Available online on 27.06.2021 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Full Text Article

Research Article

Differences in Histopathological Picture of Lung Organs in Sprague Dowley White Rats that Die Drowning in Salt Water and Fresh Water

Theza E. A. Pellondo'u.P, Marliana Nurprilinda, Yoan Marsindhy Rahayu Atuk

Medical Faculty, Univeristas Kristen Indonesia, Jakarta, Indonesia

Article Info:

Article History:

Received 04 May 2021
Review Completed 14 June 2021
Accepted 20 June 2021
Available online 27 June 2021

Cite this article as:

Pellondo'u.P TEA, Nurprilinda M, Atuk YMR, Differences in Histopathological Picture of Lung Organs in Sprague Dowley White Rats that Die Drowning in Salt Water and Fresh Water, Journal of Drug Delivery and Therapeutics. 2021; 11(3-S):163-169
DOI: http://dx.doi.org/10.22270/jddt.v11i3-S.4852

*Address for Correspondence:

Theza E. A. Pellondo'u.P, Medical Faculty, Univeristas Kristen Indonesia, Jakarta, Indonesia

Abstract

This study aimed to investigate the differences in the pulmonary histopathology of the drowned victims in salt water and fresh water. By utilizing a post-test only control design, the research used Sprague Dawley lab rat as the main subject of the experiment. The subject would be drowned in two different types of water, which were salt water and fresh water. There were fifteen male rats which divided evenly into three separate groups. The first group was subjected to salt water, and the second group was subjected to fresh water. The last group was a control group. The result from the test was analyzed by comparing the lung tissue histopathological structure of the subject for the salt water group and the fresh water group. On the outcome, there was a significant difference between interalveolar haemorrhage in the lung of a mouse drowned in salt water and submerged in fresh water. In addition, there was over-distension of the alveoli, thinning of the alveolar septal wall in both groups. The result of the experiment showed that there was a significant interalveolar haemorrhage difference in the histopathological structure between the salt water group and the fresh water group.

Keywords: pulmonary histopathology, drowning, alveolus, white rat

INTRODUCTION

Drowning is a significant public health problem that is often overlooked. In late 1990, the World Bank and WHO released the first Global Burden of Disease (GBD) study, which stated that drowning was one of the most common causes of death in the world [1].

In 2013, there were an estimated 368,000 deaths from drowning, down from 545,000 deaths in 1990. Of these deaths, 82,000 occurred in children under five years [2]. The World Health Organization (WHO) noted that in 2015 an estimated 360,000 people died from drowning. It makes drowning a significant public health problem in the world. Accidental drowning is the third leading cause of accidental injury resulting in death worldwide. Drowning accounts for 7% of all injury-related deaths (excluding those caused by natural disasters). The Global Burden and Death found that accidental deaths from drowning account for more than 90% in low- and middle-income countries. More than half of the world's drownings occur in the WHO West Pacific and WHO Southeast Asia regions. The highest incidence of drowning deaths occurred in the WHO Africa region, 15-20 times higher than Germany or the UK [3]. The incidence is highest in developing countries, especially in children aged 1-4 years. In addition to age, other factors that play a role in increasing drowning cases are gender, especially men, who have twice the mortality rate of women. In addition to consuming alcohol or abusing illegal drugs near water or while in water, unsupervised children worsen a previous medical condition (seizures) [3].

Most drowning cases occur in fresh water (rivers, lakes and swimming pools) around 90% and seawater about 10% [2]. Drowning is a form of death caused by asphyxia. Deaths due to asphyxia often occur either naturally or unnaturally, so that the police/investigators often ask for doctor's help to solve cases of deaths caused by asphyxia, especially when there is suspicion of an unnatural death [4]. Drowning is an affixed type of death due to water blocking the respiratory tract to the lungs [5]. There is no electrolyte change in the blood in other asphyxia, whereas, in drowning, these changes are present, either submerged in fresh water or submerged in salt water. The mechanism of death that may also occur is drowning due to vagal inhibition and laryngeal spasm [6].

Drowning in salt water, hemoconcentration occurs, fluid from the circulation can be pulled out up to about 42 per cent and into the lung tissue, resulting in severe pulmonary oedema in a relatively short time. While drowning in fresh water, there is the absorption of fresh water in large quantities resulting in severe hemodilution of up to 72%, which results in hemolysis. Hemodilution causes fluid in the blood vessels or circulation to become excessive. As a result, there is a decrease in systolic pressure. Ventricular fibrillation occurs within a few minutes, the heart is still beating weakly, and there is severe cerebral anoxia. It explains why death occurs so quickly [6].

The drowning case is still a dilemma in forensic medicine. For example, the murder case drowned his victim in a river, but the victim was found in the sea. The diagnosis of drowning as a cause of death can be complicated and problematic in individual cases, especially when the typical morphological signs of drowning are absent and where the body is drowning [7]. In this case, forensic medicine has a significant contribution in conducting post-mortem to determine the cause and manner of death, whether accident, homicide, or suicide, and finding the exact location of the victim's death [6]. The diagnosis of drowning can be established by considering the forensic examination results, including an external examination and an internal examination. The macroscopic examination is part of an internal examination usually carried out on the respiratory tract, looking for microscopically occurring changes [8]. Further microscopic examination, namely histopathological examination by processing the organs of the victim's body that have not been rotten to be used as preparations which aim to make a difference between death due to drowning and death due to other reasons.

