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Histopathology of Rat (*Rattus norvegicus*) Lungs in Various Freshwater Drowning Conditions

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ABSTRACT

Background: Drowning remains a significant cause of mortality, with complex pathophysiological mechanisms that are often challenging to diagnose. Histopathological examination of the lung is one of the tests that supports the diagnosis of the cause of death and manner of death in drowning cases. Objective: This study aims to analyze the histopathological features of rat lungs drowned in freshwater under conscious, unconscious, and post-mortem conditions. Methods: A descriptive experimental study by dividing the rats into three conditions; conscious, unconscious and post-mortem drowning. The lung tissues collected from the drowned rats, processed, stained with hematoxylin eosin and observed under the microscope. The results stated in descriptive form and scores. Results: In Group 1, alveolar edema (score 2), hemorrhage (score 4), inflammatory cell infiltration (score 2.2), and alveolar thickening (score 2.4) were observed. Group 2 exhibited a similar histopathological pattern to Group 1. Group 3 showed mild alveolar edema (score 1), massive hemorrhage (score 4), extensive inflammatory cell infiltration (score 4), and severe alveolar thickening (score 3). Conclusion: Histopathology of lung tissue of rats that drowned alive showed moderate alveolar edema, massive hemorrhage, moderate inflammatory cell infiltration and moderate alveolar thickening. Rats that drowned unconsciously showed moderate alveolar edema, massive hemorrhage, moderate inflammatory cell infiltration and moderate alveolar thickening. Rats that drowned after death showed minimal alveolar edema, massive hemorrhage, massive inflammatory cell infiltration and severe alveolar thickening.

1. INTRODUCTION

Drowning remains as one of the significant causes of death with complex pathophysiology and that are often challenging to diagnose. Drowning events can occur in places where water is involved such as the sea, rivers and bathrooms. Although most occur accidentally, it does not rule out the possibility that drowning becomes a way of suicide or murder (Dahlan & Trisnadi, 2019; Armstrong & Erskine, 2022). The drowning case of a University of Indonesia student in 2015 is an example of a drowning case with unclear cause and manner of death. The body of a 19-year-old boy named Akseyna was found floating in Lake Kenanga with a rock-contained backpack on his back (Kompas, 2023). In another case, the bodies of teenagers named Handi and Salsa, whose bodies were found in the Serayu River, were also found to have been involved in an accident far from the river before being dumped. However, the condition of the victims before being dumped in the river could not be confirmed as alive or dead (Detik News, 2022). Both issues could have been further investigated with an accurate histopathology examination from the forensic department.

There are 372,000 drowning deaths per year in the world. This incident ranks in the top ten causes of death in people aged 1-24 years according to WHO (2014). They also states that drowning occurs three times more in developing countries than in developed countries. According to BASARNAS Cilacap's data (2023), the total number of drowning incidents in 2021 was 52 cases with 40 deaths, in

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2022 there were 57 cases with 46 deaths, while in the January-August period in 2023 there were 33 cases with 26 people who died.

Victims lose their lives in drowning events due to asphyxia, which is when the lungs become partially or completely deprived of oxygen supply and cause respiratory disorders. When the body tries to seek more oxygen, water enters the lungs, triggering inflammation in the alveolus and eventually causing death (Dahlan & Trisnadi, 2019).

Histopathologic microscopic examination is performed by looking at pieces of lung tissue and seeing if there are changes in tissue structure under the microscope lens. Histopathologic examination is useful for finding the cause of death of drowning death cases when crime scene investigation and autopsy by experts are not accurate enough to make a diagnosis (Dettmeyer, 2014; Hadjiev et al., 2022). Syamsun et al. (2022) conducted research related to differences in the histological picture of the lung tissue of rats drowned in fresh water and sea water. The results showed a picture of edema, hemorrhage and inflammatory cell infiltration in rat lung tissue in both examination groups. This supports the statement that histopathological examination can help determine the cause (cause of death) and mechanism (manner of death) of drowning cases. Based on this, the author is interested in examining the histopathological picture of the lung tissue of rats conscious, unconscious, and post-mortem drowning. Rats were chosen as test subjects because their respiratory system shares similarities with humans in terms of lung tissue response to hypoxia and edema. This study is an original experimental descriptive research.

