

RESEARCH ARTICLE

Novel formaldehyde-free embalming fluid formulation for long-term preservation of cadavers for anatomy teaching

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ABSTRACT

Dissection of mammalian cadavers enables veterinary students to appreciate the three-dimensional relationships of these specimens. Chemically fixed cadavers are generally used for dissections owing to the impracticality of using fresh specimens in most teaching facilities. The aim of this study was to evaluate a novel embalming fluid formulation comprising 25% ethanol, 20% polyethylene glycol 400, 0.1% chloroxylenol, and 10% sodium nitrate made up to 100% with tap water to fix a series of domestic mammals for anatomical dissection. The embalmed cadavers (two cats, two dogs, two goats, and two sheep) were periodically evaluated over a 6-month period. At 1, 10, and 20 weeks of the dissection program, the cadavers were colorimetrically measured and the texture profiles, including hardness, adhesiveness, and deformation, were examined. In addition, tissue samples of the skeletal muscle, liver, lung, and intestine were collected and microbiologically analyzed at various time intervals. The embalmed cadavers closely resembled their fresh counterparts. There was negligible difference in the objective and subjective parameters, with a gradual increase in the hardness and adhesiveness of the muscle samples with time. No microbiological growth was detected in the tissue samples obtained from the embalmed specimens, except in the colon tissue from one cat and lung tissue from one sheep. These findings demonstrated the successful preservation of cadavers in the novel embalming formulation for a period of 6 months. The subjective evaluations during dissection were harmonious with the objective assessments of the embalmed cadaver.

Keywords: Anatomy; colorimeter; dissection; embalming; formaldehyde; texture analyzer

INTRODUCTION

Anatomy training forms the core of all veterinary and human medical education programs. The use of fresh cadavers during anatomy training can, in some cases, pose a health hazard to the students and staff members owing to the potential risk of zoonoses and wound infections (Ehdaivand et al., 2013; Correia et al., 2014; Hayashi et al., 2016). Embalming is an effective method to minimize the risk of disease transmission from cadavers to humans and to increase the shelf life of a carcass for ongoing progressive dissections (Jaung et al., 2011; Keough et al., 2017; Al Aiyan et al., 2018).

A fixed cadaver can pose a small risk of infection to humans because it is not possible to achieve total microbial sterility with any embalming process (Demiryürek et al., 2002; Balta

et al., 2015; Al Aiyan et al., 2018). Ideally, embalming fluids should destroy all organisms that inhabit the living body and those that reside on the decaying carcass. Additionally, the embalming fluids must not have adverse effects on the health of those working with the embalmed bodies. Fixatives such as formaldehyde and glutaraldehyde have exemplary preservative properties but are coming into disuse because of their noxious and carcinogenic nature (Kerns et al., 1983; Kriebel et al., 1993; Balta et al., 2015).

As a learning aid, the embalmed cadaver should resemble its fresh state, in terms of both color and texture. Color retention and tactile modalities help students to differentiate between the muscles and fasciae, nerves, vasculature, and other neighboring structures, thus making it appear more realistic for dissection (Whitehead and Savoia, 2008; Healy et al., 2015). Likewise, the mechanical

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properties of a cadaver should emulate the feel of a fresh cadaver dissection or live animal (Öhman et al., 2008; Liao et al., 2015).

