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Original Research Article

Efficacy of Embalming Mixture Containing Formalin, Methanol and Water on Early Stage of Decomposition: A Validation Study

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ABSTRACT

Background: Stabbing with kitchen knife is a common method of homicide in Nigeria; and embalming of decomposing stabbed cadavers has been a challenge for morticians in Nigeria, because of the impairment of embalming fluid through the damaged arteries. The aim of this study is to investigate the effectiveness of an embalming mixture containing formalin, methanol and water on stabbed early decomposing carcasses using porcine analogues.

Methods: Two infant pigs were used for this study. Animals were sacrificed and allowed to reach the early stage of decomposition before embalming. Cervical arterial and hypodermic embalming techniques were employed. Post-embalming changes were observed and scored. A Pearson correlation analysis was used to determine the relationship between the outcome of embalmment and the embalming mixture.

Results: There was a statistically significant moderate positive correlation (r = .576, n = 42, p = .001) between outcome of embalming, and duration of embalming; a statistically significant strong negative correlation (r = -.623, n = 42, p = .001) with volume of embalming fluid; and a statistically significant moderate negative correlation (r = -.528, n = 42, p = .001) with room temperature. There was a statistically insignificant very weak positive correlation (r = .186, n = 42, p = .239) between outcome of embalming and humidity; and a statistically insignificant very weak negative correlation (r = -.141, n = 42, p = .375) with atmospheric temperature.

Conclusions: This study validated the embalming methodology and fluid suitable for arresting early decomposition process of stabbed infant carcasses in Nigeria. More so, an embalming mixture containing formalin, methanol and water in equal proportion is suitable for arresting early decomposition of stabbed cadavers.

KEYWORDS: Embalming of decomposing bodies, Embalming of stabbed bodies, Funeral services, Modern embalming science, Commercial embalming, Stab wounds, Specialist embalming.

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INTRODUCTION

Embalming is the art and science of preserving human remains by treating them in its modern form with chemicals in order to forestall decomposition [1]. Embalming keeps carcasses intact, and preserves it for a long

period of time. The primary goals of embalming are sanitization, presentation, preservation, and restoration [2]. Accident victims, stab wounds, dissected bodies or anything that mutilates the body structure have been reported to influence decomposition process [3-5]. Therefore, embalming decomposing bodies resulting from stab wounds require specialist attention [6]. A study on decomposing bodies resulting from stab wounds reported that an ideal embalming fluid used for slowing down or halting decomposition should contain formaldehyde, methanol, ammonium salt, thymol and water [7]. The two active preservatives in this mixture are formaldehyde and methanol [2]. Therefore, this study carried out a validity test to investigate the efficacy of embalming mixture containing formalin, methanol and water on an early stage of decomposition using infant porcine analogues.

Formalin is a solution of formaldehyde in water which is used chiefly for preservation of bodies or specimens. It preserves proteins and cellular organelles in a stepwise process [8]. Formalin is a very toxic chemical to bacteria especially in concentrations of 200mg/L and above [9]. It is also very toxic to humans and have been reported to cause irritation to the eyes and respiratory tract. Therefore, necessary precautions must be taken when using formalin for embalming [10].

Methanol may be used routinely for fixing tissues because it gives good results [11]. It is clear liquid with polar properties, making it a good solvent. It is also highly flammable, and highly toxic to humans if ingested [12]. It is easy to use, and has a flexible fixation period [11]. Methanol is more commonly used for fixation of exfoliative cytology smears and blood films [12]. Methanol has also been reported to be one of the chemicals used in embalming putrefying bodies in some of the funeral homes in Anambra state, Nigeria [7].

METHODS

Ethical approval: The ethical approval was obtained from the ethical committee, Faculty of Basic Medical Science, College of Health, Nnamdi Azikiwe University, Nnewi Campus.

Materials: The materials used for the study include 40% formalin, 10% methanol, water, embalming gravity tank, dissecting kit, surgical gloves, steel tape, digital thermo-hygrometer, Generic digital pH and chemical tester, digital thermometer, suturing needles, 10mL and 20mL syringes, boots, cotton wool, weighing scale, methylated spirit, nose-mask, kitchen knife and digital camera.

