

INCAPABILITY OF FORMALIN BASED FIXATIVE TO KILLED MAGGOT IN MUTILATED HUMAN CARRION

Oladele, A.A. ^{*1}, Sanya, J.O. ², Raji, M.O ³, Ekundina, V.O ³.

^{*1} Department Of Histopathology And Morbid Anatomy, Obafemi Awolowo University Teaching Hospital, Ile-Ife, Osun State, Nigeria.

² Department of Physiological Science, Afe Babalola University, Ado-Ekiti, Nigeria.

³ Department of Medical Laboratory Science, Afe Babalola University, Ado-Ekiti, Nigeria.

ABSTRACT

Background: Embalming is a process used to temporarily preserve a human cadaver to forestall decomposition and make it suitable for display at funerals; thus, are agents that prevent autolysis and putrefaction. The outbreak of maggots from a heap of inadequately embalmed bodies due to deep cuts and bodies involved in inferno, necessitated the need to re-investigate the efficacy of formalin based embalming fluid and its inability to kill maggots.

Methodology: Various strength of Formaldehyde, Xylene, Kerosene, and, Lime fluid, Isopropanol, Gamalin 20, Potassium ferrocyanide, and Physiological saline as control were used in the investigation. In the present investigation, Two maggots under the same atmospheric condition were put in each of the ten selected chemical reagents/solutions, including Lime, Kerosene, and the Gamalin 20 that are naturally available were initially dispensed into ten glass universal containers. Maggot movements in each reagent solution were critically observed.

Result: Maggots death occurred within the first ten minutes in test number three groups III that contains Concentrated Formalin and Xylene and Maggots died after fifteen minute of the experiment, but maggots did not die until about eight hours after the test in two of the experiment.

Discussion: Results of this investigation showed clearly that Maggots were not killed as soon as expected by the embalmer when ordinary ten percent alcoholic formalin embalming fluid is used. Equal volume of concentrated formalin plus Xylene was found out to be effective at killing maggot instantly.

Conclusion: It is therefore advisable to use Xylene plus Conc. Formalin when preserving cadaver infested with Maggot and this could at the same time prevent the occurrence of Maggots Infestation and better preservation of mass of burnt mutilated corpses in our Mortuary.

KEY WORDS: Embalming, Fixatives, Maggot, Human Carrion, Xylene.

Address for Correspondence: Dr. Oladele, A.A., Department Of Histopathology And Morbid Anatomy, Obafemi Awolowo University Teaching Hospital, Ile-Ife, Osun State, Nigeria. Call No.: +2348088176682 **E-Mail:** ladelero45@gmail.com

Access this Article online

Quick Response code



DOI: 10.16965/ijar.2015.117

Web site: International Journal of Anatomy and Research
ISSN 2321-4287
www.ijmhr.org/ijar.htm

Received: 10 Feb 2015 Accepted: 16 Mar 2015
Peer Review: 10 Feb 2015 Published (O):31 Mar 2015
Revised: 26 Feb 2015 Published (P):31 Mar 2015

INTRODUCTION

Maggot is a larva of the fly of order Diptera is particularly related to Brachyceran flies examples of which are cheese flies, green bottle flies, blow flies, and house flies. This is unlike

larva from nematocera for example such as mosquitoes, and Crane flies. Due to the facts that larva or maggot has no limbs is scientifically referred to as Grub [1].

Most flies are not very friendly to human because of their process of transmitting large number of communicable diseases, and production of filthy maggot by most of the flying insects. They are commonly found in filthy and dirty environment. Accordingly they are always signals that indicate the presence of dead decaying bodies around. This spans through insects, and animals. Almost all the gravid female adult flies belonging to Green bottle fly species, metaphorize from egg laying to Maggots (larvae) production. They almost appear as creamy-white structure and conico-cylindrically in shape. Essentially in average they are about 15 – 20mm length [2,3,4]. Maggots are usually seen on decomposing matter such as garbage and on dead multicellular organisms especially on dead birds, animals, and human organism, therefore serve as an essential component of the ecosystem [5].