Although the quality of an embalmed cadaver depends on its visual and haptic properties and its state of preservation, anatomists are yet to reach a consensus regarding the objective measures to be employed for assessing the quality of embalming. Recent studies have used colorimetric methods such as the L, a, b, and RGB technologies to measure color changes in the muscle and fat of the cadaver over the course of ongoing progressive dissection (Jaung et al., 2011; Li et al., 2011). The colorimetric assay used in this study measured the color of the muscles' tissues using the following three parameters: L, a, and b. The L scale measures the darkness of the sample and ranges from 0 to 100, with values closer to 0 denoting black and those closer to 100 denoting lightness or white. A change in color, such as darkening of the tissue during putrefaction, is recorded as an increase in the "L" value. The "a" value measures the color range between red (increasing value of a) and green (decreasing value of a) of the color spectrum. Increase in the "a" value indicates increasing redness, whereas negative values show a change toward the green end of the spectrum. The "a" value is the proxy measure for preservation and demonstrates higher values in fresh samples. Putrefaction with ensuing oxidative browning of the muscles results in a gradual reduction of the red color of muscle samples. This is indicated by a decrease in the "a" value in the colorimetric scale. Any icteric tinge in the cadaver fascia is objectively measured using the colorimetric "b" value. A positive value of "b" implies a shift toward the yellow end of the spectrum, whereas a negative value indicates a migration toward the blue end. Although many studies on embalming employ a subjective assessment of the embalmed samples through photographs (Jaung et al., 2011; Zarb et al., 2017), we employed a more objective and precise colorimetric method that is routinely followed in food technology (Li et al., 2011). Turan employed a similar approach to assess the level of preservation of the cadaver (Turan et al., 2017).

Postmortem autolysis and putrefaction cause changes in the mechanical properties of the tissues (Huidobro et al., 2005; Juárez et al., 2012). These changes in mechanical properties due to histomorphologic changes are easily measured in bones, tissues, and tendons using shear wave elastography or tools such as uniaxial extensometer or food texture analyzer (Joy et al., 2015; Liao et al., 2015). In the present study, the level of putrefaction was objectively measured using a texture analyzer to determine the hardness, resilience, adhesive force, recoverable work, and recoverable force. Tissue softness, as seen in putrefaction and dehydration during preservation, is detected by performing hardness

analysis. The flexible and pliable nature of tissue is assessed by measuring the resilience and recoverable deformation (Juarez et al., 2012). Adhesiveness increases with the level of putrefaction. Putrefaction leads to an increase in the softness of the tissue and decrease in the pliability; dehydrating fixatives such as formaldehyde have the opposite effect (Hayashi et al., 2016). These measurable changes that occur during putrefaction or fixation have been exploited to test the putrefactive decay of embalming fluid (Fessel et al., 2011; Turan et al., 2017). Similarly, the flexibility and softness of musculoskeletal tissues can be measured by the range of movements in joints. A rigid joint indicates the hardness of the surrounding muscles and tendons (Jaung et al., 2011; Haizuka et al., 2018).

In the current study, a novel less-hazardous embalming fluid formulation was evaluated for use in anatomical dissection and surgical training. A series of small domestic animal embalmed cadavers were used to obtain subjective and objective measurements immediately after fixation and at intervals over a 6-month period.

MATERIAL AND METHODS

Animals, euthanasia, cadaver preparation, and fixation

This study adhered to the recommendations by the United Arab Emirates University (UAEU) Research Ethics Committee and was approved by the Animal Research Ethics Committee (A-REC) at the UAEU (ERA_2019_5854). Animal cadavers, which included those of two cats (3.5, 4 kg), two dogs (15, 20 kg), two goats (35, 37 kg), and two sheep (35, 37 kg), routinely used for the Basic Anatomy Course during the Veterinary Graduate Program were utilized in this study. The goats and sheep were euthanized by a veterinarian in accordance with the research ethics code of the UAEU. The dogs and cats used in this study had died or were euthanized for medical reasons at the veterinary clinic in the Dubai Municipality. The euthanized animals were perfused within 1 to 24 hours of death. The left common carotid artery was catheterized in all the cadavers for perfusion with the newly formulated embalming fluid.

This embalming solution comprised ethyl alcohol (25%), polyethylene glycol PEG400 (20%), chloroxylenol (0.1%), and sodium nitrate (10%) was made up to 100% by volume by adding tap water.

Perfusion was performed using a peristaltic pump (MA-1450 Perfu-Tek, Medis GmbH, Germany) at pressures of 10–20 PSI and continued till the embalming fluid started to foam out of the cadaver's nasal cavity through the nostrils. The animals were then immersed in the embalming fluid in a cold room.