Location of the study: This study was carried out at the Gross Anatomy laboratory of the Department of Anatomy, Nnamdi Azikiwe University, Nnewi campus, Nigeria.

Hypothesis

Null Hypothesis (H_0): An embalming mixture containing formalin, methanol and water can halt the early decomposition process of stabbed pigs.

Alternate Hypothesis (H_1): An embalming mixture containing formalin, methanol and water cannot halt the early decomposition process of stabbed pigs.

Experimental animals: The experimental animals used for the study were two (male and female) infant domestic pigs (*Sus scrofa domestica*) because of the ethical issues related to the mode of death of the animals. The animals were procured from a pig farm located very close to the study location. Animals were in healthy condition.

Experimental procedure: The study adopted a single-case experimental study design. The concept used for the research procedure was formulated by the researchers. Data on the observable decomposition changes of the animals were collected by the researchers. The peri-mortem and post-mortem body temperatures of the pigs were documented.

Animals were sacrificed via multiple stab wounds on the trunk. Animal death was confirmed when no heart beat was recorded using stethoscope and observation of the pupillary reflex. The exact time of death was recorded. Early visible signs of decomposition (algor mortis, rigor mortis, pallor mortis, livor mortis) were monitored for eight hours. The choice of this timeline is because it is the usual timeline for reporting or delivering of victims of stab wounds to the funeral homes

in Anambra state. It is also the timeline for early decomposition as indicated by a study on decomposition timeline in this region [13]. After eight hours, the animals were embalmed via cervical arterial embalming and hypodermic embalming methods. Embalming activities were completed when all the body parts were completely fixed. Animals were re-embalmed fourth day and seventh day.

The atmospheric temperature, room temperature, and humidity were recorded from the time of death till the last day of the study. The post embalmment changes were monitored morning, afternoon and night for two weeks and subsequently monitored morning and night till the end of the study. The body structures that were completely fixed where scored whereas the body parts not fixed were not scored.

Method of data collection for daily climate readings: The thermo-hygrometer was placed on the slabs of the dissecting room and the wire extended outside the room via its window. The time was set on the equipment to GMT (+1) to ensure accuracy in documenting the readings. The lowest atmospheric temperature of the day was recorded between 3am and 7am; and the highest atmospheric temperature of the day recorded between 11am and 3pm. The lowest humidity of the day was recorded between 11am and 3pm; and the highest humidity of the day recorded between 3am and 7am.

Method of embalming: The methanol, water and formaldehyde were measured using a measuring cylinder each 1000ml. Water was first measured, and this was followed by the measurement of formalin and methanol. Methanol was measured last because of its high evaporation property. Single point method of arterial embalming technique was first used via the internal carotid artery (on the neck); and was followed by hypodermic embalming technique in order to ensure that the embalming fluid circulated to all the body parts affected by the stab wounds.

Scoring Method: The researchers developed a scoring method for the completely fixed parts of the carrions post-embalmment. The completely fixed parts were scored '1', whereas the unfixed parts were either scored '0' or not scored at all. The head and neck body structures that were scored are crown, two ears, two eyes, oral region, snout, dorsal aspect of the neck and ventral aspect of the neck. The body structures of the trunk that were scored are tail, umbilical region, thorax, dorsal aspect of the trunk and the anorectal region. The four limbs were scored individually.

Experimental control / precaution: Animals were procured from a farm close to the research facility in order to ensure that there was no change in body thermal condition. Animals were procured very early in the morning between 5am and 6am, and allowed to rest and acclimatize for a period of 1 hour before sacrifice.

The study avoided parallax error when reading the animal weight on the analogue weighing scale. The thermometer was cleaned with cotton wool and methylated spirit, and dried after every rectal reading to ensure accuracy in data collection.

The researchers avoided direct contact of the embalming chemicals to the skin and eyes. Protective nose masks were worn, and the rooms were well-ventilated.

Statistical analysis: The statistical tool used for this study was SPSS IBM series version 25. The data were descriptively and inferentially analysed and represented in tables.

Pearson correlation was used to test the relationship between outcome of embalmment (represented as body scores – TBS) and embalming fluid (represented as volume of embalming fluid – VEF).