Embalming is a way of temporarily disturbing the ecosystem by preserving a human cadaver to forestall decomposition and make it suitable for display at a funeral; and, Post Mortem, and Forensic study in crime investigation. This is a form of pre-treatment of corpses with special preservatives mainly chemicals in order to prevent decomposition. Perfume or fragrance, even eyelashes and false eyes are added to the bodies when it comes to funeral presentation. Thus, agents responsible for autolysis and putrefaction are almost halted. This eventually prevented maggot infestation, because noxious smelling debris produced by putrefying Bacteria and autolysis had been stopped. However, whenever the opposite happened then there will be maggot infestation as long as flies are not prevented from landing on the dead matter. Formaldehyde, Alcohol, Phenol, Glycerol and water are the major chemical agents used in most embalming technique today, except in the case of Thiel embalming technique which is based on 4-chloro 3- methylenephenol for fixation, boric acid for disinfecting and ethylene glycol for preservation of tissue plasticity [6]. Formalin is an organic compound with formulae CH_2O . It is a simplest form of aldehyde. It is widely used as it is always readily available [7, 8].

Formalin fixes tissues by forming a methyl cross

linkages with protein thereby replacing the normal colloidal fluid in the cells with a jelly-like rigid compound. The latter effect exhibits the coagulation properties of formaldehyde. Tissue and bacterium cells are made of protoplasm and as such, contain large amounts of moisture. The introduction of formaldehyde into the tissue coagulates proteins in the protoplasm living ambient water, the expected dryness that may follow initial embalming is normally arrested by injecting the corpse with additional two percent (2%) formalin and this process killed the cell. In terms of embalming practices, this is a perfect situation as the formaldehyde not only disinfects the tissue but replaces the tissue cell water with a rigid gel thus allowing the embalmed tissue to maintain its contour. Additionally, the "new" cell structure will resist further bacterial attacks as its composition now contains a formaldehyde-based compound with the tissue elements thereby denature the tissue. Formaldehyde is a potent disinfectant and anti-bacterial agent [9,10] and used for fumigation(s), less effective as a fungicide, insecticide, or larvicide [9].

Formalin fixes tissue very slowly when compared with alcohol and acetic acid but penetrate tissue very fast [11]. A tissue of 4mm in thickness will be well penetrated in less than one hour. This relates to the binding time necessary for formaldehyde to fix a tissue, a 90% threshold binding takes not less than 24 hours at 22C and 18hours at 37C respectively. The tissue does not die until it is fixed and the process is reversible if withdrawn before the cell death (s) [8]. The rate of penetration of formalin is said to be $d = k \times \text{square root of time}$, where d is the depth of penetration, k is the coefficient of diffusion which is between 2.0 – 3.6 for concentrated formalin, 0.78 for 10% formalin [12]. The first layer is penetrated at the rate of 90mm per hour. The 4mm tissue block would be fully penetrated in less than 1 hour [13].

Phenol also known as phenic acid prevents the growth of moulds in embalming solution [14].

Refrigeration can also be used as a means of preserving human remains after death. However it has been reported that refrigeration only retard the development of maggot from the larvae of flies but does not kill them [15,16,17].

Recently we found out that Maggot breakout from a mass of bodies that were burnt beyond recognition from a ghastly road Traffic Accident (RTA) that resulted into a huge inferno. The bodies were brought to the our Hospital Mortuary and were treated as usual by formalin based fixative hoping that the bodies will remain intact till some days prior their release to relatives to be taken away for burial in this case mass burial is usually done. But to our dismay we found out that the bodies have been infested with numerous number of flies maggots, after embalming them and put them in an enclosed room with air conditioner as practiced. This is what actually encourages us to carry out the present investigation. This idea now suggests that maggots were likely presented that they have capability to resist the formalin base fixation.

In view of the above curiosity, some chemicals carefully selected in our Laboratory and were put to test. One of the selected chemical mixtures was Physiological saline which was used as negative control and we found out that of some the chemical tested against living maggots were killed immediately they were put into certain mixture, while it took some days before maggots placed in some other chemicals were killed. Our observation shows that some of the mixtures were very effective at killing the Maggots while in some the chemicals are found out to be poorly effective at killing maggots placed in such mixture. The present investigation discovered some chemical mixtures that kill Maggots at a faster rate than others in the groups investigated.

MATERIALS AND METHODS

Formalin fixed and injected Mass of human carrion but still infested with numerous maggots was used in obtaining actively living maggots for the experiment. Glove hand was used to pick the maggots. This was transferred into the laboratory very close to the Morgue. In the laboratory four maggot each were put into each of the solution /mixture into each of the 10 universal bottles using a long blunt end forceps to picked maggot and dropped into the universal bottles.

