

The Investigation of Potential Preservative Effect of Boric Acid on Formalin Fixed Striated Muscle Tissues

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ABSTRACT

The formalin solution is the best fixative and preservative chemical for the cadavers. However, it has harmful and carcinogenic effects on human health. Furthermore, boron is an essential element for humans and it has no known harmful effects on human health. Boric acid has also antiseptic features. In this study, it was aimed to measure the preservative effects of boric acid on formalin fixed striated muscle tissues thus decreasing the formalin's exposure time in the laboratories. Firstly fresh bovine striated muscle tissues were fixed in the 10% formalin solution for a week and then divided and stored into 0.1%, 0.2%, 0.4%, 5%, 20% boric acid, 0.9% saline and 10% formalin solutions during seven months. After this period, tissues were again stored in the 10% formalin solutions for one month. Macro-anatomical and histopathological inspections were performed on the muscle tissues. Moreover, aerobic mesophilic bacteria, yeast, and mold proliferation were controlled in all solutions. It was observed that the high concentration of boric acid had corrosive effects on the striated muscle tissue whereas its low concentration did not have any protective effect against the microorganisms' proliferation and proteolysis. In conclusion, boric acid concentrations that using in this study were observed that may not appropriate as a preservative agent for the cadaver protection. The future studies using at different concentrations of boric acid solutions with other chemicals should be planned.

Keywords: Boric acid, Fixation solution, Formalin, Striated muscle tissue, Histopathology.

Formalin ile Tespit Edilmiş Çizgili Kas Dokuları Üzerine Borik Asit Muhtemel Koruyucu Etkisinin Araştırılması

ÖZ

Formalin, kavrılar için en iyi tespit solüsyonu ve koruyucu kimyasaldır. Bununla birlikte insan sağlığı üzerine zararlı ve kansinojenik etkilere sahiptir. Diğer yandan bor ise; insanlar için gerekli olan ve insan sağlığı üzerine zararlı etkileri olmadığı bilinen bir elementtir. Borik asit aynı zamanda antiseptik özelliklere sahiptir. Bu çalışmada formalin ile tespit edilmiş çizgili kas dokuları üzerine borik asit koruyucu etkilerini ölçerek laboratuvarlarda formaline maruz kalma süresinde azalma amaçlandı. İlk olarak taze sığır çizgili kas dokuları bir hafta boyunca %10'luk formalin solüsyonunda tespit edildi ve daha sonra parçalanarak sırasıyla borikasit % 0.1, % 0.2, % 0.4, % 5, % 20'lik solüsyonlarında, % 0.9'luk tuz çözeltisi ve % 10'luk formalin solüsyonlarında 7 ay boyunca muhafaza edildi. Bu sürenin sonunda, dokular % 10'luk formalin solüsyonlarında 1 ay boyunca tekrar muhafaza edildi. Kas dokularında makroanatomik ve histopatolojik incelemeler gerçekleştirildi. Ayrıca aerobik mezofilik bakteri, maya ve küf birikimi tüm solüsyonlarda kontrol edildi. Borik asit antiseptik dozlarının çizgili kas dokuları üzerinde tahriş edici etkiye sahip olduğu, buna karşın antiseptik olmayan dozlarının ise mikroorganizmaların üreme ve proteolitik etkilerine karşı herhangi bir koruyucu etkisinin olmadığı gözlemlendi. Sonuç olarak bu çalışmada kullanılan borik asit konsantrasyonlarının, kavrıların muhafazası için istenen sonuçları vermediği; bundan sonra yapılacak olan çalışmalarda diğer kimyasallar ile farklı konsantrasyonlarda borik asit solüsyonlarının test edilmesinin uygun olacağı sonucuna varıldı.

Anahtar Kelimeler: Borik asit, Tespit solüsyonu, Formalin, Çizgili kas dokusu, Histopatoloji.