Based on the data above, the authors would like to study differences in pulmonary histopathological features in rats drowned in salt water and fresh water. Based on the background described above, the main problem is as follows "Are there differences in the histopathological picture of the lungs in Sprague Dawley white rats that drowned in salt water and fresh water?" with the aim of research to determine differences in the histopathological features of the lungs of white rats that drowned in salt water and fresh water.

LITERATURE REVIEW

Drowning is the immersion of part or all body into the water, which causes death due to a lack of oxygen when water fluid enters the body through the respiratory tract. In drowning, the whole body does not have to be submerged in water. As long as the nostrils and mouth are below the water's surface, it is sufficient to meet the criteria as a drowning event. Based on this understanding, drowning events can not only occur in the sea or rivers but can occur in a sink or bucket filled with water [9].

The lungs are conical in shape according to the space defined by the pleural cavity. Each lung has a base and apex, costal and medial surfaces, anterior and inferior margins, and a hilum (the entry point for blood vessels, nerves and bronchial components on the medial surface). The lung base is concave and conforms to the dome shape of the diaphragm. The right dome of the diaphragm is located higher than the left dome of the diaphragm. Therefore, the right lung is shorter than the left lung. Because the heart occupies a position slightly to the left of the midline, the suitable lung base is wider than the left lung base. The apex of the lung forms a prominent pulmonary dome above the first rib and clavicle, under the fascia covering the muscle in the neck (scalenus muscle) [10].

The right lung is more comprehensive and shorter (than the left lung), having three distinct lobes; 12 superior lobes, middle lobes and inferior lobes. The left lung is divided into two lobes: the superior lobe and the inferior lobe. In addition, the lungs have two serous sacs that surround and protect them called the pleura. Each pleura consists of two layers: the parietal layer, which covers the thoracic wall, the thoracic surface of the diaphragm and the lateral surface of the mediastinum extending to the base of the neck; the visceral layer, which covers the entire outer surface of the lung and extends into the interlobar fissures [11].

The bronchi, pulmonary connective tissue, and visceral pleura receive blood from the bronchial arteries, branches of the descending aorta. The bronchial veins drain into the azygos and hemiazygos veins. The alveoli receive deoxygenated blood from the terminal branches of the pulmonary artery. Oxygenated blood leaves the alveolar capillaries and finally empties into the two pulmonary veins, leaving the pulmonary roots of each lung to empty into the left atrium of the heart [12].

The pulmonary plexus contains parasympathetic and sympathetic nerves to the bronchial and vascular branches of each lung. The parasympathetic fibres of the vagus nerve are preganglionic and secretomotor to the bronchial mucosal glands. Sympathetic postganglionic fibres are vasomotor for the arterial system [13]. The respiratory system can be divided into the upper respiratory and lower respiratory systems. The lungs and chest wall are elastic structures. Under normal circumstances, there is a thin layer of fluid between the lungs and the chest wall so that the lungs easily slide against the chest wall. The pressure in the space between the lungs and the chest wall is below atmospheric pressure [14]. The physiological process of respiration, namely the oxygen being transferred from the air into the tissues and carbon dioxide released into the exhaled air, can be divided into three stages: ventilation, cellular respiration, or internal respiration.

The mechanism of respiration consists of the processes of inspiration and expiration. When inspiration (air enters the lungs), there is a contraction between the muscles and the ribs and is lifted to increase chest cavity volume. Otherwise, the pressure in the chest cavity becomes smaller than the air pressure in the lungs. As a result, air from outside into the lungs so that the lungs will expand, the volume becomes more prominent while the pressure becomes smaller than the free air pressure [15]. On the other hand, during expiration (air comes out of the lungs), the muscles between the ribs will return to their original position (relaxation), causing the ribs to be pulled to their original position so that the volume of the chest cavity will decrease while the pressure increases. This tremendous pressure causes the lung cavities to shrink due to the pressure on the lung walls, which increases the air pressure in the lung cavities. This situation causes the air in the lung cavity to be pushed out.

Alveoli are small pouch-like evaginations that open on one side in the respiratory bronchioles, alveolar ducts and alveolar sacs. Inside this bowl-like structure, the exchange of O2 and CO2 between the air and the blood occurs. The structure of the alveolar walls is specialized to facilitate and facilitate diffusion between the external and internal environment. Generally, each wall is located between two adjacent alveoli, so it is called an interalveolar septum [16]. The interalveolar septum consists of a single layer of squamous alveolar cells, fine connective tissue fibres and fibroblasts, and many capillaries located in the thin interalveolar septum. The thin interalveolar septum allows the capillaries to be adjacent to the flattened alveolar cells in the adjacent alveoli [20].

