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HISTOLOGY OF LUNG AND KIDNEY ORGANS OF WHITE RATS (Rattus norvegicus) THAT DIED FROM DROWNING AND FORCED DROWNING IN SEAWATER

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ABSTRACT

This study aims to analyze histological changes in the lung and kidney organs of white rats (Rattus norvegicus) that died from drowning and being submerged in seawater. The method used in this study included microscopic observation with hematoxylin-eosin staining of lung and kidney tissue. The parameters identified included alveolar edema, inflammatory cell infiltration, and blood vessel bleeding in the lungs, as well as tubular necrosis and glomerular congestion in the kidneys. The results of the analysis showed that there were significant differences in the histological parameters of the lungs and kidneys between the treatment groups and postmortem time. Vascular bleeding in the lungs showed a statistically significant difference ($p = 0.009$). The submerged white rat group (P1) showed faster pathological changes in lung and kidney tissue compared to the drowned white rat group (P2). The conclusion of this study is that there are histological changes in the form of alveolar edema, inflammatory cell infiltration, and blood vessel bleeding in the lung organs, while in the kidneys there are histological changes in the form of glomerular congestion and tubular necrosis, so these results have the potential to provide important information in determining the cause of death due to drowning and submersion and support the development of forensic science.

Keywords: Forensic, Kidney, Histology, Lung, Drowning

INTRODUCTION

Forensic science plays an important role in law enforcement efforts as it collaborates with various scientific disciplines to uncover events related to crimes or unnatural deaths. Through a scientific approach, forensic science is able to provide objective evidence that can be legally accounted for, whether it involves biological, chemical, or physical analysis[1]. One of its applications in solving problems in the field of forensics is the autopsy, which is a postmortem examination aimed at investigating sudden, suspicious, mysterious, unwitnessed, unclear, unexplained, or unexpected deaths, as well as deaths legally considered criminal, work-related, or medical-related. The results of forensic autopsies are a crucial part of the evidence-gathering process, providing accurate information to support legal decisions[2] The pursuit of truth in specific case investigations is carried out through forensic biology.

Forensic biology makes a major contribution to uncovering scientific evidence through biological approaches in criminal cases, especially by analyzing biological samples[3]. In death cases, branches of forensic biology play an important role in investigations, including forensic pathology, forensic entomology, and molecular examinations such as DNA analysis[4]. One type of death case requiring forensic biology is drowning. Histopathological analysis has been proven to support forensic diagnosis in such cases, as it reveals tissue changes caused by hypoxia experienced by the victim.

Several previous studies emphasize the importance of examining vital organs to detect specific signs in drowning cases. Lunetta et al. reported alveolar edema and pulmonary hemorrhage as key indicators. [5]Meanwhile, Zhang et al. examined kidney organs, particularly identifying glomerular congestion and tubular necrosis as bodily responses to hypoxia. [6]

Drowning can cause pathological changes in organs, which are important to analyze for forensic purposes. According to Budiyanto (1997), drowning is defined as an incident in which fluid enters the body through the respiratory tract, leading to asphyxia due to oxygen deprivation. In drowning events, the entire body does not need to be submerged in water—if the head, particularly the nose and mouth, is submerged, it is already considered drowning[7]

The World Health Organization (WHO) reported that approximately 0.7% of global deaths each year are caused by drowning[8]. Drowning-related deaths remain frequent. Considering Indonesia's geography—surrounded by water and with an extensive coastline—this may contribute to the high rate of drowning deaths. According to data from

Sanglah General Hospital (RSUP) between 2010–2012, there were 97 drowning cases, dominated by male victims[9]

Drowning can occur in various types of water, such as freshwater (rivers, lakes, or pools) and saltwater (sea)[10]. Victims may be found in either freshwater or saltwater, but the impacts differ. In freshwater drowning, fluid is absorbed significantly because its electrolyte concentration is lower than blood, leading to hemodilution and water entering alveolar capillaries, which may cause hemolysis. In contrast, saltwater drowning causes hemoconcentration, as the higher electrolyte concentration draws water out of the pulmonary circulation into the interstitial space of the lungs. This results in hypovolemia, increased blood magnesium levels, heart failure, and may lead to death[11]

According to Budiyanto, several terms are associated with drowning, including: wet drowning, in which fluid enters the victim's respiratory tract; dry drowning, which occurs when fluid does not enter the respiratory tract due to laryngeal spasm; secondary drowning, where victims die several days after being rescued due to complications; and immersion syndrome, in which death occurs due to vagal reflex after immersion in cold water[12]

Drowning-related deaths are common; therefore, examinations of drowning victims are crucial. One type of examination is the analysis of the lungs and kidneys, as these organs are key indicators to determine whether death was truly caused by drowning, forced drowning, or murder followed by submersion. The most common method is tissue-level examination, known as histology. To observe damage and changes in the lungs and kidneys, histopathology is applied[13]

In forensic science, histopathology serves as an important diagnostic method through microscopic analysis of cells and tissues to reveal pathological changes[14] Histopathological testing is highly important because it helps determine whether a victim truly died from drowning by examining pathological changes that occur postmortem—whether by drowning, forced drowning, or homicide followed by drowning

Based on comparisons with previous studies, it is evident that the main focus has generally been on lung examinations or diatom testing, while histopathological studies of drowning victims are still rare. Moreover, many studies distinguish between freshwater and saltwater drowning, though research could focus on just one type. Therefore, research on the histological features of lungs and kidneys in white rats (Rattus norvegicus) in drowning cases—identifying pathological changes at different time intervals—is important. Such studies can enrich forensic literature and provide supporting evidence for diagnosing drowning in seawater.