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INTRODUCTION

Fixation and preservation process is a crucial point in biomedical sciences (Onyije and Avwioro 2012, Thavarajah et al. 2012, Brenner 2014). The fixatives are allowed the scientists to make histopathological evaluations on the biopsy tissue and preservation of the specimens or cadavers for the lessons (Thavarajah et al. 2012, Brenner 2014). There are four major groups of fixatives and formaldehyde is placed under one of these groups (Thavarajah et al. 2012, Brenner 2014). Formaldehyde has a wide usage all over the world because of its high efficiency and accuracy (Thavarajah et al. 2012, Brenner 2014). Moreover, the formalin fixed tissues have a long lasting time (Thavarajah et al. 2012, Brenner 2014).

Unfortunately, formaldehyde has been classified as a carcinogenic chemical recently (Duong et al. 2011, Onyije and Avwioro 2012, Brenner 2014). Humans are exposed to this chemical from indoor inhalation (Duong et al. 2011, Onyije and Avwioro 2012, Nielsen et al. 2013). Students, technicians and scientists in the anatomy and pathology laboratories are exposed to this threat (Nielsen et al. 2013). Formaldehyde has harmful effects especially on developmental, reproductive, and respiratory systems (Nielsen et al. 2013). Meanwhile, the researches for the safe and healthy fixative solutions have already been continued (Thiel 1992, Turan et al. 2017).

However, boron is a safe and essential element for humans (Penland 1994, Kabu and Akosman 2013). Except boron mines, it already exists in fruits and vegetables in the nature. People consume this element daily with diet (Kabu and Akosman 2013). It is beneficial for human body and plays role on many metabolic activities (Penland 1994, Kabu and Akosman 2013). From this point of view, in the present study, it was aimed to measure the preservative effects of boric acid on formalin fixed muscle tissues thus decreasing the formalin's exposure time in the laboratories. Thus, it was tried to restrict the formalin's indoor inhalation.

MATERIAL AND METHODS

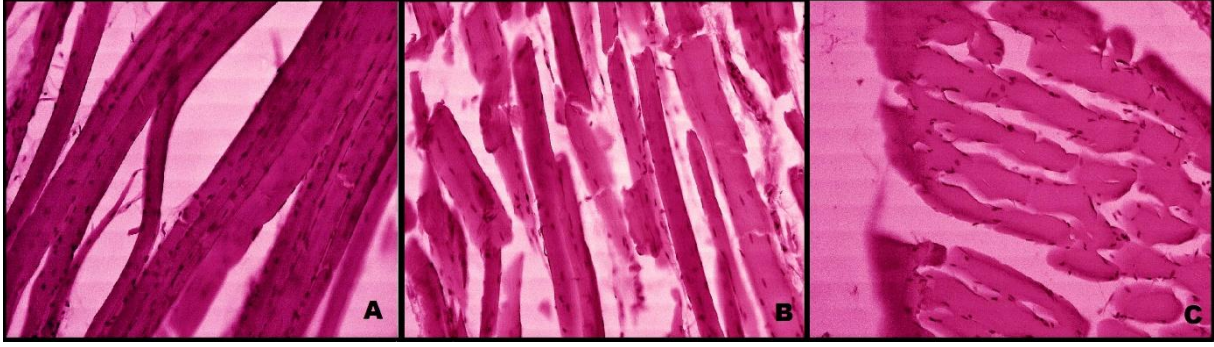
Six pieces of fresh bovine striated muscle tissues were sliced into 1cm³ and fixed with 10% formalin for one week. Then, all muscle tissues were divided and stored into 0.1%, 0.2%, 0.4%, 5%, 20% boric acid (Eti Mine Industrial Company General Management Kırka/Turkey (Na₂B₄O₇·10H₂O)), 0.9% saline and 10%

formalin solutions for seven months at the room temperature. All solutions were prepared by using distilled water in the laboratory. For microbiological analysis, dilutions of samples were used for inoculation by using spread plate technique in order to determine the total number of mesophilic aerobic bacteria, yeast and mold (ISO 2003). Mesophilic aerobic bacterial counts were performed using a standard plate count agar (Merck 1.05463, Darmstadt, Germany), which were incubated at 35°C for 2 days (Halkman 2005). After counting, results were expressed as CFU/mL (colony-forming units per milliliter).