Dissection and sample collection

The embalmed carcasses were removed for dissection 1 week after initial fixation and used twice weekly over the next 6 months. The carcasses were used to dissect the musculoskeletal system by a group of students throughout the 6-month period. Since the third week onward, the same animals were used in parallel by a separate group of students to dissect the internal organs and associated structures as follows: weeks 3–6, head; weeks 7–11, thorax; and weeks 12–20, abdomen and pelvis.

Tissue samples (0.5 cm³) were collected for microbiological examination from a superficial muscle (*latissimus dorsi*), deep muscle (*longissimus lumborum*), the left caudal pulmonary lobe, and the caudate process of the liver; in addition, samples (0.5 × 0.5 cm × 0.5 cm in size) were collected from the jejunum and descending colon. All samples were collected at 2 months and 4 months post-fixation.

Muscle samples (diameter, 6.5 cm; thickness, 1 cm) were obtained from one cat, one goat and 2 dog carcasses, 1 week after initial fixation for colorimetric analysis. Each sample was repeatedly tested after 10 and 20 weeks for color parameters. The samples were preserved in the embalming fluid and aired twice a week for 2 h to simulate air exposure during the dissection course.

Texture analysis was conducted on nine deep muscle samples (1 cm³, *longissimus lumborum* muscle) obtained from one cat, one goat and 2 dog carcasses during the first week of dissection. Three out of the nine samples were measured immediately during the first week, whereas the remaining six were preserved and aired as described for colorimetric analysis. Subsequently, three samples were measured after 10 weeks and three after 20 weeks. Each sample cube was used only once for measurement.

The microbiological tissue samples were stored in normal saline and cultured in appropriate media (Nutrient Agar, Potato Dextrose Agar, EMB Agar, MacConkey Agar, or Baird Parker) on the same day of sampling for aerobic and anaerobic bacteria and fungi. A Vitek2 Automated Microbiology System was used for the cultures to identify the organisms via the phenotypic identification methods.

Subjective evaluation

The color, tissue pliability, ease of dissection, ease of separating tissue planes, definition of nerves, and vasculature and flexibility of the joints of the cadaver were observed and determined by the students and instructors during each dissection exercise. A checklist of the parameters was provided, and the observations were audio recorded during dissection to be documented at a later stage.

Muscle colorimetry and texture analysis

Three colorimetric readings of the L, a, and b values of each sample were obtained using the HunterLab Colorimeter (Figure 5) (Hunter Associates Laboratory, Inc. Virginia, USA). The samples were measured for hardness, adhesiveness, and deformation using the TexturePro CT V1.3 Build 15 texture analyzer (Figure 6) (Brookfield Engineering Labs, Inc., Middleborough, USA). The parameter settings were as follows: target value, 30%; target load, 6.8 g; and test speed, 1.00 mm/s. A cylindrical probe having a diameter of 12.7 mm and a length of 13 mm was used. Three samples were tested at each time interval. Because the samples could be used only once, nine samples from the same muscle were assessed for texture at three different time intervals (weeks 1, 10, and 20).

The results obtained from the colorimetric and texture analyses were analyzed using SPSS 25 (IBM/SPSS, Armonk, New York, United States). The data were tested for significant changes in the colorimetric and texture parameters using the repeated-measurement analyses of variance. Multiple readings from three samples of the same carcass at a given period of time formed a group. ANOVA was calculated among samples taken at 1, 10 and 20 weeks for each carcass. Only those measurements with a p-value of <0.001 were considered as significant.

RESULTS

During dissection, the instructors and students observed that the appearance and feel of the cadavers embalmed for 3–6 months closely resembled those of a freshly dead animal. On dissection, the skin did not adhere to the underlying fascia and was easy to separate in all the animals. In the cat and goat cadavers, the subcutaneous fat was yellower than that in the dogs and sheep. Furthermore, the cutaneous muscles were easily separated from the underlying deeper muscles during dissection. All muscles were soft, pliable, moist, shiny, and easy to dissect. They were pale red, soft, and pliable in the sheep, goats, and cats, but darker and a little firmer in the dogs. The joints were flexible and easy to manipulate, and the tendons and fasciae were readily identifiable and. Likewise, the accompanying blood vessels were flexible and. The nerves supplying the muscles were easy to identify, both visually and haptically. The thoracic organs were well-preserved; the lungs were soft and spongy in texture and appeared a shade darker than those observed in fresh carcasses.

