International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

The Effect of Adding Linalol to Formalin on the Effectiveness of Embalming of Gastrocnemius Muscle

Setyo Trisnadi[a*], Eni Widayati[b], Dymas Rachma Kumala[c]

[a]*Department of Forensic Science Legal Medicine, Faculty of Medicine, Universitas Islam Sultan Agung, Jln. Raya Kaligawe KM. 4 Semarang, 50112*

[b]*Department of Chemistry, Faculty of Medicine, Universitas Islam Sultan Agung, Jln. Raya Kaligawe KM. 4 Semarang, 50112*

[c]*Student of Faculty of Medicine, Faculty of Medicine, Universitas Islam Sultan Agung, Jln. Raya Kaligawe KM. 4 Semarang, 50112*

[a]*Email: trisnadisetyo@gmail.com*

[b]*Email: eniwidayati@unissula.ac.id*

[c]*Email: kumalarachman@gmail.com*

Abstract

Formaldehyde has been a standardized embalming agent. However, it has a strong odor and potential adverse health effects. Linalool, a natural fragrance, has been shown to have an antibacterial activity. This study aimed to determine the effect of adding linalool to formaldehyde on the effectiveness of embalming rat *gastrocnemius* muscle. In this posttest only control group design, 25 Wistar rats were randomly divided into 5 groups. Forty eight hours postmortem, the first group injected with 10 ml formaldehyde was used as control, the remaining three groups were injected with 10 ml formaldehyde in combination with 2.5 ml, 5ml or 67.5 ml or 10 ml of linalool respectively. Forty eight hours post mortem, the histologic features of the gastrocnimeus muscle of rats was assessed. The data were tested for normality and homogeneity followed by Kruskal Wallis test. Mean number of post mortem autolytic cells of gastrocnimeus muscles for the four groups were 1.88±3.67, 10.58±11.70, 15.00±12.65, 16.31±27.31, 4.49±2.93 respectively. There was no significant difference ($p>0.05$). Adding linalool to formaldehyde has no effect on the effectiveness of embalming of rat gastrocnimeus muscle.

Keywords: Linalol; Formalin; Embalming; Gastronemius.

---

* Corresponding author.

1. Introduction

Embalming dead body has been intended for preservation and prevent it from decomposition [1]. Formalin has been commonly used for corpse preservation [2]. Despite its effectiveness, formalin has a very pungent odor that can cause irritation of the respiratory tract, nausea and eye mucosal inflammation. Adding deodorant has been proposed to reduce odor [3]. One of the deodorant chemicals, linalool, has the properties as a solvent that if mixed with formalin it will not change the structure and nature of formalin and is proposed to be an effective preservative agent [4]. Preservation of dead bodies is said to be successful when both the organ and tissue experience minimal shrinkages and damages [5]. There has been no studies on the effect of adding Linalol on formalin for embalming fluid. Formalin is a harmful organic compound and highly reactive and volatile at room temperature [6]. Although formalin does not damage tissue of corpses, it has the property of causing a pungent odor and the resulting vapor stimulating the mucous membranes in the nose, throat and burning sensation. In fact, long term inhalation of formalin will cause respiratory failure [7].

A research conducted by Turan and his colleagues [8] at the Faculty of Veterinary Medicine, Turkey's Adnan Menders University has successfully produced a new corpse preservative without stinging odor consisting of a mixture of ethanol, liquid soap and citric acid . The new preservative has been tested to preserve goats. Ethanol serves as a fixative agent and is used to replace formalin in histological tissues preservation. One component of liquid soap is glycerine which will work with ethanol as a fixative agent and prevent hardening of the corpse. Citric acid serves to prevent the growth of bacteria and fungi, so the three ingredients are used as a new preservative to replace formaldehyde. There has been no study on the effect of adding linalool on the effectiveness of embalming. This study aimed to investigate the effectiveness of embalming of male wistar rat because it has no significant different from human in term of physiology, metabolism and anatomy [9]. The body part of the Wistar rats to be observed in this study was the gastrocnionus muscle seen microscopically. Gastrocnimeus muscle is selected because in post-morterm (after death), the organ undergoes a rapid decareasing pH so leading to a faster rate of autolysis [10].