The number of alveoli reaches 300 million pieces. In the presence of the alveolus, the surface area of the entire alveolus is estimated to be up to 100 times larger than the body surface area [17]. The walls of the alveoli contain blood capillaries that allow the diffusion of gases to occur. Type I alveolar cells (also called type I pneumocytes or squamous alveolar cells) are fragile cells that line the surface of the

alveoli. The primary function of these cells is to form a barrier of minimal thickness through which gases can pass easily. Type II alveolar cells (type II pneumocytes) are distributed between type I alveolar cells by tight junctions and desmosomes connecting them to the cells. In addition, type II cells display a characteristic vesicular or foamy cytoplasm. These vesicles are due to the presence of lamellar bodies that are preserved and present in the tissue. These lamellae bodies produce material that spreads over the alveolar surface in the form of pulmonary surfactant, forming an extracellular layer that reduces surface tension.

According to WHO, several factors cause drowning, including age, gender, flood disaster, travelling by water transportation, other risk factors. There are several types of drowning, including a) Wet Drowning - The entry of fluids into the respiratory tract after the victim drowns [18]; b) Dry Drowning - In this type, water does not enter the lungs but is the result of laryngeal spasm due to the flow of water into the nasopharynx or larynx. Thick muscle, foam and others may occur, resulting in blockage. It is seen in 10-20% of immersion cases; c) Secondary Drowning - In this type, death occurs from half an hour to several days after resuscitation. Electrolytes are disturbed, and metabolic acidosis occurs. Death occurs due to anoxia with irreversible brain damage; and d) Immersion Syndrome - Death resulting from heart failure caused by vagal obstruction [19].

More than 85% of drowning accidents involve the aspiration of water into the lungs. Another 15% of cases may be due to lung injury. The most important contributing factors to morbidity and mortality from drowning are hypoxemia and acidosis and the multiorgan effects of this process. Central nervous system damage can result from hypoxemia that occurs during a drowning episode (primary injury), or it can result from arrhythmias, ongoing lung injury, reperfusion injury, or multiorgan dysfunction (secondary injury), especially with prolonged tissue hypoxia [20].

The further respiratory effort may lead to the formation of negative pressure (noncardiogenic) pulmonary oedema. Changes in intrathoracic pressure can cause an increase in intrathoracic blood volume and pulmonary artery pressure and a decrease in pulmonary interstitial pressure. This shift in pressure can cause fluid to flow into the interstitium from the blood vessels, which, when large enough, overflow into the alveoli [21].

Aspiration of fluid into the lungs destroys or weakens surfactant, disrupts the alveolar-capillary membrane and damages the pneumocytes, causing atelectasis. It increases the area of very low or zero ventilation-perfusion ratio (V/Q) and a decrease in compliance. At this time, the victim is unable to breathe, causing oxygen depletion and carbon dioxide retention. As the oxygen tension in the blood falls further, the victim gasps, hyperventilates, may aspirate varying amounts of fluid [22].

It leads to further hypoxemia. Depending on the degree of hypoxemia and the resulting change in acidosis in the acid-base balance, the person may develop myocardial dysfunction, electrical instability, cardiac arrest, and central nervous system ischemia. Therefore, fluid aspiration of at least 11 mL/kg is required for changes in blood volume to occur, and aspiration of more than 22 mL/kg is required before significant electrolyte changes occur.

Fresh water is more hypotonic when compared to blood [15]. When immersed in fresh water, the blood plasma is hypotonic. Aspiration of fresh water into the lungs will be rapidly absorbed from the alveoli so that it draws the fluid from the alveoli into the pulmonary capillaries causing intravascular hypervolemia, allowing hyponatremia and hypokalemia, and severe hemodilution up to 72%, which results in hemolysis This hemolysis can interfere with the delivery of oxygen to the tissues, resulting in severe anoxia in the myocardium. Hemodilution inactivates surfactant in the alveoli. It changes the alveolar surface tension and can lead to alveolar collapse and pulmonary atelectasis. Ventilation/perfusion mismatch caused by perfused but not ventilated lung areas causes hypoxemia, which can be severe. It can be reinforced by water in the alveoli and interstitium, which acts as a diffusion barrier. This intrapulmonary shunting and blood oxygenation barrier further impede tissue oxygenation. Hemodilution also causes fluid in the blood vessels or circulation to become excessive, a decrease in systolic pressure occurs, and within a few minutes, ventricular fibrillation occurs. The heart is still beating weakly for a while, and there is severe cerebral anoxia. It explains why death occurs so quickly [5].

Seawater has high Na, Mg, and Ca levels, attracting plasma fluid from the pulmonary capillaries to the alveoli because of its hypertonic nature. Drowning in seawater, hemoconcentration occurs, fluid from the circulation can be pulled out up to about 42%, and into the lung tissue, resulting in severe pulmonary oedema in a relatively short time. Electrolytes withdrawal from salt water into the blood results in an increase in the hematocrit and an increase in sodium and chloride levels. Suppose significant enough can cause a decrease in intravascular fluid volume. Ventricular fibrillation does not occur. The occurrence of anoxia in the myocardium and accompanied by an increase in blood viscosity will cause heart failure. There is no hemolysis but hemoconcentration. The systolic pressure will settle within a few minutes. 5 Salt water is less damaging to surfactants, so atelectasis does not occur [23].