Visually, all the abdominal organs in the fixed cadavers closely resembled those of a freshly dead animal (Figure 1). However, over time, the organs became pale with the ongoing immersion in the preservation

fluid and exposure to air during dissection. Structures associated with the intestine, including the blood vessels, lymph nodes, and mesenteries, were well-preserved and structurally distinct. The small intestine was easy to distinguish from the connective tissues and blood vessels. The large intestine became softer and considerably friable toward the end of the anatomy course. Repeated observation after 4 months showed that the small intestinal segments were preserved better than the large intestinal segments, both in color and texture. The bulk of the liver lobes were firm but their periphery was soft and supple.

In goats and sheep, the thoracic/abdominal organs had fragile adhesions to the pleura, pericardium, and peritoneum. There were some salt depositions on the internal organs, especially on surfaces that had adhered to the other organs or peritoneum. These deposits were limited to the adherent areas at the apex of the heart between the diaphragm and the liver and parts of the stomach that were in contact with the diaphragm. The adherent surfaces appeared to be rough.

Despite negligible differences in the subjective assessments of the muscle parameters, objective measurements using texture analysis showed a gradual increase in hardness and adhesiveness of the muscle samples with time (Table 1).

Colorimetric analysis of the muscle samples showed a slight but statistically significant change in L value across all species (Table 2 and Figure 2). The L value increased in Goat and Dog carcass, while it decreased in the other Dog and Cat samples over the period of 6 months. The goat cadaver samples presented with a significant shift from the green end of the spectrum to the red, indicating excellent preservation. However, with time, the dog muscle samples showed a

steady decline in redness (Table 2 and Figure 3). The icteric tinge observed in the cadaver fascia was measured using the colorimetric parameter “b.” All specimens, except one, showed a significant increase in the intensity of yellow color of the muscles (Table 2 and Figure 4).

The embalmed cadavers were sterile with no microbiological growth in tissue samples from the superficial muscles, deep muscles, lungs, liver, colon, and small intestine in all specimens, except for the colon tissue from one cat (*Enterococcus faecalis*) and lung tissue from one sheep (*Sphingomonas paucimobilis*).

DISCUSSION

The embalming formulation used in this study demonstrated excellent tissue preserving properties. The carcasses that were immersed in the embalming fluid throughout their preservation had a long shelf life. The texture profiles of the cadavers in terms of hardness, adhesiveness, and deformation did not show any extensive putrefactive change. The students and faculty were satisfied with the odor, color, and feel of the embalmed cadavers during dissection.

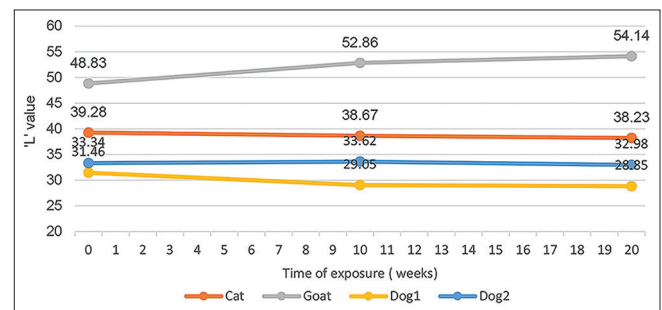


Fig 2. Changes in the darkness of muscle tissue immersed in embalming solution over a period of 20 weeks.

The darkness of the tissue was measured using the “L” parameter of the HunterLab Colorimeter. Lower values of “L” denote increased darkness. The changes were statistically significant in all the species.