Four maggots each of between (8-16mm in length) from the heap or pile of burnt human bodies after embalming were put into each of the following solutions: - Physiological saline, 10% formal saline, Concentrated formalin plus Xylene (Xylol), Concentrated formalin only, Xylol only, Gamalin 20, Lime plus concentrated formalin, Kerosene plus concentrated formalin, Saturated solution of Potassium ferrocyanide, and finally Concentrate Isopropanol. All mixture/ Solutions were dispensed in 30ml equal volume into glass universal bottles arranged on a laboratory working bench in a fully illuminated laboratory for easy and correct observation. The time it took for the maggots to die in each of the test and controls were carefully observed and recorded immediately (Dated 05-10-2014, 9:42am – 6:00pm).

RESULTS

The maggots in group III (Conc. Formalin + Xylene) died within the first 10minutes, but those maggots in groups II, (10% Formalin), VII (Lime + Conc. Formalin, and VIII (Kerosene) died at 15minutes interval ,however , maggots in group IV (Conc. Formalin) died in the next 20minutes intervals after the commencement of the experiment..

Maggots in Groups I (Physiological saline), V (Xylene + 10% Formalin), and VI (Gamalin 20), were deadly sluggish at 40 minutes interval, IX (Saturated Potassium Ferrocyanide) and X (Isopropanol) groups were sluggish at 40 minutes at the same time.

The maggot in groups I, V, and VI were still deadly sluggish even after 3hours post experiment, but finally died at 8hours interval post experiment. The maggot in groups XI and X though were sluggish at three hour, but finally died at 8th hour interval of the experiment.

In the investigation, it was observed that, out of the entire reagents used for the experiment, the maggot in group III (Concentrated Formalin + Xylene) died within the first 10minutes followed by group II. , VII, and group VIII Kerosene in which the Maggots in each test died after a period of fifteen minute of the experiment.

Activities of some chemicals on maggots in the histopathology laboratory.

Test/time	0-5 mins	5-10 mins	10-15 mins	15-20 mins	20-25 mins	25-30 mins	30-35 mins	35-40 mins
I Physiological saline	+++	+++	++	++	+	+	+	+
II 10% Formalin	+++	++	‡	‡	‡	‡	‡	‡
III Concentrated Formalin+ Xylol	+++	‡	‡	‡	‡	‡	‡	‡
IV Formalin only	+++	+++	+	‡	‡	‡	‡	‡
V Xylol only	+++	++	++	++	+	+	+	+
VI Gamalin 20	+++	+++	++	++	++	+	+	+
VII Lime juice+Conc. Formalin	+++	+++	‡	‡	‡	‡	‡	‡
VIII Kerosene+ Formalin	+++	++	‡	‡	‡	‡	‡	‡
IX Saturated Pot. Ferrocyanide	+++	+++	+++	+++	+++	+++	+++	++
X Isopropanol	+++	+++	+++	+++	+++	+++	+++	++

ACTIVE MOVEMENT +++ , SLUGGISH MOVEMENT ++ , DEADLY SLUGGISH MOVEMENT + , DEAD (ZERO MOVEMENT ‡

DISCUSSION AND CONCLUSION

Xylene, Kerosene, and formal- Lime were found to be very actively toxic to maggot, the four maggots each died within first 3- 5minutes. Xylene, kerosene, and Lime formol had not been used. The maggots were able to live longer in 10% formalin, equal volume of formalin and alcohol for 1hr30min and 1hr respectively. Kerosene and Xylene proved to be more lethal to maggots as both mixtures containing Formalin killed maggots within 5 minutes. The longer period that maggots stayed alive in formalin support the facts that formalin is less effective at destroying Maggots or Grubs of most fly larvae.^[9] Formalin only penetrates tissue very fast but fixes slowly [12]. And death only occurs when a tissue is fixed. Maggots used in this experiment were about 15mm in length and 4mm in width. This signified that formalin penetration would be achieved in less than 30 minutes, calculating from the formula $d = k \times \text{square root of time}$, $d = 4\text{mm}$ width of the maggot, constant k is taking to be 0.78. Fixation which is the formation of methyl cross linkage converting the tissue constituent to irreversible gel may takes a lot of time after penetration as 90% threshold binding may take 24 hours at 22°C and 18 hours at 37°C. This vindicates the ability of maggot to live for such a long time when immersed in a formalin solution.

We decided to use Xylene with equal volume of concentrated formalin is because of the facts that using Xylene alone may burn the tissues

rather than preserving the tissues, which could even burn a human skin if come in contact. It has never been used as parts or component of embalming fluid. to Its our thinking that xylene may act as a form of Fungicide and that inability of formalin at killing Maggot might be that Grubs tissue contain certain substance that prevent formaldehyde penetration . We hope that future researchers can a further study on this problem. The fastest death of maggot occurred in solution/mixture of formalin plus Xylene, Kerosene and lime respectively. In tandem with current investigation it is pertinent to use any of Xylene, Kerosene or Lime juice whenever outbreak of Grub infestation is suspected especially bodies brought into the Mortuary due road traffic accident.