2. METHOD

Tools and Materials

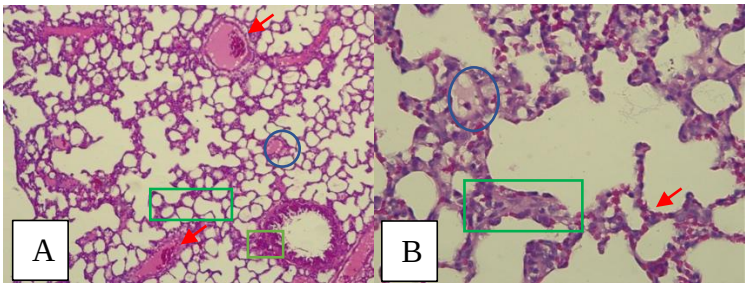
The tools needed for this study are two 60x30x30 cm rat cages for the acclimatization period, three 20x15x23 cm rat cages for the drowning of experimental animals, light microscope, embedding cassette, 60 cc sterile urine pot, object glass, cover glass, scalpel, anatomical tweezers, 1 cc syringe, 26Gx1/2 “syringe, microtome and tissue processor. The materials needed for this study are 15 rats (Rattus norvegicus) with the inclusion criteria of male white rats, aged 2-3 months, with a body weight of 150-200 grams and healthy which are divided into 3 groups, each group consisting of 5 rats, AD-II experimental animal feed, husks, black plastic, cardboard, hematoxylin dye, eosin dye, paraffin wax 1 kg, formalin buffer NBF 10% 2 L, ketamine 100 ml, immersion oil, canada balsam, ethanol or alcohol, aquadest and xylene.

Research steps

a. Test Animals and Aclimatization
b. Selecting rats according to the inclusion criteria, acclimatizing rats for 3 days in the laboratory, and providing nutrition to rats twice a day every morning and evening to maintain the body weight and health of the rats.
c. Drowning Procedure
d. Group 1 test animals were not given special treatment, group 2 test animals were anesthetized with ketamine intramuscularly at a dose of 0.2 mL, injected on the lateral side of the thigh while test animals in group 3 were euthanized via cervical dislocation to ensure death before drowning. Furthermore, the group of rats was put into a small cage and drowned by dipping and then pulled repeatedly for 10 minutes until the test animals died completely.
e. Sample collection and staining
f. Histopathological Analysis

3. RESULT AND DISCUSSION

Result

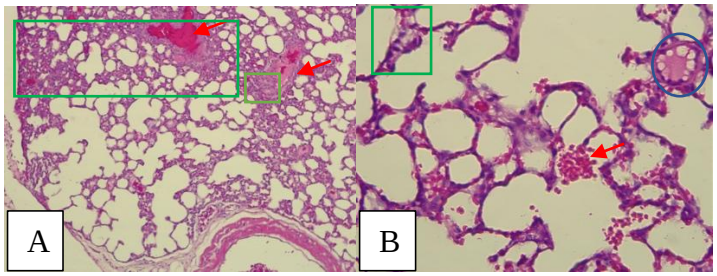


Picture 1. Photographs of histopathological preparations of lung tissue of group 1 rats, H&E staining, (A) 100x and (B) 400x magnification, showing intraalveolar edema (blue circle), hemorrhage and vascular congestion (red arrow), inflammatory cell infiltration (green box) and thickening of alveoli septa (yellow box).

Table 1. Group 1's Score

	Intraalveolar edema	Haemorrhage / vascular congestion	Inflammatory cell infiltration	Alveoli thickening
Group 1 (Conscious)	2	4	2	2
	2	4	2	2
	2	4	2	2
	2	4	3	4
	2	4	2	2
Score Average	2	4	2,2	2,4

Based on Table I, test animals in group 1 showing image of intraalveoler edema around 11-40%, bleeding or capillary congestion >81%, infiltration of inflammatory cells 11-40% and thickening of alveoli 11-40% in lung tissue. This is in accordance with research by Ibrahim et al. (2022) which states that rats drowned alive in fresh water will show a histopathological picture of lung tissue in the form of a little alveolar edema, a little bleeding, infiltration of rare inflammatory cells and a slight thickening of the alveolar septa.