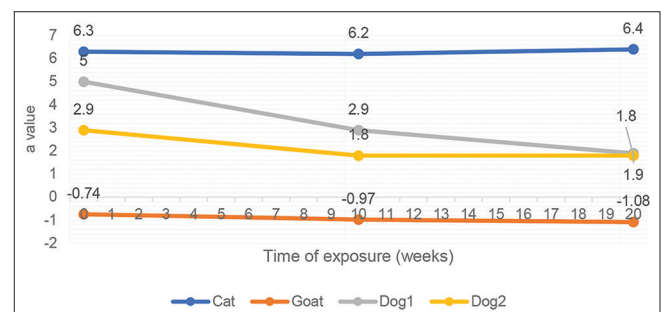


Fig 3. Changes in the redness of muscle tissues immersed in embalming solution over a period of 20 weeks.

The redness of the tissue was measured using the “a” parameter of HunterLab Colorimeter. A higher value of “a” denotes increased redness. The changes were statistically significant in all species, except the cat.

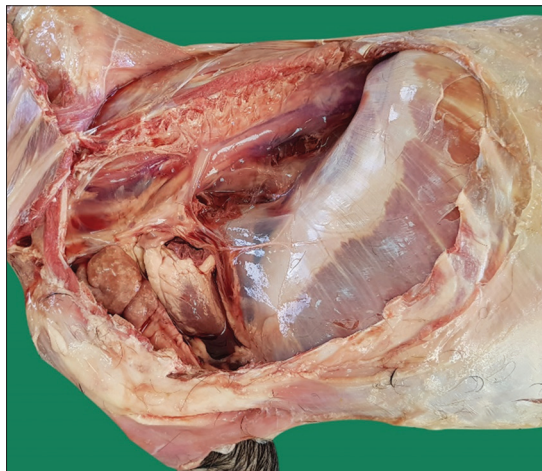


Fig 1. Thorax organs submerged in embalming solution showing well-preserved color of goat carcass after 6 weeks of embalmin.

Table 1: Muscle texture analysis of cat, goat, and dog carcasses showed a gradual change in parameters with time

Parameter	Texture Analysis											
	Cat 1			Goat 1			Dog 1			Dog 2		
	1 week	10 week	20 week	1 week	10 week	20 week	1 week	10 week	20 week	1 week	10 week	20 week
Hardness (g)	154.6	569.3	750.6	505.5	688.1	694.8	691.5	777.5	934.3	201*	905.1*	952.8*
Recoverable work (mJ)	0.4	5.44	2.43	0.95	0.68	0.36	5.49	11.17	7.27	0.68*	2.45*	2.97*
Recoverable deformation (mm)	1.29	1.6	1.44	1.14*	1.17*	1.73*	1.96	2.14	2.14	1.35	1.35	1.52
Adhesive force (mJ)	3.83	3.67	4.5	4.33	4	3.33	3.33	3.5	3.5	3.83	3.17	3.83
Resilience	0.27	0.26	0.24	0.25	0.25	0.25	0.33	0.36	0.32	0.27	0.29	0.27

Each value represents the mean of three readings from three sample pieces of the same muscle of the animal carcass. These measures were repeated from the same muscle specimen, at week 10 and week 20. *statistically significant change in parameters with a p value of <0.01

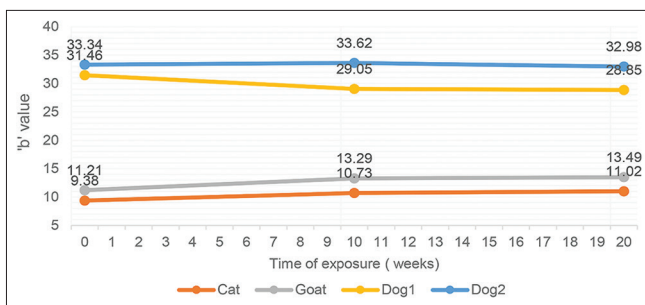


Fig 4. Changes in the intensity of the yellow color of the muscle tissue immersed in embalming solution over a period of 20 weeks. The yellowness of the tissue was measured using the “b” parameter in the HunterLab Colorimeter. Higher values of “b” denote an increase in the intensity of yellow color. The changes were statistically significant in all species.