Duration of research: This study lasted for a period of 42 days (from April 2021 to May 2021).

RESULTS

Body statistics of experimental animals: Result obtained from the peri-mortem body statistics of the animals showed that the animals were infants (about two months old). The body temperatures of the animals were at optimal levels (Table 1).

The body weight of the animals slightly decreased after eight hours (Table 2). The body

temperature of the animals significantly decreased after eight hours (Table 2); and there was a significant decrease in body weight of the animals at the end of the study (42nd day post-mortem).

Visible post-embalming changes: The visible changes within the first eight hours after death are algor mortis, rigor mortis, putrid odour, and presence of fly activities on the stab wounds (Table 3; Table 4). Decomposition progressed from the fresh stage of decomposition and was halted at the bloat stage. By the fourth day, the four limbs, ventral aspect of the trunk, and the snout were still decomposing. Other structures of the pig's body were completely fixed (Figure 2; Table 5).

At the seventh day, the decomposition of almost all the structures was arrested except the umbilical region which showed signs of decay (Figure 3; Table 6). However, by the eighth day, the entire body structures of all the animals were fixed with no putrid odour. The animals remained fixed till the last day of the study (Figure 4; Table 7).

Relationship between the outcome of embalmment and independent variables: The dependent variable for this study is the outcome of embalmment. The independent variables for this study are the factors that influence the rate of decomposition which in turn could affect the outcome of embalmment. The independent variables include room temperature, humidity, atmospheric temperature, volume of embalming fluid and duration of post-embalming (Table 8).

Pearson correlation analysis showed that there was a statistically significant moderate positive correlation (r = .576, n = 42, p = .001) between outcome of embalming and duration of embalming; a statistically significant strong negative correlation (r = -.623, n = 42, p = .001) between outcome of embalming and volume of embalming fluid; and a statistically significant moderate negative correlation (r = -.528, n = 42, p = .001) between outcome of embalming and room temperature. However, Pearson correlation analysis showed that there was a statistically insignificant very weak positive correlation (r = .186, n = 42, p = .239) between outcome of embalming and humidity; and a statistically insignificant very weak negative correlation (r = -.141, n = 42, p = .375) between outcome of embalming and atmospheric temperature.

Fig. 1: Day 1 – After embalming.

Fig. 2: Day 4 Post-embalming changes.

Fig. 3: Day 7 Post-embalming.

Fig. 4: Day 42 Post-embalming.

Table 1: Peri-mortem body statistics.

BODY STATISTICS	PIG 1	PIG 2
Weight (Kg)	14	13
Body Temperature (^{0}C)	39.5	39.8
Recumbent Length (cm)	64	60
Chest Circumference (cm)	41	40
Waist Circumference (cm)	40	38
Atmospheric temperature at death (^{0}C)	27.4	27.4
Time of death	8:44	8:45

Table 2: Post-mortem body statistics.

BODY STATISTICS	PIG 1	PIG 2
Weight after 8 hours (Kg)	13.5	12.5
Body temperature after 8 hours (^{0}C)	32	32
Weight at day 42 (Kg)	6	5

Table 3: DAY 1 Data – 10 Minutes Periodic observations after Death.

TIME	BT (^{0}C)	AT (^{0}C)	RT (^{0}C)	VISIBLE CHANGES	Insect Activities
8:44am	39.5	27.4	28.1	No pupillary reflex.	Nil
8:54am	39.5	27.5	28.1	A clear fluid exited the anus.	Nil
9:04am	39.3	27.4	28.2	Algor mortis starts with movement of exposed intestine.	Nil
9:14am	38.6	27.7	28.3	Body temperature drops.	Nil
9:24am	37.3	28.5	28.6	Body temperature continues to drop.	Nil
9:34am	36.7	28.9	28.7	Body temperature continues to drop.	Nil
9:44am	36.6	29.2	28.9	Body temperature continues to drop.	Nil

BT. Body temperature, AT. Atmospheric temperature, RT. Room temperature

Table 4: DAY 1 Data – Hourly Observation.