Drowning can lead to acute asphyxial cardiac arrest, which stems from hypoxemia that precedes the development of ischemia. It begins with an early cessation of gas exchange followed by worsening hypoxia and finally cardiac arrest. Hypoxemia is the leading cause. Myocardial dysfunction may result from ventricular dysrhythmias, pulseless electrical activity, and asystole from hypoxemia, hypothermia, acidosis, or electrolyte abnormalities. In addition, hypoxemia can directly damage the myocardium, decreasing cardiac output [24].

Pulmonary hypertension may result from the release of pulmonary inflammatory mediators, increasing right ventricular afterload and decreasing pulmonary perfusion and left ventricular preload. However, although cardiovascular effects may be severe, they are usually temporary, unlike severe central nervous system injuries. Swimming can serve as a trigger for arrhythmias and produce a dive reflex, which can lead to autonomic instability. The dive reflex is elicited by facial contact with cold water and consists of breath, bradycardia, and intense peripheral vasoconstriction [25].

Hypoxic-ischemic brain injury is a dangerous sequel to the asphyxial heart attack associated with drowning. The extent of CNS injury remains a significant determinant of long-term survival and morbidity in drowning. Two minutes after immersion, a child will lose consciousness. Irreversible brain damage usually occurs after 4-6 minutes. Most surviving children were found within 2 minutes of immersion. Most of the children who died were found after 10 minutes [25].

The high-risk areas of the brain are subcortical tissue that is metabolically active, and that has limited perfusion.

Widespread brain injury occurs in hypoxaemia and low flow conditions resulting in energy failure, lipid peroxidation, free radical production, inflammatory processes, and release of excitotoxic neurotransmitters. Neuronal and glial functions are impaired. Asphyxial cardiac arrest causes selective neuronal injury [25].

Primary central nervous system injury is initially associated with tissue hypoxia and ischemia. However, if the period of hypoxia and ischemia is brief or if the person is a very young child who rapidly develops core hypothermia, the primary injury may be limited. In addition, the patient may recover with minimal neurologic sequelae, even after more extended immersion. In contrast, drowning associated with prolonged hypoxia or ischemia is likely to result in significant primary and secondary injury, particularly in older patients who cannot rapidly achieve core hypothermia [23].

RESEARCH METHOD

This research is pure experimental research using a post-test only control design. Data collection was carried out only at the end of the study after the treatment by comparing the results in the fresh water treatment group with the salt water treatment group with the control group. This research was conducted at the Forensic Laboratory and Anatomical Pathology Laboratory, Faculty of Medicine, the Christian University of Indonesia, in December 2017-February 2018. The population is partly taken from the entire object under study and is considered to have the entire population. The target populations in this study were white male rats of the Sprague Dawley strain. The samples of this study were female white rats of the Sprague Dawley strain kept in the Forensic Laboratory of the Faculty of Medicine, Christian University of Indonesia, as many as 15 tails. Rats were divided into three groups; each group consisted of 5 rats. The rat cage must be strong enough and not easily damaged and the animal visible from the outside. The bottom is given cardboard as a base and given wood dust to absorb the rat urine to remain dry, and the rat feels comfortable. Before being treated, the rats were adapted for approximately one week in a quiet room with good air ventilation so that during the treatment, the rats did not experience stress and were easy to handle. Rats were fed and bottled ad libitum. The data obtained in the form of histopathological description of the rat lung in each group (fresh water group, salt water group and control group), then analyzed descriptively by comparing the histopathological structure of the lung.

RESULT AND DISCUSSION

This study is an experimental study using a post only control design [29]. The white rats used in this study were male Sprague Dawley strain rats because their biological conditions were stable compared to female rats whose biological conditions were influenced by the estrus cycle period. The treatment group was submerged in salt water, the treatment group was submerged in fresh water, and the control group consisted of 5 individuals in each group. The data taken are in the form of macroscopic and microscopic observations. Microscopic observations were made by reading microanatomical preparations from rat lungs. The results of observations made in this study are presented descriptively.

The maintenance of the test animals took place at the Forensic Laboratory, Faculty of Medicine, the Christian University of Indonesia, for one week. The test animals are placed in 3 strong cages not easily damage from rat bites. Besides that, the cage must also be visible from the outside. At the bottom, cardboard is given as a base, and wood dust is given to absorb rat urine so that sometimes the rats stay dry and the rats feel comfortable. Rats were fed and bottled ad libitum

White rats were divided into three groups: the salt water treatment group, the fresh water treatment group, and the control group. In the treatment group, the rats will be submerged in 2 types: fresh water and salt water, while the control group will undergo cervical dislocation. After the rat was declared dead, the rat operated from the abdomen up to the chin using a scalpel. First, take the rat lung organ by separating the rat lung from the other rat organs. Then macroscopically, observe the lung morphology of the three groups; after that, the lungs were put into small bottles that had been given a 10% buffered formalin solution.

