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RESEARCH ARTICLE

The mixture of liquid foam soap, ethanol and citric acid as a new fixative–preservative solution in veterinary anatomy

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ABSTRACT

The present study investigates the efficiency of liquid foam soap, ethanol, citric acid and benzalkonium chloride as a fixative–preservative solution (a soap-and ethanol-based fixing solution, or SEFS). In this study, ethanol serves as the fixative and preservative, liquid foam soap as the modifying agent, citric acid as the antioxidant and benzalkonium chloride as the disinfectant. The goat cadavers perfused with SEFS (n = 8) were evaluated over a period of one year with respect to hardness, colour and odour using objective methods. Colour and hardness were compared between one fresh cadaver and the SEFS-embalmed cadavers. Histological and microbiological examinations were also performed in tissue samples. Additionally, the cadavers were subjectively evaluated after dissection and palpation. The SEFS provided the effectiveness expected over a 1-year embalming period for the animal cadavers. No bacteria or fungi were isolated except for some non-pathogenic *Bacillus* species. Visible mould was not present on either cadavers or in the surrounding environment. The cadavers maintained an appearance close to their original anatomical appearance, with muscles having good hardness and elasticity for dissection.

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1. Introduction

The discovery of formaldehyde by the German chemist August Wilhelm von Hofmann in 1869 was a milestone to embalming. Formaldehyde (used to embalm cadavers to the present day) became a common fixing agent thanks to several desirable characteristics, including its impact on the denaturation reactions of proteins as well as its antioxidant activity and ability to prevent microbial growth (Brenner, 2014; Balta et al., 2015). However, formaldehyde is associated with several health risks. A registered carcinogen (NTP, 2010), formaldehyde has irritant effects on the airways, skin and eyes. It has also potential adverse effects on foetal development (Saillenfait et al., 1989; IARC, 2006). Precautions such as the use of gas masks, reduction of formaldehyde concentrations in fixing solutions, rinsing off of cadavers in water before handling (Janczyk et al., 2011) and proper improvements in laboratory ventilation (Hammer et al., 2012) have been recommended to minimize the harmful effects of formaldehyde. Nevertheless, evaporation of formaldehyde from formalin-preserved cadavers has been suggested to be carcinogenic even with the use of personal protective equipment in the gross anatomy laboratory (Balta et al., 2015).

With the recognition of these harmful effects has come the awareness of the need for new embalming alternatives in both veterinary and human medicine. Recently, the effectiveness of pickling salt supplemented with antioxidants (Friker et al., 2007) and nitrite pickling salt with ethanol and glycols (Janczyk et al., 2011) was reviewed, with both items reported to be sufficient fixatives and preservatives. The European Association of Establishments for Veterinary Education (EAEVE) recommended a method devised by Friker et al. (2007) as a non-toxic, easy-to-handle alternative fixation method to preserve macroscopic anatomic specimens (EAEVE, 2010). However, Friker's method has not proven useful in the preservation of the whole cadaver, in spite of it being good for conserving limbs (Janczyk et al., 2011). With another method proposed by Janczyk, the internal organs have been of distorted consistency and colour when the cadaver is conserved with a closed abdominal cavity (Janczyk et al., 2011).

Embalming fluids used in preservation of gross anatomy are expected to provide good long-term structural preservation of organs and tissues, proper tissue stiffness for dissection and preservation of the colours of tissues and organs (Coleman and Kogan 1998; Brenner 2014; Balta et al., 2015). These fluids should also

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Table 1
The mixing ratios of the chemicals used to prepare the SEFS.

Name	Ratio
Liquid foam soap (Perle Foam-Ready®, İduna)[a]	55 (L)
Ethanol (96%, Teksol®, Tekkim)	35 (L)
Citric acid (citric acid®, Jungbunzlauer)	10 (kg)

[a] The chemical components of the soap; Sodium Laureth Sulfate <5%, Cocamidopropyl Betain <5%, Coco Glucoside <5%, Cocamide DEA (and) Glycerin <5%, preservatives, perfumes <5% (Safety data sheet for Perle Foam-Ready- According to Regulation (EC) No 1272/2008).

inhibit desiccation, fungal and bacterial growth. New chemicals are needed for use as optimal fixatives that have these characteristics. The present study aimed to develop an alternative fixative solution with liquid foam soap, ethanol, citric acid and benzalkonium chloride that allows easy dissection, is non-irritating to the skin, eyes and airways, and facilitates the long-term storage of cadavers.