TIME	BT (^{0}C)	AT (^{0}C)	RT (^{0}C)	VISIBLE CHANGES	Insect Activities
10:44am	34.4	30.4	29.7	Rigor mortis starts.	Few flies on the evaginated intestine.
11:44am	33	33.4	30.5	Fluid discharge from the anus.	Few flies on the chest.
12:44pm	32	34.1	31.5	Increase in intra-abdominal pressure with discharge of faecal matter.	Nil
1:44pm	32	36.4	32.3	Algor and rigor mortises progressed, with increase in intra-abdominal pressure.	Few flies on the chest.
2:44pm	32	36.5	33.4	Algor and rigor mortises progressed, with increase in intra-abdominal pressure.	Nil
3:44pm	32	35.9	34	Algor and rigor mortises progressed, with increase in intra-abdominal pressure. Presence of putrid odour.	Increased fly activity at exposed body parts (wounds) and orifices.
4:44pm	32	37.7	33.1	Algor and rigor mortises progressed, with increase in intra-abdominal pressure. Putrid odour intensifies.	Swarm of flies at exposed body parts (wounds) and orifices.

BT. Body temperature, AT. Atmospheric temperature, RT. Room temperature

Table 5: DAY 4 Post-embalming visible changes.

Time	Head & neck visible changes	Trunk visible changes	Limbs visible changes
Morning (6:15am)	The snout was decomposing with discharge of a foamy substance. The crown, two ears, the whole neck were completely fixed.	The umbilical region continues to appear bloated and decomposing. The dorsal aspect of the trunk, tail and anorectal region were completely fixed with no discolouration.	The limbs were bloating. The forelimb was discoloured to dark-brown.
Afternoon (12:05pm)	Decomposition of the snout progressed. The crown, two ears, the whole neck were completely fixed.	Decomposition of the umbilical region and chest progressed. A clear fluid discharged from the stitched umbilical region. The dorsal aspect of the trunk, tail and anorectal region were completely fixed.	Decomposition progressed with all the activities observed in the morning.
Evening (6:09pm)	Decomposition on the snout progressed with presence of fly. Foamy discharge from the snout reduced. The crown, two ears, the whole neck were completely fixed.	Greenish discolouration of the umbilical region and chest. Flies on the umbilical region. The dorsal aspect of the trunk, tail and anorectal region were completely fixed with no discolouration.	Decomposition progressed with all the activities observed in the morning and afternoon.

Table 6: DAY 7 Post-embalming visible changes.

Time	Head & neck visible changes	Trunk visible changes	Limbs visible changes
Morning (6:00am)	All the structures of the head and neck region were completely fixed.	Decomposition slows down at the umbilical region. Other aspects of the trunk were completely fixed.	All the limbs were completely fixed.
Afternoon (12:00pm)	No visible changes.	Decomposition continues to slow down at the umbilical region.	No visible changes.
Evening (6:09pm)	No visible changes.	Decomposition continues to slow down at the umbilical region.	No visible changes.

Table 7: DAY 42 Post-embalming visible changes.

Time	Head & neck visible changes	Trunk visible changes	Limbs visible changes
Morning (6:12am)	All structures of the head and neck were completely fixed.	All structures of the trunk were completely fixed.	All the limbs were completely fixed.
Evening (6.00pm)	No visible changes.	No visible changes.	No visible changes.

Table 8: Pearson Correlation between the outcome of embalming and all the independent variables used for the study.

		Days of Embalming	Average Atmospheric Temp.	Average Humidity	Average Room temp.	Average Total Post-Embalming Body Score	Average VEF
Average Total Body Score (Outcome of embalming)	Pearson Correlation	.576**	-0.141	0.186	-.528**	1	-.623**
	Sig. (2-tailed)	0	0.375	0.239	0		0
	N	42	42	42	42	42	42

*. Correlation is significant at the .05 level (2-tailed).
**. Correlation is significant at the .01 level (2-tailed).

KEY TO QUALITY OF RELATIONSHIP
0.80 – 1.00 Very strong positive
0.60 – 0.79 Strong Positive
0.40 – 0.59 Moderate positive
0.20 – 0.39 Weak positive
0.00 – 0.19 Very weak positive

Table 9: Post-embalming body scores (outcome of embalming) of the pigs and Volume of embalming fluid applied.