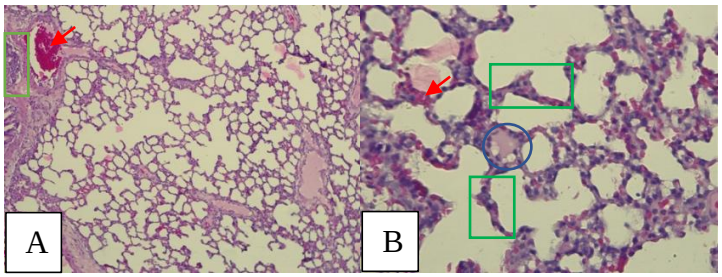


Picture 2. Photographs of Histopathology Preparation of Lung Tissue of Group 2 Rats, H&E staining, (A) 100x and (B) 400x magnification, showing intraalveolar edema (blue circle), hemorrhage and vascular congestion (red arrow), inflammatory cell infiltration (green box) and thickening of alveoli septa

Table 2. Group 2's Score

	Intraalveolar edema	Haemorrhage / vascular congestion	Inflammatory cell infiltration	Alveoli thickening
Group 2 (Unconscious)	2	4	2	2
	2	4	2	2
	2	4	2	2
	2	4	3	4
	2	4	2	2
Score Average	2	4	2,2	2,4

Based on Table 2, a picture of intraalveoler edema is obtained around 11-40%, bleeding or capillary congestion >81%, inflammatory cell infiltration 11-40% and thickening of the alveoli 11-40% in lung tissue.

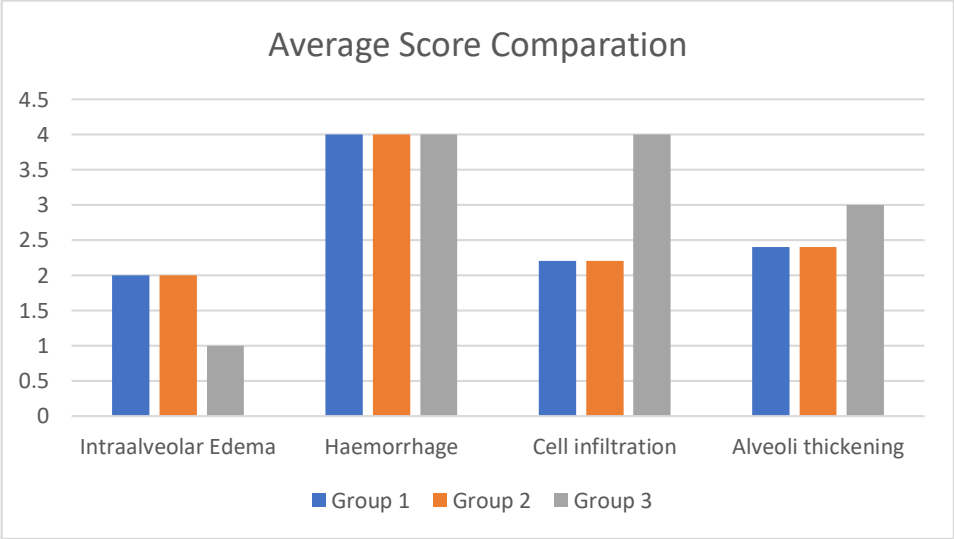


Picture 3. Photographs of Histopathological Preparations of Lung Tissue of Group 3 Rats, H&E staining, (A) 100x and (B) 400x magnification, showing intraalveolar edema (blue circle), hemorrhage and vascular congestion (red arrow), inflammatory cell infiltration (green box) and thickening of alveoli septa (yellow box).

Table 3. Group 3's Score

	Intraalveolar edema	**Haemorrhage / vascular congestion**	**Inflammatory cell infiltration**	**Alveoli thickening**
Group 3 (Post-mortem drowning)	1	4	4	3
	1	4	4	3
	1	4	4	3
	1	4	4	3
	1	4	4	3
Score Average	1	4	4	3

From Table 3, we could conclude that post-mortem drowning rats will have <10% intraalveolar edema, >81% capillary hemorrhage or congestion, >81% inflammatory cell invasion dominated by macrophage cells and 41-80% thickening of the alveoli membrane in the lung tissue.