2. Materials and methods

2.1. Animals and fixation

This study was conducted in the Anatomy Laboratory of the Faculty of Veterinary Medicine with the approval of the Animal Ethics Committee of Adnan Menderes University. For this investigation, 9 healthy male Saanen goats (7 months old; body weight range, 22–28 kg) were used. The animals were obtained from the goat breeding unit of the Faculty of Agriculture at University of Adnan Menderes. General anaesthesia was induced with the intramuscular administration of xylazine (0.1 mg/kg; Alfazyne®; Ege Vet) and ketamine (22 mg/kg; Alfamine®; Ege Vet). The animals' entire bodies were shaved using an electric shaver (Favorita II®, Aesculap). Under general anaesthesia, the animals exsanguinated through a cannula inserted in the right common carotid artery; next, about 300-mL diluted (1%) antiseptic (10% benzalkonium chloride, Zefirolum®, Kimpa) was perfused via the same vessel in 8 goats; the ninth goat was not perfused for the purpose of creating a control cadaver for comparison purposes. Fifteen minutes after this process, the SEFS was perfused with a maximum pressure of 0.22–0.26 bar, over a 6-h period via a tank that was 2 m in height, with the influence of gravity being used to perfuse the compound. The animals were perfused simultaneously by using eight delivery tubes controlled by valves. The used perfusion apparatus was adapted from Tompsett (1956). The amount of fixative solution perfused for eight cadavers was up to 35–40 L. The SEFS was also injected into both the abdominal and thoracic cavities of each goat cadaver, as much as 500 mL for each animal. The mixing ratios of the chemicals used to prepare the SEFS were shown in Table 1. The cadavers were washed with the same antiseptic (10% benzalkonium) after the fixing process, then kept and rotated in a cold chamber (+4 °C) over a 24-h period. Subsequently, they were transferred into tanks with a hinged lid containing the SEFS and maintained at the same temperature throughout the study. In one animal the fixative solution was not perfused so that the colour and hardness data for the cadavers could be compared between a fresh cadaver and the eight cadavers embalmed with SEFS. Five 1-cm^3 samples were collected from the liver and quadriceps muscle of the fresh cadaver, and colour and hardness measurements were immediately obtained. Additionally, tissue samples from the muscle, lung, liver, and small intestine were obtained from the fresh cadaver and fixed after 24 h with 10% neutral buffered formalin (NBF) for the purposes of comparison with the SEFS-embalmed cadavers. The remaining fresh cadavers were immersed in the SEFS separately and stored at +4 °C.

Please add the following footnote under the table 1.[a]The chemical components of the soap; Sodium Laureth Sulfate <5%, Cocamidopropyl Betain <5%, Coco Glucoside <5%, Cocamide DEA (and) Glycerin <5%, preservatives, perfumes < 5% (Safety data sheet for Perle Foam-Ready- According to Regulation (EC) No 1272/2008).

2.2. The use of cadavers for dissection

The eight SEFS-embalmed cadavers were dissected by students and their lecturers according to an anatomy practice lesson program over a period of one year. Before each lesson, the tables were cleaned with the same antiseptic. SEFS was added into the tanks when it was determined that the amount had declined. The cadavers were not removed from the tanks except for during lessons and when tissue samples needed for objective tests were obtained from the cadavers as part of the lessons.

2.3. Collection and evaluation of data

The cadavers were evaluated throughout the study using objective methods with respect to histological and microbiological characteristics, hardness, colour and odour. Samples were taken from eight fixed cadavers for a total of four sampling stages separated by four-month intervals; that is, sampling stage 1 was at the beginning of the study, sampling stage 2 at month 4, stage 3 at month 8, and stage 4 at month 12, or the end of the study. Additionally, the cadavers were photographed and monitored by observation and palpation throughout the year of anatomical dissections.

Histological samples for analysis (quadriceps femoris muscle, liver, lung, jejunum) were collected from each fixed cadaver. These samples were kept separately throughout the study in the SEFS to avoid damaging the tissues. The same tissue samples were initially obtained from the fresh cadaver (that is, the control) after 24 h of being fixed with 10% NBF. For each sampling stage, these tissue samples were used for routine tissue processing and paraffin blocking (Presnell and Schreibman, 1997). The samples were taken at 6-μm thick serial transversal sections with a 50-μm interval from the prepared paraffin blocks. Two of these sections were stained using the Crossman triple staining method for histological analysis of muscle, liver, lung and jejunum (Bancraft and Stevans, 1990), and with the periodic acid–Schiff (PAS) staining method for the demonstration of glycogen in hepatocytes and goblet cells in the villus intestinalis (Cook, 1990). An image analysis system (Leica Q Win Standard®, Leica Microsystems Imaging Solutions Ltd., Cambridge, UK) connected with a light microscope (Leica DMLB®, Leica Microscopy Systems Ltd., Heerbrugg, Switzerland) was used for histological analysis.

The bacteriological and fungal examinations were performed at the microbiology department of the Bornova Veterinary Control and Research Institute, Izmir, Turkey according to standard procedures (Winn et al., 1997). The swab samples of the superficial muscle (surface of the muscle) and deep muscle (taken at least 1 cm below the surface) tissues were collected from the quadriceps femoris muscle of two different cadavers at every sampling stage and analysed for the presence of fungi as well as aerobic and anaerobic bacteria.

The analyses of quadriceps femoris muscle and liver hardness were performed using the CT3 Texture Analyzer® (Brookfield AMETEK, Inc., Massachusetts, USA) device according to the user manual. The settings of the device were: target value, 3 mm; test speed, 1 mm/s; tissue samples were prepared at a size of 1 cm^3 .

Colour measurements of the quadriceps femoris muscle and liver samples were performed using a Colorflex EZ® device (Hunter Associates Laboratory, Inc., Virginia, USA). Measurements were achieved in the referential CIE 1976 L*a*b*, where L* denotes lightness on a 0–100 scale from black to white; a* corresponds to the indication of red when its value is positive and of green when neg-

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