2. Methods

The research was designed as post-test only control group design. The independent variable in this study was the adding of linalool to formaldehyde, while the dependent variable was the effectiveness of gastrocnimeus muscle embalming. Linalool was added to formaldehyde to a mixture of various compositions, ie composition 1(a mixture of 10 ml of formaldehyde plus 2.5 ml linalool), a composition of 2 (mixture of 10 ml of formalin plus 5 ml linalool) , composition 3 (a mixture of 10 ml of formalin plus linalool 7.5 ml), composition (a mixture of 10 ml of formalin plus 10 ml linalool) then each injected hypodermally on the whole body of rats after terminated with cervical dislocation and confirmed to be dead. Fourth eight hours post-mortem, gastrocnimus muscles of the right foot of the rats were microscopically observed. The effectiveness of embalming was assessed by observing the histopathological feature of the right gastrocnemius muscle by counting the number of autolytic cells. Muscle cells undergoing autolysis were characterized by changes in the cell nucleus including karyolylysis ,pyknosis or karyorrhexis and a ruptured plasma membrane and a more pink cytoplasmic cell [11]. The population of the study was male wistar

rats bred at the Biology Laboratory of the Faculty of Medicine, Islamic University of Sultan Agung Semarang. While the research sample was in accordance to WHO criteria of at least 5 rats per group of a total of 25 male rats meeting the inclusion criteria. The inclusion criteria included male Wistar rats aged 2-3 months, weighing 200-250 grams, active, not disabled, normal appetite and there are no external injuries. The exclusion criterion was a unhealthy rat (inactive motion). The rat meeting these criteria were terminated with a cervical dislocation and confirmed to be dead and hypodermically injected mixture of formalin- linalool throughout the body mouse. Gastrocnionus muscle sample was taken 48 hours post-mortem.From the medial and lateral part. The upper limit of the organ cut was on the top of the medial condylus and the lateral condylus lateral and the lower limit of the cut was the calcanei tendo. The muscle was then merged into the formalin solution and was histopathological prepared for evaluation in laboratory. The number of necrotic muscle cells in gastrocnimeus divided by all muscle cells gastrocnimeus multiplied by 100%. The observation was in 5 visual fields using light microscope at 40 x 10 magnifications.The data were analyzed was using SPSS. Test the normality of data using was analyzed using Shapiro-wilk test and data variance test using Leuvene statistic test. Since the data distribution was not normal and data variance were not homogenous non parametric test of *Kruskal Wallis* was applied to determine whether the research results was significant or not. In Kruskal Wallis test obtained p value> 0. 05 meaning there wasno difference and tested with Mann-Whitney.

3. Result

Table 1

	GROUP				
	I	II	III	IV	V
Mean±SD(%)	1.88±3.67	10.58±11.70	15.00±12.65	16.31±27.31	4.49±2.93
Shapiro Wilks	0,001	0.108	0.287	0.005	0.573
Transformation	0,023	0.712	0.444	0.316	0.543
Levene Test	0,039				
Transformation	0,347				
Kruskal Walls	0,133				

Group IV showed the highest percentage of histopathologic damage of gastrocnemius muscle (16.31%), whereas group I showed the lowest percentage of histopathologic damage of gastrocnemius muscle (1.88%). The distribution of data on microscopic feature of gastrocnemius muscle in all five groups was normal (p> 0.05), except for group I and IV as indicated by the Shapiro wilk test (p <0.05). The data variance data analyzed by levene test was not homogeneous with p value = 0.039 (p> 0.05). The result of normality test on transformed data showed that group I remained abnormal (p <0.05), although the data variant was homogeneous (p> 0,05), so the difference of percentage of histopathologic damage of gastrocnemius muscle was tested by nonparametric significance with Kruskall wallis and resulted in p= 0.133, meaning that there was no difference in histopathology of gastrocnemius muscle among the five groups meaning that the addition of linalool to formalin had no effect on the effectivness of embalming of male wistar rats .