The embalming fluid for human corpses with open wound should contain more phenol when total immersion is not possible.

Conflicts of Interests: None

REFERENCES

- [1]. Wikipedia. Maggot. Retrieved 5th June, 2013 from <http://en.m.wikipedia.org/wiki/maggot>.
- [2]. Major, R. Decomposition: Fly Life Cycle and Development Times. 2009; Retrieved from www.ausralianmuseum.net.au/Decomposition on 5th May, 2014.
- [3]. Anderson, G.S. Minimum and maximum development rates of some forensically important Calliphoridae (Diptera). *Journal of Forensic Sciences*. 2000; 45: 824-832.

- [4]. O'Flynn, M.A. (1983). The succession and rate of development of blowflies in carrion in southern Queensland and the application of these data to forensic entomology. *Journal of the Australian Entomological Society*. 1983; 22: 137-148.
- [5]. New World Encyclopedia. Maggot. 2008; Retrieved from www.newworldencyclopedia.org/maggot on 5th May, 2014.
- [6]. Ingrid K., Tom V.H., Piet P. and Katharina D. Endogent: Centre for Anatomy and Invasive Technique International journal of experimental and clinical anatomy. 2008; 2: 28 – 33. (thiel).
- [7]. Carson, F.L. *Histotechnology*. 2nd ed. Chicago: ASCP Press; 1997
- [8]. Helander, K.G. Formaldehyde binding in brain and kidney: A kinetic study of fixation. *Journal of Histotechnology*. 1999; 22: 317-318.
- [9]. Thomas, B. Waltzek, M.S. and Ronald, P. Hedrick. Koi Herpesvirus. 2004; Update 2004 Retrieved from <http://www.oocities.org/koifla/FishHealth.htm> on 5th May, 2014.
- [10]. Lischka, M.F., Wewalka, G., Stanek, G. and Kramar, E.B. Comparison of disinfectant activities of 3 different embalming fluid on cadavera for anatomical study. *Anat. Anz.* 1979; 146(3): 295 – 306.
- [11]. Ingrid E.C., Jeffrey T., Timothy, J. and Carol, B. Elevated pressure improve the rate of formalin penetration while preserving the tissue morphology. *Journal of cancer*. 2010; 1:178 – 183.
- [12]. Rolfs, G.O. Fixation and fixative (2) – factors influencing chemical fixation, Formaldehyde and Glutaraldehyde. Leica Biosystem. Wetzlar, Germany; 2012.
- [13]. Baker and Helander. Formalin Fixation Protocol. 1994; Retrieved 20th August, 2004 from <http://www.abrn.net/pdf/Formalin%20fixation%20protocol%20August04.pdf>
- [14]. Sehee, J. Green Burial: It's Only Natural, PERC Reports, Winter; 2007. Retrieved on 6th June, 2013
- [15]. Catts, Paul E, and Neal H. *Entomology and Death: A procedural guild*. South Carolina: Joyce print shop Inc.; 2005
- [16]. Hungtington, T.E., Higly, L.G. and Baxedale, F.P. Maggot development during morgue storage and its effect on estimating the post-mortem interval. *J. Forensic Sci.* 2007; 52(2): 453- 8.
- [17]. Thevan, K., Ahmad, A. H., Md. Rawi, C. S and Singh B. Growth of chrysomia macephala (Fabricius) maggot in a morgue cooler. *J. Forensic Science*. 2010; 55: 1656- 1658.
- [18]. Adams, Z.J.O. and Hall, M.J. Methods used for killing and preservation of blowfly larvae and effect on post mortem larvae length. *Forensic Science International*. 2003; 138, 50 – 61.
- [19]. Leong, A.S.Y. Fixation and fixatives. In Woods AE and Ellis RC eds. *Laboratory histopathology*. New York: Churchill Livingstone. 1994; 4.1-1 - 4.1-26.
- [20]. Medawar P.B. The rate of penetration of fixative. *J. Royal micros soc.* 1941; 61:46 -57.

How to cite this article:

Oladele, A.A., Sanya, J.O., Raji, M.O., Ekundina, V.O. INCAPABILITY OF FORMALIN BASED FIXATIVE TO KILLED MAGGOT IN MUTILATED HUMAN CARRION. *Int J Anat Res* 2015;3(1):922-926.
DOI: 10.16965/ijar.2015.117