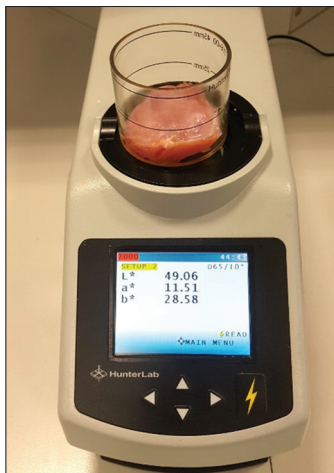


Fig 5. Colorimetric assessment of muscle samples. HunterLab Colorimeter (Hunter Associates Laboratory, Inc. Virginia, USA) used to measure L, a, b values

The colorimetric assay showed a significant change in the redness of the muscle tissues. Oxymyoglobin, deoxymyoglobin, and metmyoglobin in the muscles contribute to the red, purple, and brown colors, respectively, in a fresh carcass (Li et al., 2011; Warner et al., 2017). This



Fig 6. Texture analyzer for texture analysis of muscle samples. TexturePro CT V1.3 Build 15 texture analyzer (Brookfield Engineering Labs, Inc., Middleborough, USA) used to measure hardness, recoverable work, recoverable deformation, adhesive force and resilience.

postmortem oxidation and other putrefaction-related biochemical changes influence the gradual change in the color of the muscle from red to pale brown. Ideally, the fixative should minimize the oxidative browning of the muscles and other tissues. These changes are hastened by atmospheric exposure during dissection (Coleman and Kogan, 1998). The embalming fluid, in the current study, was effective in delaying the color change in the muscles and internal organs. This property was remarkably attributed to sodium nitrite in the embalming solution. Sodium nitrite is a popular food preservative used for canning and curing meat. It works as a mild anti-oxidant; it exerts its preservative effect by inhibiting lipid oxidation and improving the color of the meat by combining with myoglobin (Coleman and Kogan, 1998). Thus, these effects of color preservation and anti-oxidation are expected in an embalming fluid containing sodium nitrite. However, chemicals such as nitrite salts and glyoxals increase the icteric tint in all organs, especially the adipose tissue (Jaung

Table 2: Colorimetric analysis of cat, goat, and dog carcasses showed a gradual shift in the L, a, and b parameters with time

Parameter	Colorimetric Assay											
	Cat 1				Goat 1				Dog 1			
	1 week	10 week	20 week	1 week	10 week	20 week	1 week	10 week	1 week	10 week	20 week	1 week
L	39.28 ± 0.28	38.67 ± 0.20	38.23 ± 0.05	48.83 ± 0.56	52.87 ± 0.61	54.14 ± 0.72	31.46 ± 0.39	29.05 ± 0.24	28.85 ± 0.49	33.34 ± 0.20	33.62 ± 0.06	32.98 ± 0.22
a	6.31 ± 0.11*	6.30 ± 0.03*	6.32 ± 0.076*	-0.73 ± 0.11	-0.98 ± 0.025	-1.08 ± 0.05	5.04 ± 0.26	2.88 ± 0.12	1.91 ± 0.04	2.93 ± 0.02	1.85 ± 0.11	1.87 ± 0.05
b	9.38 ± 0.07	10.73 ± 0.12	11.02 ± 0.19	11.21 ± 0.08	13.29 ± 0.24	13.49 ± 0.14	4.96 ± 0.05	4.96 ± 0.072	5.66 ± 0.15	-2.45 ± 0.08	0.77 ± 0.16	1.56 ± 0.20

The three readings were taken at intervals of 10 weeks. The values showed a statistically significant difference and change in parameters with a P value of <0.01 in all species, except the ones denoted by an asterisk. "L" measures darkness, "a" measures redness and "b" measures yellowness of the sample. Lower values of "L" denote increased darkness. A higher value of "a" denotes increased redness. Higher values of "b" denote an increase in the intensity of yellow color.

et al., 2011; Brenner, 2014). Hence, the increase in the intensity of the yellow color in the samples of this study may be attributed to the nitrite salts in the formulation.