After the lungs were immersed in 10% buffered formalin, the lungs were then taken to the Anatomical Pathology Laboratory to be made into preparations to be used for microscopic examination. Differences in lung morphology were found between the control and treatment groups. In the control group, the lungs were dark red, and this happened because the author did the cervical dislocation incorrectly, causing bleeding in the lungs of the test animals.

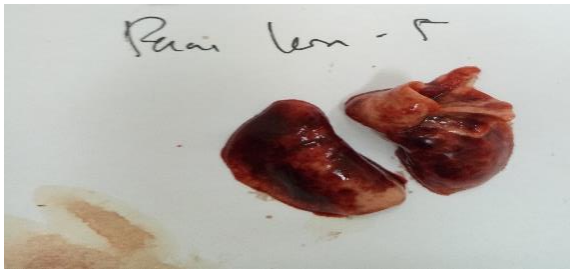

Figure 1: Bleeding in the control lung

In the salt water submersion treatment group, the morphology of the lungs was found to have bleeding spots reddish.

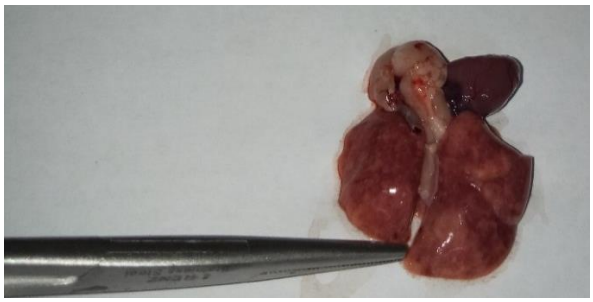

Figure 2: Salt water lungs

The results of observations in the treatment group in fresh water mean that the lungs are pale red and bleeding spots.

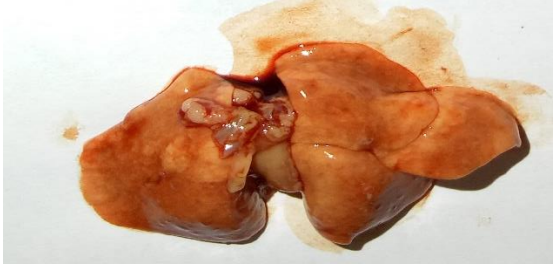

Figure 3: Fresh water lungs

Table 1: Results of Microscopic Observations of Rat Lungs

Macroscopic parameters	Control (n=5)	Fresh water (n=5)	Salt water (n=5)
Intraaveolar hemorrhage	1/5 = 20 %	5/5 = 100%	5/5 = 100%
Alveolar distension/dilation	1/5 = 20 %	5/5 = 100%	5/5 = 100%
Alveolar septal wall thinning	0/5 = 0%	5/5 = 100%	5/5 = 100%

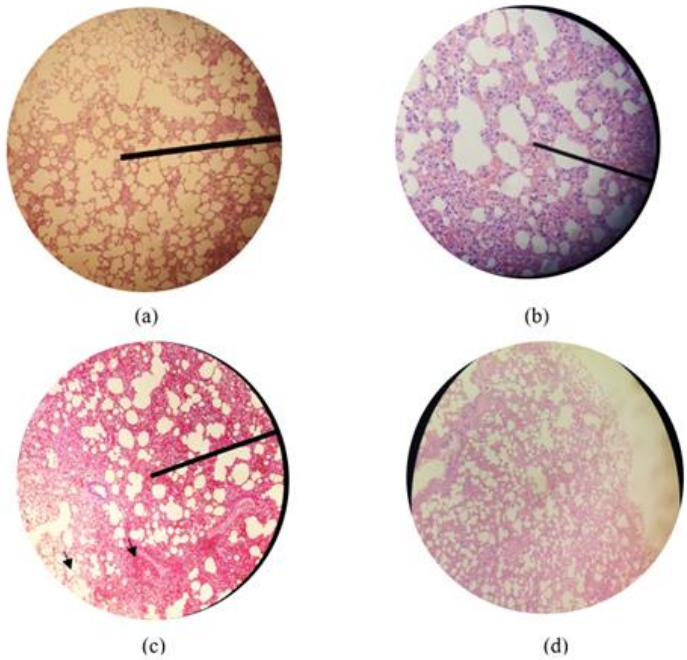

Figure 4: is a histopathological picture of the control rat lung. (a) The alveolus experienced dilation (overdistension) magnification 100x. (b) No effacement of the alveolar septum and alveolar septal wall rupture, magnification 400x. (c) & (d) Intraalveolar hemorrhage, magnification 100x