4. Discussion

This present study showed that adding linalool to formalin did not affect the effectiveness of embalming. This

finding supports the review by SIDS [4] that linalool is a solvent. Mixing linalool to formalin did not change formalin structure and properties and has been shown to be effective as a preservative agent. In Ancient Egypt linalool has been used as a mummification along with other essential oil herbs (known as mastic*)* such as verbenone , α-terpineol , and Pentacyclic triterpenes because it has antiseptic and antimicrobial activity [12]. Utilization of essential oils having antiseptic or antimicrobial activity in embalming can help to inhibit decomposition or decay [13]. Formalin reacts with albumin to dissolve into the cell and turns into albuminoids or gel along with the occurrence of bacterial destruction thus including delaying autolysis gastrocnemius muscle [2]. The amount of damage to the group given formalin was 1.88% while the group treated with mixed linalool and formalin in various doses varied 4.49% - 16.31% at 48 hours post-mortem. Percentage of gastrocnemius muscle cell damage between groups treated with formaldehyde was smaller than that of in the mixed group of formalin and linalool. Formalin has been standardized as an embalming agent. The descriptive analysis showed that the addition of linalool to formalin in the process of embalming increases gastrocnemius muscle damage but its effect was not statistically significant meaning that linalool does not affect the effectiveness of gastrocnemius muscle embalming [14]. The lowest number of cell damage was found in group 5 (449%) followed by group 2(10.58%) , group 3 ((15.00%) and group of 4 (16.31%). In group 5, added linalool added has the highest number had the lowest level of damage compared to other mixed groups. Brenner's research [13] states that linalool, antiseptic or antimicrobial essential oil in embalming can help inhibit decomposition or decay. The more linalool added, the growth of bacteria and fungi will also be much inhibited, but the damage occurring in the group of 4 (10 ml of formalin + 7.5 ml linalool) was higher than that in the group of 1 (10 ml formalin) linalool as an antibacterial agent. Further research is needed on antiseptic or antimicrobial activity of linalool. Linalool has the chemical formula of $C_{10}H_{18}O$ has a longer carbon chain than formalin (CH_2O), the difference in the length of this carbon chain that causes linalool is difficult to dissolve in formalin. Formalin is polar (hydrophilic) whereas linalool is non polar (lipophilic). Mixing between linalool and formalin using Tween 80 emulsifier was to increase the solubility of both. Tween 80 (polyoxyethylene 20 sorbitan monooleate) is a sorbitanpolyoxyethylene fatty acid ester used as a surfactant to increase the solubility of the substance [15]. Based on the mixing results, when the mixture is let to rest for a few moments there is two separate layers, so that the distribution of mixtures that may affect the gastrocnemius muscle is uneven and as a result inhibition of the autolysis process in the gastrocnemius muscle also occurs unevenly. According to Giroud *and his colleagues* [16] emulsion main problem is often not completely stable and after a while they will tend to progressively split into two phases [16]. Use of other emulsifiers such as propylene glycol may be further tested, as propylene glycol is an excellent emulsifier and can be used to prepare liquids.This study is the first study on the effect of adding linalool to formaldehyde in the embalming process. The results obtained can occur due to limited resources on how to mix between linalool and formalin. Another limitation of this study was the absence of negative control group treated with only linalool to determine the effect of linalool alone on histopathology of the post-mortem gastrocnemius muscle