Yeasts and molds were enumerated in potato dextrose agar (Merck 1.10130) (pH 3.5) with 10% added tartaric acid (Koburger and Marth 1984). Incubation was performed at 25 °C for 5 days. After counting, results were expressed as CFU/mL (colony-forming units per milliliter). Also the samples from all solutions were cultured to count for the proliferation of the total aerobic mesophilic bacteria, yeast, and molds at the end of the seven months (Dokuzlu 2004). Because the tissue samples stored in 0.1%, 0.4%, 5%, and 20% boric acid were not appropriate for tissue processing, only the samples in 0.2% boric acid, 0.9% saline and 10% formalin were used. These samples were again stored into 10% formalin solution for one month, embedded into paraffin, sectioned at 5µm thickness, and stained with haematoxylin-eosin (H&E). Finally, sections were blindly analyzed by a pathologist under the light microscope (Olympus BX51 and DP20 attached Microscopic Digital Image Analyze System, Tokyo, Japan).

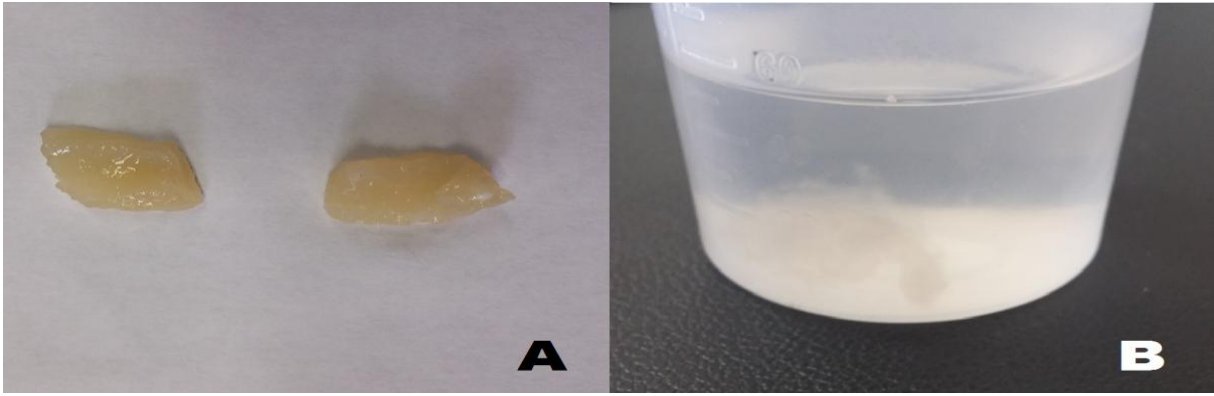
RESULTS

At the end of the seven months, corrosion with discolouring in 5% and 20% boric acid solutions and advanced melting in the muscle tissues in 0.1% and 0.4% boric acid solutions were observed. The tissues in the 0.2% boric acid, 10% formalin and 0.9% saline were protected their normal morphological texture. However, histopathologically against to the 10% formalin fixation (Figure 1-A), a cut-up appearance in the muscle fibres stored in 0.9% saline (Figure 1-B) solution was detected. In addition to this appearance, in 0.2% boric acid solution (Figure 1-C), slightly swollen muscle fibres were noticed.



Şekil 1. Kas hücrelerinin histopatolojik görünümü. Tüm şekiller H&E ile boyanmıştır. Orijinal büyütme oranı olarak 20x kullanılmıştır. Şekil 1A: %10'luk formalin solüsyonu. Şekil 1B: %0.9'luk tuz solüsyonu. Şekil 1C: %0.2'lik Borik asit solüsyonu.