Turan (2017) employed the same method to evaluate color changes in goat cadavers embalmed with liquid foam soap, ethanol, and citric acid and reported an identical increase in intensity of the yellow color along with better preservation of the redness. However, a more marked increase in L values was noted in their study than that noted in our study, although the duration of preservation was same in both the studies. This shows that the SEFS solution (Soap and ethanol based fixative solution) resulted in a more darkened carcass than that with the formulation used in this study (Turan et al., 2017).

Postmortem changes in texture usually accompany color changes. Although the changes in texture were not observed by students and instructors, the texture analysis showed otherwise. Hardness is the optimal indicator of dehydration during embalming. In the present study, no statistically significant increase in hardness was noted in all the samples, except in one dog sample. This could be attributed to the lack of power of the testing method in detecting any change for a short duration of 6 months. Similar results were observed in the study by Turan (2017) when the hardness of goat carcass was tested using a texture analyzer; a significant change was detected only at the end of 12 months.

The reason for the increase in hardness can be attributed to the dehydrating embalming ingredients such as ethanol. Ethanol has a hygroscopic effect and shrivels up all the soft tissue it comes in contact with (Stefan et al., 2010). This loss of water alters the mechanical properties of the soft tissues and bones and simultaneously causes shrinkage of all the tissues (Stefan et al., 2010; Joy et al., 2015; Rocha Ferreira et al., 2017). Polyethylene glycol, a polymer of ethylene oxide that is widely used as an osmotic laxative, counters this desiccating effect. The ability of polyethylene glycol to delay desiccation was exploited by Janczyk in his pilot study (Janczyk et al., 2011). The formulation used in the present study consisted of ethanol as the sole desiccant, the effect of which was easily annulled by polyethylene glycol.

The inclusion of ethanol and chloroxylenol, both known to have antibacterial and antimycotic effects, was successful in preserving the carcasses with credible sterility. On continued preservation for 4 months, no positive bacterial growth was observed, except in a sample from cat colon (*E. faecalis*) and a sample from sheep lung (*S. paucimobilis*). Chloroxylenol is known to be non-toxic without any chronic effects (Benckiser, 2019). However, it might cause mild irritation to the eyes and rarely to the skin, thus

making protective wear indispensable for students during dissection.

Enterococcus faecalis is the most prevalent enterococcus species in the normal colonic microflora of cats. Decomposition in the digestive tract is rapid and is due, in part, to its pre-existing microbial population (Rocha Ferreira et al., 2017). This is the most likely explanation for bacterial isolation in the gut samples. Increased resistance to antibiotics and antimicrobial agents in the gut microflora is an alarming trend in humans and animals (Bengtsson-Palme et al., 2015; Casals-Pascual et al., 2018). This heightened ability of the gut microflora to thrive in bactericidal solutions should be considered when assessing the germicidal potential of the current embalming solution. The ability of enterococci to survive in a similar formulation with nitrite pickling salt has been previously reported (Janczyk et al., 2011).

Isolates of the aerobe *S. paucimobilis* from a sheep lung sample in this study suggest putrefactive microbial contamination. This aerobic bacteria is commonly found in soil and water, but it rarely infects humans (Maragakis et al., 2009; Ryan and Adley, 2010).

Some adhesions were observed between organs and the adjacent coelomic wall, particularly in the goats and sheep of this study. In addition, small salt deposits were detected on the internal organs, especially on surfaces that adhered to the other organs or peritoneum. Because these deposits appeared to be limited to the adhesions, it most probably indicated incomplete perfusion of the embalming fluid on these surfaces. Similar observations using an embalming solution containing nitrite salts were reported by Janczyk (2011).

CONCLUSIONS

In this study, we demonstrated the use of a safe embalming formulation with impressive preserving properties. Additionally, we attempted to develop a method that can be repeatedly used to objectively evaluate the safety and preserving qualities of embalming fluids using colorimetry and texture analysis.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' contributions

Preetha Menon: methodology, laboratory analysis, writing – review and editing; Adnan Aldarwich and Layaly Hamdan: laboratory analysis and data collection; Maha Hammoud: chemical analysis, toxicological analysis and consultation; Ahmad Al Aiyan: conceptualization, supervision, methodology, writing – review and editing.

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