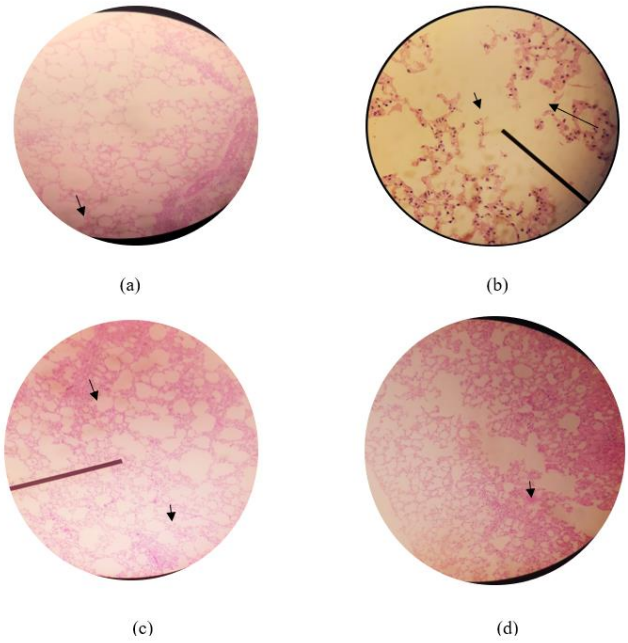

Figure 5: It is a histopathological picture of the lungs treated by immersion in salt water. (a) The alveolus is enlarged (overdistension) at 100x magnification. (b) rupture of the alveolar septum and alveolar septal wall rupture, magnification 400x. (c) & (d) Intraalveolar hemorrhage, magnification 100x

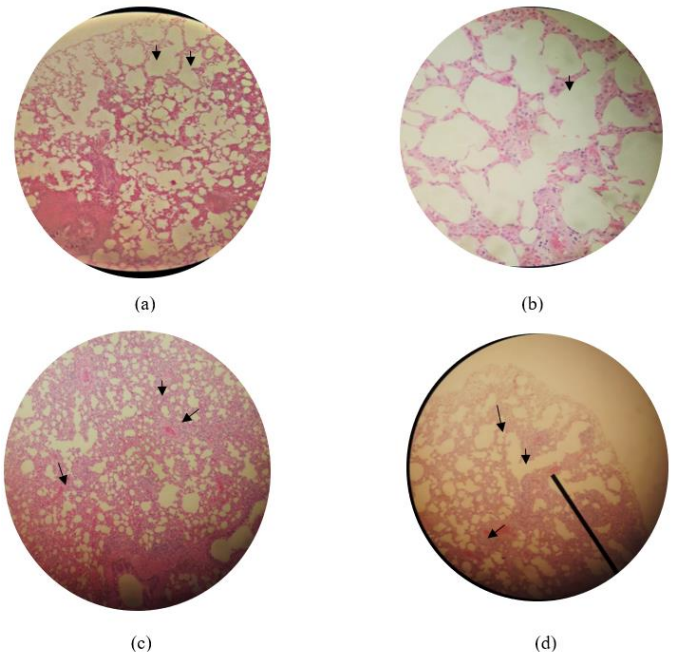

Figure 6: It is a histopathological description of the lungs treated by submersion in fresh water. (a) The alveolus is enlarged (overdistension) at 100x magnification. (b) Thinning of the alveolar septum and rupture of the alveolar septal wall, magnification 400x. (c) Intraalveolar haemorrhage, magnification 400x. (d) Intraalveolar haemorrhage, magnification 100x

In the lungs of both groups, Paltauf's spots were found due to tearing of the alveolar septum. In the salt water treatment group, oedema was found because salt water can rinse the surfactant covering the alveoli, but some surfactants cover the alveoli remain intact not to reduce surface tension ability to rinse surfactant by salt water is not comprehensive. In addition, salt water has a hypertonic nature. Therefore, fluid from the intravascular will be drawn into the alveolus resulting in a massive backflow into the intrapulmonary and pulmonary oedema will occur. In a study conducted by Samuel et al., it was found that seawater can rinse out intact lung surfactants. However, this flushing is not complete for all surfactants.33 Found in seawater or hypertonic fluids, 2.5 – 3.5% results in increased hemoconcentration of serum sodium and potassium and osmolality [26].

On histopathological observation of salt water lung, it was found that 5 out of 5 samples studied had alveolar widening and rupture of the interalveolar septal wall. Due to the hypertonic nature of salt water, which draws the fluid from the intravascular into the lungs and pulmonary oedema occurs, it can cause hyperinflation and rupture of the septal wall interalveolar. In addition, Knierim and Hartmann wrote in their study that rupture of the bronchiolar sphincters often causes significant dilatation of the alveoli, creating large spaces filled with edematous fluid [27].

In fresh water, the tonicity is lower than the extracellular fluid in the body, so that fresh water is absorbed more quickly from the alveoli through the capillaries located on the basement alveoli membrane to the intravascular. Blood volume and extracellular electrolyte concentrations immediately alter the secondary hydrophilic material produced by Pneumocystis II cells in the alveoli. It causes ion balance disruption in the surfactant so that the function of the surfactant to reduce the surface tension of the alveoli is reduced and causes collapse of the alveoli [28]. In a study conducted by Samuel et al., it was found that fresh water damages the lung surfactant layer that lines the alveolar cell walls.