5. Conclusion

There is no additional effect on the effectiveness of linalool in formaldehyde embalming gastrocnimeus muscle strain Wistar male rats. Microscopic feature of gastrocnimax muscle in male Wistar rats either given 10 ml

formalin after 48 hours post-mortem (after death) were lowest (1.88 ± 3.67%), whereas for microscopic images of gastrocnemius muscle in a mixture of 10 ml formalin and linalool 2, 5 ml of 10.58 ± 11.70%, on a mixture of 10 ml formalin and 5 ml of linalool at 15.00 ± 12.65%, on a mixture of 10 ml formalin and 7.5 ml of linalool of 16.31 ± 27.31%, and on the mixture of 10 ml of formalin 10 ml and linalool at 4.49 ± 2.93%. The most effective composition of the mixture as a preservative is the composition 4 (formalin 10 ml + linalool 10 ml) at 48 hours post-mortem via histopathology of the gastrocnionus muscle. Futhur studies need to include treatment group given linalool alone and use propylene glycol as an emulsifier to prepare the mixture of formalin and linalool.

6. Ethics Approval

This study has obtained permission (ethical clearance) from the Health Research Ethics Committee (IEC) Sultan Agung Islamic University, Faculty of Medicine with (Registration number 2151 / VII /Komisi Bioetik).

7. Conflicts of Interest: None

References

[1] Zulham. Histoteknik dasar. 1st ed. Medan: Departemen Histologi 2009; FK USU

[2] Edmund G, Brown JR. Information and instructions for embalmer licensure. 2011. [Accessed on 26 Januari 2016] Available from: http://www.cfb.ca.gov

[3] Atmadja DS. Tatacara dan pelayanan pemeriksaan serta pengawetan jenazah pada kematian wajar. Jakarta: Bagian Kedokteran Forensik dan Medikolegal FKUI / RSUPN Cipto Mangunkosumo. 2002. [diakses pada 26 Januari 2017] diunduh dari: http://www.tatacaraembalming.com.

[4] SIDS Initial Assessment Report, George Dr.. 11 February 2002; Switzerland

[5] Coleman et al. An improved low-formaldehyde embalming fluid to preserve cadavers for anatomy teaching. Journal of anatomy.1998 : 443-446

[6] Eells JT, Mc Martin KE, Black K, et al. Formaldehyde poisoning: Rapid metabolism to formic acid. JAMA 1981; 246: 1237-38

[7] Abdollahi M, Hosseini A. Encyclopedia of toxicology Ed 3. Formaldehyde. 2014 : 653–656

[8] Turan, et al. The mixture of liquid foam soap, ethanol and citric acid as a new fixative–preservative solution in veterinary anatomy. Jurnal Annals of Anatomy – Anatomischer Anzeiger 2016; 209: 11-17

[9] Srinivasan K, Ramarao P. Animal models in type 2 diabetes research : an overview. Indian J Med Res 2007; 125 : 451-472

[10] Murray, R., Granner, D., Rodwell, V., 2006, Harper's Illustrated Biochemistry, 27th Edition, The McGraw-Hill Companies, United States of America, 207.

[11] Kumar, V., Cotran, R.S., Robbins, S.L. Buku Ajar Patologi, Edisi 7, Volume 1, 2007; EGC, Jakarta, 189

[12] Maksoud, Gomma Abel and El-Amin, Abdel Rahman. A Review On Material Used During The Mummification Process In Ancien Egypt. Mediterranean Archaeology and Archaeometry 2011; 11(2) : 129-150

[13] Brenner, E. Human body preservation – old and new techniques. Journal of Anatomy 2014; 224(3) : 316–344. http://doi.org/10.1111/joa.12160

[14] Andrew J Connolly, Walter E. Finkbeiner, Philip C. Ursell, Richard L. Davis. Autopsy Pathology. Philadelphia : Elsevier. 2016: 26

[15] Rowe RC, Sheske PJ, Quinn ME. 2009. Handbook of Pharmaceutical Excipients. Lexi-Comp: American Pharmaceutical Association, Inc. p. 418, 685.

[16] Giroun C. Mariangela DC. Aurelie B., Vincent V., Nicolas CL., Bernard B. E-Cigarettes: A Review of New Trends in Cannabis Use. Int J Environ Res Public Health 2015;12 (8):9988-10008