissue, particularly soft tissue, which has an effect on joint flexibility. Our study is in accordance with (20) who reported that formalin crosslinks several proteins chemically by adding a methylene bridge (–CH2–), resulted in fixation or a tanning-type action. He also suggested various embalming methods to reduce the formalin concentration. Our study proves that SSS-embalmed cadavers have a joint flexibility comparable with that of fresh cadavers. Our results showed that the joints of SSS-embalmed cadavers tended to be softer than those of formaldehyde embalmed (10 % formaldehyde) had harder tissue. Our findings are in line with (21) stated that a rigid cadaver were obtained by the use of formaldehyde high concentrations in other solutions, a long life time and highly flexible cadaver were produced by the introduction of salts along with an extended duration of time, and the use of salts containing low level concentrations of formaldehyde produced a cadaver flexible and conserved for a shorter period of time. The formaldehyde produces rigid cadavers are therefore not appropriate for anatomical and surgical training. In our study, FS failed to keep tissue coloration and cadavers were intensely red in contrast to SSS, did not alter the coloration of cadaver, which was fairly similar to that normally found in living animals. Our results are in accordance with Silva et.al.2014 that also showed similar findings. He also compared LS and MLS- LS was without formalin and it retained good color. The soft consistency, color and flexibility, especially at joints of specimens preserved in SSS, were found to be suitable for dissection, demonstration and display purposes. According to the questionnaire circulated among the students, their opinion about the preservative fluid was supportive for SSS as it caused little skin irritation. Our study is in accordance to the Tandon.2014 who also showed that smell and mucosal irritation is suspected at lowest concentration of formaldehyde and similar features of mucosal irritation were experienced by our students. Students also found experience with cadavers preserved in SSS was very pleasant as compared to the offensive odor of formaldehyde. SSS is a suitable alternative for the preservation of specimens. However, efforts have to be made to reduce or replace the use of formaldehyde as a primary fixative. In this method of preservation, we also conclude that preventing blood coagulation, to allow a perfect bleeding and perfusion with SSS and 10% formaldehyde until it exits the nasal openings (22). We believe that removing all of the blood is essential to allow salt to reach the tissue. Our study is similar to (23). In contrast other authors such as hayashi et al. (2014), did not remove blood from corpses before perfusing SSS because their formula was reported to liquefy the blood. In our study we used the embalming method consisted of perfusing a fixative or embalming fluid through an artery while preventing the any outflow, allowing the fluid to fix body tissue during the maceration body period. Some authors infuse up to 25 litre of fixative inside a human corpse, as described by Hayashi et al. (2014).or even up to 8 Gal (30 litre), as reported as reported by Whitehead & Savoia (2008), resulting in uniform swelling of the cadaver. In conclusion, the SSS method is simple and cheap with a low risk of infection. For encouraging the diffusion and uptake of surgical skill trainings, many economical, technical, and hygienic difficulties still remain as unsolved issues. The SSS method will become one of the bases of the spread of SST using cadavers.

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