Picture 4. Average Score Comparation

Discussion

The presence and severity of histological changes differed among the groups. Rats drowned while alive or unconscious showed similar profiles, indicating that respiration is necessary for the typical drowning pathology to develop. In contrast, post-mortem submersion

resulted in different histopathological patterns, marked by cellular necrosis and increased macrophage activity due to the absence of oxygen circulation and immune regulation.

According to Orlowski et al. (1987), drowning in fresh water will cause direct damage to the alveoli. When hypotonic water is aspirated into the respiratory tract, water will diffuse through the semi-permeable membrane into the intracellular due to decreased hydrostatic pressure in the interstitial. The entry of water into the cell causes hydropic degeneration characterized by a picture of swollen cytoplasm so that the cell looks pale. Hemorrhage or vascular congestion in this test group was found in all preparations in a massive condition, characterized by a picture of dilated capillaries filled with erythrocytes and a homogeneous mass of reddish color. Congestion may occur due to rapid inflow of fresh water into the respiratory tract. The presence of edema and capillary congestion will trigger infiltration of lymphocyte inflammatory cells. Lymphocyte inflammatory cells can be found in areas of alveolus and peribronchovascular thickening. Thickening of the alveoli septum appears on the medial part of the lung tissue, especially around the bronchi. This can occur due to inflammation so that the interstitial tissue is filled with swollen cells. (Pellondo'u *et al.*, 2021; Arsmstrong & Erskine, 2018; Chaudhari *et al*., 2016).

Suresh *et al.* (2024) wrote in their study that the histopathology of lung tissue due to drowning there was massive congestion and edema yet only half of the cases had bleeding. When rats are anesthetized, they will be put in an unconscious condition but the body still functions normally. Therefore, there is still aspiration of water through the nose and mouth which led straight to the lungs. In this group's slides, images of lymphocyte cell infiltration and alveoli thickening were seen around the bronchi.

Dead organism's body can longer function physiologically. After rats are terminated by cervical dislocation, the connection between the brainstem and spinal cord is severed so that death occurs rapidly. The tissues of the dead organism will not have an oxygen supply resulting in tissue hypoxia which later turns into anoxia. This condition causes massive cell necrosis. Cells that have been damaged and die will be phagocytized by macrophage cells so that they appear around the alveoli in lung tissue preparations (Abbas *et al.,* 2020). Thickening of the alveoli can occur due to edema, fibrosis, or contact with pollutants. Irritation may occur due to the ingress of chaff particles into the respiratory tract of rats. If particles from chaff are aspirated into the lungs, the first-line innate immune system will be activated by summoning alveolar macrophages. This could also be the reason why many macrophage cells were found in the preparate (Hu & Christman, 2019; Malainou *et al.*, 2023; Ma'rufi, 2016). Differences in the histopathological preparation of lung tissue can also be caused by differences in the duration of death of the rats. At the time of termination there was one rat that did not die immediately after cervical dislocation. The post mortem rats before drowning affects the physiology of the rat's body which later results in changes in the histopathological picture of lung tissue.

These findings align with previous studies highlighting the forensic value of lung histopathology in distinguishing antemortem from postmortem drowning. The observed differences can aid forensic pathologists in confirming whether submersion occurred before or after death.

4. CONCLUSION

In rats drowned consciously, moderate intraalveolar edema, massive vascular bleeding or congestion, moderate inflammatory cell infiltration and moderate thickening of alveoli septa were observed. Rats drowned in the unconscious state showed moderate intraalveolar edema, massive vascular hemorrhage or congestion, moderate inflammatory cell infiltration, and moderate thickening of alveoli septa. In rats drowned in the dead state, there was mild intraalveolar edema, massive bleeding or vascular congestion, massive inflammatory cell infiltration and relatively much thickening of the alveoli septa. For further research, it is recommended to consider making healthy rat controls as a comparison with other groups, replacing husk material with other materials that cannot irritate organs, terminating rats to the maximum, weighing lung organs and

the results are used as one of the comparison parameters for each treatment and binding rat bronchioles before immersion in formalin for a more optimal histopathological picture.

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