On histopathological observations of fresh water lungs, it was found that 5 of the five samples studied had widening of the alveolus, as well as rupture of the interalveolar septal wall. According to Hong et al., this lung enlargement is caused by substantial fluctuations in air pressure in the airways due to a closed glottis. According to Layon, fluid aspiration when submerged, whether fresh or salty, can cause acute lung injury. Aspiration of fresh water destroys surfactants, resulting in alveolar collapse and atelectasis. Hypotonic fluids also directly affect alveolar and vascular endothelial cells, leading to interstitial and alveolar oedema. In all types of drowning, bronchospasm occurs due to the entry of fluid into the airways. In addition, acute emphysema may result from alveolar rupture due to fluctuations in airway pressure with ventilation of a closed glottis [29].

In addition to pulmonary oedema, alveolar distention and thinning of the alveolar walls, the lung preparations of both treatment groups were found to have intra-alveolar bleeding. It was found that in salt water, 5 out of 5 samples had mild interalveolar bleeding, while in fresh water, 5 out of 5 samples studied had severe interalveolar bleeding. Drowning is the cause of acute lung injury/acute respiratory distress syndrome associated with changes in pulmonary capillary permeability and alveolar diffusion capacity and an increase in intrapulmonary shunt. Acute Lung Injury/Acute Distress Respiratory Syndrome will cause activation of inflammatory mediators such as Tumor Necroting Factor, Interleukin-1, Interleukin-6, Neutrophils which will damage lung tissue, change endothelial permeability, and damage microcirculation [30]. Activation of neutrophils and other inflammatory mediators leads to loss of integrity of the alveolar-capillary membrane leading to extravasation of red blood cells into the alveoli, resulting in interalveolar haemorrhage on observation.

The difference in the bleeding severity in the observations of the two treatment groups may be due to the salinity level in the water aspirated into the lungs. Lung has a direct toxic effect on the alveolar and vascular endothelial

cells [31], so it was found in the observations that intra-alveolar bleeding in fresh water is heavier than salt water. Based on the observations that have been made in this study, it can be concluded that there are significant results between interalveolar bleeding found in the lungs of rats drowned in salt water and drowned in fresh water. Where in fresh water, the bleeding is heavier than in salt water.

CONCLUSION

This study concludes that descriptively there are significant results between interalveolar bleeding in the lungs of rats drowned in salt water and drowned in fresh water. However, given the limitations and shortcomings of this study, further research is needed. Namely similar studies with higher-level experimental animals or similar studies with differences in post mortem examination times, or with different research designs and data analysis techniques so that they can be used further. In determining the location of death so that it can be applied to determine the location of death more accurately.

REFERENCES

[1] van Beeck EF, Branche CM, Szpilman D, Modell JH, Bierens JJ. A new definition of drowning: towards documentation and prevention of a global public health problem. Bulletin of the World Health Organization. 2005; 83:853-6.

[2] Byard RW. Immersion deaths and drowning: issues arising in the investigation of bodies recovered from water.

[3] Hossain MJ, Biswas A, Mashreky SR, Rahman F, Rahman A. Epidemiology of adulthood drowning deaths in Bangladesh: Findings from a nationwide health and injury survey. F1000Research. 2017;6. https://doi.org/10.12688/f1000research.10980.1

[4] Mount K. Falling into the River with Albert, Madeleine and James. Performance Research. 2013 Aug 1; 18(4):151-9. https://doi.org/10.1080/13528165.2013.814349

[5] Armstrong EJ. The Forensic Pathological Aspects of Deaths Due to Drowning and Bodies Recovered from Fluid Environments. InWater-Related Death Investigation 2021 May 18 (pp. 267-300). CRC Press. https://doi.org/10.4324/9780367332297-9

[6] Locali RF, Almeida MD, Oliveira-Júnior IS. Use of the histopathology in the differential diagnosis of drowning in fresh and salty water: an experimental model establishment in rats. Acta cirurgica brasileira. 2006 Aug; 21(4):203-6. https://doi.org/10.1590/S0102-86502006000400003

[7] Fortes FJ, Perez-Carceles MD, Sibon A, Luna A, Laserna JJ. Spatial distribution analysis of strontium in human teeth by laser-induced breakdown spectroscopy: application to diagnosis of seawater drowning. International journal of legal medicine. 2015 Jul; 129(4):807-13. https://doi.org/10.1007/s00414-014-1131-9

[8] Schultz M. Paleohistopathology of bone: a new approach to the study of ancient diseases. American Journal of Physical Anthropology: The Official Publication of the American Association of Physical Anthropologists. 2001; 116(S33):106-47. https://doi.org/10.1002/ajpa.10024

[9] Armstrong EJ, Erskine KL. Investigation of drowning deaths: a practical review. Academic forensic pathology. 2018 Mar; 8(1):8-43. https://doi.org/10.23907/2018.002

[10] Basmajian, John V., Slonecker, Charles E. Grant anatomi klinik. Jakarta: EGC, 2012.

[11] Ilangovan G, Jain M. A glance of pleura from a radiologist eye. European Congress of Radiology-ECR 2019.