DAY	TPBS 1	TPBS 2	CTPBS	ATPBS	VEF 1(ml)	VEF 2 (ml)
1	0	0	0	0	1300	1500
2	5	1	6	3	0	0
3	5	2	7	3.5	0	0
4	8	11	19	9.5	400	400
5	12	11	23	11.5	0	0
6	15	15	30	15	0	0
7	17	15	32	16	600	0
8	18	18	36	18	0	0
9	18	18	36	18	0	0
10	18	18	36	18	0	0
11	18	18	36	18	400	0
12	18	18	36	18	0	0
13	18	18	36	18	0	0
14	18	18	36	18	0	0
15	18	18	36	18	0	0
16	18	18	36	18	0	0
17	18	18	36	18	0	0
18	18	18	36	18	0	0
19	18	18	36	18	0	0
20	18	18	36	18	0	0
21	18	18	36	18	100	0
22	18	18	36	18	100	0
23	18	18	36	18	100	0
24	18	18	36	18	0	0
25	18	18	36	18	0	0
26	18	18	36	18	0	0
27	18	18	36	18	0	0
28	18	18	36	18	0	0
29	18	18	36	18	0	0
30	18	18	36	18	0	0
31	18	18	36	18	0	0
32	18	18	36	18	0	0
33	18	18	36	18	0	0
34	18	18	36	18	0	0
35	18	18	36	18	0	0
36	18	18	36	18	0	0
37	18	18	36	18	0	0
38	18	18	36	18	0	0
39	18	18	36	18	0	0
40	18	18	36	18	0	0
41	18	18	36	18	0	0
42	18	18	36	18	0	0

TPBS 1. Total post-embalming body score for Pig 1
TPBS 2. Total post-embalming body score for Pig 2
CTPBS. Cumulative total post-embalming body scores
ATPBS. Average total post-embalming body scores
VEF 1. Volume of embalming fluid used to fix Pig 1
VEF 2. Volume of embalming fluid used to fix Pig 2

Table 10: Climatic factors that influence decomposition.

DAY	HAT (^{0}C)	LAT (^{0}C)	HH (%)	LH (%)	HRT (^{0}C)	LRT (^{0}C)
1	38.4	27.8	92	63	33.9	28.1
2	30.1	22.4	98	77	33.4	24.1
3	30.1	22.5	92	72	30	24.5
4	32.6	24	94	65	30.6	25.8
5	32.4	24.8	96	65	31.9	26
6	33.4	25.4	92	52	32.2	26.9
7	33.4	26.5	88	65	31	28.3
8	34.2	23.6	93	74	31.1	24.1
9	29.4	24.1	95	74	29	23.6
10	30.3	23	94	62	29.7	23.4
11	33.4	24.4	96	70	31.1	23.7
12	34.6	23.2	94	69	31.2	24.1
13	40	23.6	90	65	32.4	23.4
14	39.4	24.5	93	53	33.5	23.9
15	33.5	22.3	97	71	31.2	23.5
16	33.3	23.6	97	69	31.2	23.2
17	34	23.1	92	66	32.3	23.8
18	33.9	22.9	98	76	30.9	23.1
19	34.8	24.1	94	68	31.5	23.7
20	34	22.7	95	66	30	23.9
21	31.2	21.5	99	77	23.5	23.1
22	31.6	22.7	93	74	29.8	24.8
23	30.6	22.2	96	77	28.9	24.1
24	32	22.1	97	76	29.9	23.4
25	31.6	23.4	95	72	30.8	23.9
26	35.4	23.7	98	69	32.3	24.1
27	26.8	21.2	99	87	25.7	23.1
28	32.1	22.1	99	72	29.8	24.6
29	32.5	24.4	97	70	30.4	29
30	32.2	24.7	94	72	30.2	26.2
31	32.7	24.8	91	69	30.1	26.1
32	32.5	25.4	92	77	29.7	26.7
33	27.5	24.4	99	91	28.5	25.8
34	31.2	25.1	99	90	30.1	26.2
35	27.6	22.1	92	88	27.1	24.4
36	31.5	23.5	95	77	29	24.6
37	26.8	21.8	89	80	26.2	23.4
38	30.9	23.4	97	75	29.4	24.4
39	33.2	24.4	84	68	30.5	26.1
40	31.8	26.3	98	75	30.7	27.4
41	31.9	24.5	92	80	29.9	27.1
42	30.8	24.5	95	79	29.9	26.2