Figure 1. The histopathological appearance of the muscle cells. All figures weres tained with H&E. The original magnification was x20. Figure 1A: 10% formalin solution. Figure 1B: 0.9% saline solution. Figure 1C: 0.2% Boric acid solution



Şekil 2. Borik asitin kas dokuları üzerine etkisi. Şekil 2A: Kas dokularındaki bozulma (%5'lik borik asit solüsyonu) Şekil 2B: Kas dokularının proteolitik enzimler tarafından parçalanması (%0.1'lik borik asit solüsyonu)

Figure 2. Effect on the muscle tissues of the boric acid. Figure 2A: Corrosion in the muscle tissues (5% Boric acid solution) Figure 2B: The muscle tissues digested by proteolytic enzymes (0.1% Boric acid solution)

DISCUSSION

Fixation of the specimens or cadavers with formalin is generally preferred at the anatomy and pathology laboratories. Cadaver preservation is also important for the anatomy lessons while biopsy and specimen collection are also important for the pathology laboratories (Duong et al. 2011, Thavarajah et al. 2012, Nielsen et al. 2013). Recently, the scientists have noticed the harmful effects of the formaldehyde and searched for new preservative solutions (Nielsen et al. 2013). Because of the harmful effects of the formalin, the formaldehyde's solitary usage does not appropriate for human health (Brenner 2014). The scientists try to combine the formalin

with other fixative fluids and decrease the formalin amount in the solution (Brenner 2014). Boron is released to the nature as boric acid (Turkez and Geyiklioglu 2010). Boric acid has been classified as a preserving agent previously (Turkez and Geyiklioglu 2010, Brenner 2014). It has antiseptic features like fungicidal and bactericidal, and, in the agricultural areas, it combined with the fertilizers (Turkez and Geyiklioglu 2010, Yilmaz 2012, Kabu and Akosman 2013). In one of the safe fixative solutions prepared by Thiel (Thiel's embalming solution), 3% boric acid was added as an antiseptic but a corrosive damage on the muscle proteins was observed (Benkhadra et al. 2011). The scientists were suspected that this

deformation of the muscle fibres may due to the corrosive effect of the boric acid (Benkhadra et al. 2011). Recently, it has been offered that the boric acid should be phased out from the embalming solutions (Brenner 2014).

The microbiological condition of fixation cadavers is the other major problem with the use of cadavers for pathological and anatomical training purposes. Fixation solution have to disinfect the cadaver to decrease any chances of infection (Davidson and Benjamin 2006, Trompette and Lemonnier, 2009). No doubt, cadavers and tissues could be infectious materials for both students and teachers when used for dissection (Shoja et al.,2013). Mould on the surface of the skin and organs is a common problem in cadavers embalmed with formaldehyde (Janczyk et al.,2011). Janczyk et al. (2011) reported that their nitrite pickling salt,

ethanol and glycols embalming fluid were not observed in muscle samples any fungal and yeast growth. In the current study, corrosive effect on the muscle proteins of the boric acid was observed in 20% and 5% solutions (Figure 2-A). Microorganisms (aerobic mesophilic bacteria, yeast, and mold) were proliferated and digested the muscle tissues by its proteolytic enzymes in the 0.4%, and 0.1% solutions (Figure 2-B). Moreover, no aerobic mesophilic bacteria proliferation was observed and muscles morphological structure was protected in the 0.2% boric acid solution. However, yeast and mold ratio were in spoiling limit in the 0.2% boric acid solution (Table 1) and that may cause the spoilage with histopathologically cut up and swallowing appearance in the muscle fibres (Figure 1-C).

Table 1. Aerobik mezofilik bakteri, maya ve küf proliferasyonları
Table 1. Aerobicmesophilicbacteria, yeast, andmoldproliferations

Sample	Total Aerobic Mesophil Bacteria Quantity (log CFU/ml)	Yeast and Mold Quantity (log CFU/ml)
0.1% BoricAcid	log<2	2.51
0.2% BoricAcid	log<2	3.59
0.4%Boric Acid	log<2	4.47
5% BoricAcid	log<2	log<2
20% BoricAcid	log<2	log<2
0.9%Saline	2.14	4.48
10% Formaldehyde	log<2	log<2

CONCLUSIONS

In conclusion, in this study, preservative feature of the boric acid on formalin fixed striated muscle cadavers was tested. It was observed that the high concentration of boric acid had corrosive effects on the striated muscle tissue whereas its low concentration did not have any protective effect against the microorganisms' proliferation and proteolysis. The future studies using at different concentrations of boric acid solutions with other chemicals should be planned.

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