[12] Elawad AA. The Study of Pulmonary Vessels in Patients with Pulmonary Embolism using Computed Tomography (Doctoral dissertation, Sudan University of Science and Technology).

[13] Jänig W, McLachlan EM. Neurobiology of the autonomic nervous system. Autonomic Failure: A Textbook of Clinical Disorders of the Autonomic Nervous System Oxford. 2013; 13:21-34. https://doi.org/10.1093/med/9780198566342.003.0003

[14] Stenqvist O, Gattinoni L, Hedenstierna G. What's new in respiratory physiology? The expanding chest wall revisited!. Intensive care medicine. 2015 Jun; 41(6):1110-3. https://doi.org/10.1007/s00134-015-3685-5

[15] Fry DL, Hyatt RE. Pulmonary mechanics: a unified analysis of the relationship between pressure, volume and gasflow in the lungs of normal and diseased human subjects. The American journal of medicine. 1960 Oct 1; 29(4):672-89. https://doi.org/10.1016/0002-9343(60)90100-5

[16] Sorokin SP. Properties of alveolar cells and tissues that strengthen alveolar defenses. Archives of internal medicine. 1970 Sep 1; 126(3):450-63. https://doi.org/10.1001/archinte.126.3.450

[17] Tsuda A, Henry FS, Butler JP. Gas and aerosol mixing in the acinus. Respiratory physiology & neurobiology. 2008 Nov 30; 163(1-3):139-49. https://doi.org/10.1016/j.resp.2008.02.010

[18] Eroschenko, Victor P. Atlas histologi di fiore dengan korelasi fungsional. Edisi 9. Jakarta: EGC, 2003

[19] Mescher AL. Histologi dasar junqueira, teks dan atlas, edisi 12. EGC. Jakarta. 2011.

[20] Wu MY, Yiang GT, Liao WT, Tsai AP, Cheng YL, Cheng PW, Li CY, Li CJ. Current mechanistic concepts in ischemia and reperfusion injury. Cellular Physiology and Biochemistry. 2018; 46(4):1650-67. https://doi.org/10.1159/000489241

[21] Ruhago GM. Economic evaluation and equity impact analysis of interventions for maternal and child health in Tanzania. Evidence for fair and efficient priority setting.

[22] Fletemeyer JR. 2 Legal Concepts, Drowning, and Lifeguard Effectiveness.

[23] Layon AJ, Modell JH. Drowning. Anesthesiology 2009; 110: 1390-401 https://doi.org/10.1097/ALN.0b013e3181a4c3b8

[24] Cachalia T, Joseph A, Harwood T. Hypothermia and near-drowning associated with life-threatening injuries: A remarkable recovery: A case report. Trauma case reports. 2016 Aug 1; 5:1-6. https://doi.org/10.1016/j.tcr.2016.09.001

[25] Kaczmarek J, Reichmuth C, McDonald BI, Kristensen JH, Larson J, Johansson F, Sullivan JL, Madsen PT. Drivers of the dive response in pinnipeds; apnea, submergence or temperature?. Journal of Experimental Biology. 2018 Jul 1; 221(13). https://doi.org/10.1242/jeb.176545

[26] Mason RJ, Broaddus VC, Martin TR, King TE, Schraufnagel D, Murray JF, Nadel JA. Murray and Nadel's Textbook of Respiratory Medicine E-Book: 2-Volume Set. Elsevier Health Sciences; 2010 Jun 9.

[27] Knieriem, A., M. Gracia Hartmann. Comparative histopathology of the lung from by-caught Atlantic white-sided dolphins(Leucopleurus acutus). Aquatic mammals, 2001; 27(2):73-81

[28] Fitzgerald ML, Xavier R, Haley KJ, Welti R, Goss JL, Brown CE, Zhuang DZ, Bell SA, Lu N, Mckee M, Seed B. ABCA3 inactivation in mice causes respiratory failure, loss of pulmonary surfactant, and depletion of lung phosphatidylglycerol. Journal of lipid research. 2007 Mar 31; 48(3):621-32. https://doi.org/10.1194/jlr.M600449-JLR200

[29] Van Pelt DR, Wingfield WE, Hackett TB, Martin LG. Application of Airway Pressure Therapy in Veterinary Critical Care: Part II: Airway Pressure Therapy. Journal of Veterinary Emergency and Critical Care. 1993 Jul; 3(2):71-81. https://doi.org/10.1111/j.1476-4431.1993.tb00104.x

[30] Meegan JE, Yang X, Coleman DC, Jannaway M, Yuan SY. Neutrophil-mediated vascular barrier injury: role of neutrophil extracellular traps. Microcirculation. 2017 Apr; 24(3):e12352. https://doi.org/10.1111/micc.12352

[31] Klein SG, Serchi T, Hoffmann L, Blömeke B, Gutleb AC. An improved 3D tetraculture system mimicking the cellular organisation at the alveolar barrier to study the potential toxic effects of particles on the lung. Particle and fibre toxicology. 2013 Dec; 10(1):1-8. https://doi.org/10.1186/1743-8977-10-31