HAT. Highest atmospheric temperature
LAT. Lowest atmospheric temperature
HH. Highest humidity
LH. Lowest humidity
HRT. Highest room temperature
LRT. Lowest room temperature

DISCUSSION

The slight decrease in body weight of all the animals after eight hours before embalming could be as a result of loss of blood from stab wounds. Khalid e*t al.* also reported that weight loss occurs during slaughter of animals [14]. The odour of the blood and exposed intestine attracted insects to the animals at about one hour after death; but this is contrast with a study which reported that decomposition process and arthropod succession are not influenced by absence or presence of wounds [15]. The significant decrease in body temperature after eight hours is referred to as algor mortis which is one of the early signs of decomposition. Livor and

Pallor mortises were not observed because of the skin colour of the animals. In addition, the animals were very dirty which impaired the observation of certain early visible decomposition changes. This means that clean white porcine models should be used to carry out this experiment in order to observe all visible decomposition changes. However, it can be deduced from the visible changes that decomposition progressed from fresh stage to early bloat stage of decomposition before it was embalmed. The animals did not actively decay due to embalming.

The significant decrease in body weight means that the animals were completely fixed and decomposition halted. Formalin has been reported to dry cadavers and prevent microorganisms and arthropods when used for embalming [8]. More so, decomposition was completely arrested on the eight day which explains that a mixture of formalin, methanol and water is very potent in arresting early decomposition of stabbed carcasses. Bradbury and Hoshino also reported that methanol prevents the polymerization of formaldehyde when used in a formalin-based solution; and also establish the proper density of the solution [16].

The outcome of embalming was positively affected by the duration of embalming which means that as duration progressed, the animals became more fixed. The outcome of embalming was negatively influenced by volume of embalming fluid and room temperature. This means that the embalming mixtureis very reliable because little amount is needed to arrest decomposition of stabbed carcasses. The constituents of this mixture have been reported to have strong preservative effects on decomposing bodies [2, 7, 8, 17]. More so, the result showed that lower (optimum) room temperature aids the preservation of stabbed carcasses compared to higher or extreme temperatures. The outcome of embalming was not influenced by atmospheric temperature and humidity.

CONCLUSION

Arterial and hypodermic embalming techniques are suitable methods for embalming stabbed carcasses. Post-embalming management (re-embalmment via hypodermic technique) is also required to achieve quicker and successful result. Since, porcine models are acceptable analogues for taphonomic research, an embalming mixture containing formalin, methanol and water in equal proportion is suitable for arresting early decomposition of stabbed cadavers.

RECOMMENDATIONS

Based on the findings from this study, the following are recommended:

1. Further studies should be carried out with mature porcine models to validate the efficacy of this mixture.

2. Further studies should be carried out to validate the efficacy of this mixture on bloat and active decay stages of decomposition.

More animals should be approved by the Animal protection index of Nigeria in order to ensure accuracy of the reports.

Author Contributions

This work was carried out in collaboration of all authors; and all authors read and approved the final manuscript.
Author Onyejike, Darlington Nnamdi (ODN) conceptualized the study, designed the study, wrote the experimental protocol, supervised the experiment, and wrote the first draft of the manuscript.
Author Akukwu, Darlington Cyprain (ADC) reviewed and edited the draft manuscript.
Author Ijike, Odinakachi (IO) carried out the experiment.
Author Onyejike, Ifeoma Miracle (OIM) managed the literature searches.
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Author Okubike, Emeka Ambrose (OEA) acquired and managed the animals.
Author Ezenwatu, Emmanuel Nzube (EEN) assisted author OIM to manage the literature searches.
Author Ojemeni, Gloria Chinenye (OGC) assisted author AUS to curate the data.
Author Okeke, Somadina Nnamdi (OSN) carried out the statistical analysis.
Author Agulanna, Ambrose Echefulachi (AAE) assisted author IO to carry out the experiment.

Conflicts of Interests: None

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