In this research, experiments were conducted on laboratory animals—white rats (Rattus norvegicus)—that were subjected to drowning or forced drowning in seawater. Histological examination of these rats was carried out to determine whether the subjects truly died from drowning, forced drowning, or homicide followed by drowning. Lung and kidney samples were collected for histopathological testing to observe pathological changes in these organs after drowning or forced drowning in seawater.

MATERIAL AND METHODS

This study employed an experimental design using a posttest-only control group design. A total of 40 male white rats (*Rattus norvegicus*), aged 2–3 months and weighing 200–300 g, were used as experimental animals. The animals were divided into four groups: (1) seawater drowning group, (2) forced-drowning group, (3) positive control group (sacrificed by cardiac puncture without drowning), and (4) negative control group (sacrificed by cardiac puncture followed by submersion in seawater). The research was conducted over three months (March–May 2025) at the Science Laboratory of Dhyana Pura University for animal handling and treatment, while histological preparations of the lungs and kidneys were processed at the Histopathology Laboratory, Veterinary Center, Denpasar.

Seawater samples were collected from Jasri Beach, Karangasem, with a salinity range of 30–35 ppt. Experimental animals in the drowning and forced-drowning groups were submerged in seawater until death was confirmed, after which necropsy was performed to collect lung and kidney samples. All specimens were fixed in 10% buffered formalin and processed using standard histopathological techniques, including fixation, dehydration, clearing, embedding in paraffin, sectioning at 10–20 µm, and hematoxylin-eosin (HE) staining. Prepared slides were examined under a light microscope at 400x magnification to assess pathological changes.

Histological assessment focused on identifying alterations in lung and kidney tissues. Lung pathology was evaluated for alveolar edema, inflammatory cell infiltration, and vascular hemorrhage, while kidney pathology included glomerular congestion and tubular necrosis. Observations were scored semi-quantitatively on a scale of 0–3 (0 = no change, 1 = focal change, 2 = multifocal change, 3 = diffuse change). Data were analyzed descriptively and statistically using the Kruskal–Wallis test with SPSS version 25 to compare tissue damage among treatment groups.

RESULT

a. Lung Histology

Histological examination was carried out on lung tissues from all treatment groups to assess the degree of damage resulting from the drowning process. The assessment was performed using a scoring system based on three main parameters: alveolar edema, inflammatory cell infiltration, and vascular hemorrhage. Scores were assigned as follows: 0 (no change), 1 (focal change), 2 (multifocal change), and 3 (diffuse change). The microscopic observations of lung tissue preparations from each group are presented

From the scoring data above, results can be summarized for each treatment group. Overall, the four groups demonstrated histological changes in lung tissues that reflect postmortem alterations due to drowning. These findings are supported by microscopic observations of lung tissues

b. Kidney Histology

Histological examination of kidney tissues from all treatment groups was conducted to evaluate postmortem changes. The assessment was based on two main parameters: glomerular congestion and tubular necrosis, observed at postmortem intervals of 1 to 3 hours. Scoring was carried out on a scale of 0 to 3, where 0 = no change, 1 = focal change, 2 = multifocal change, and 3 = diffuse change.

The histological examination of kidney tissues revealed variable changes across treatment groups at different postmortem intervals (1–3 hours). In the positive control (K+), glomerular congestion increased gradually from moderate (score 2) at 1 hour to severe (score 3) at 3 hours, while tubular necrosis progressed from absent (score 0) to moderate (score 2) within the same interval. In the negative control (K–), mild glomerular congestion and tubular necrosis were observed consistently, with scores ranging from 0 to 1 at 1 hour and progressing to 1–2 by 3 hours. In the forced-drowning group (P1), both parameters showed more pronounced damage, with glomerular congestion and tubular necrosis reaching diffuse changes (score 3) by the 3-hour interval. Similarly, in the drowning group (P2), marked glomerular congestion (score 3) and tubular necrosis (score 3) were evident at 2–3 hours postmortem. Overall, the findings indicate that both glomerular congestion and tubular necrosis are consistently present in drowned and forced-drowned animals, with severity increasing over time, particularly in the treatment groups compared to controls.

c. The Effect of Treatments on Lung and Kidney Histology

To evaluate whether there were significant differences among the treatment groups for the tested and observed parameters, statistical analysis was performed using the Kruskal–Wallis test. This test was selected because the observational data did not meet the assumptions of normal distribution and homogeneity. The analysis was intended to determine whether the treatments had a significant effect on the observed histological parameters. The results of the analysis for both lung and kidney tissue samples are presented in Table 1. below:

Table 1. Kruskal–Wallis Test Results

Organ	Alveolar Edema	Inflammatory Cell Infiltration	Vascular Hemorrhage	Tubular Necrosis	Glomerular Congestion
Lung	0.178	0.127	0.009	–	–
Kidney	–	–	–	0.672	0.185

Based on the Kruskal–Wallis test results for lung tissue histology, the p-values varied across parameters: alveolar edema ($p = 0.178$), inflammatory cell infiltration ($p = 0.127$), and vascular hemorrhage ($p = 0.009$). According to the significance criterion ($p < 0.05$), only vascular hemorrhage showed significant differences between treatment groups, while alveolar edema and inflammatory cell infiltration did not. Meanwhile, in kidney tissues, tubular necrosis ($p = 0.672$) and glomerular congestion ($p = 0.185$) both had p-values > 0.05, indicating no significant differences among treatment groups.

DISCUSSION

Lung Histological Changes

This study examined histopathological alterations in drowning cases, focusing on alveolar edema, inflammatory cell infiltration, and hemorrhage. In forced drowning (P1), alveolar edema appeared early and progressed rapidly to diffuse forms, driven by seawater hypertonicity and aspiration, which damaged alveolar structures. Natural drowning (P2) showed milder and more localized edema, while the trauma group (K+) developed edema from direct injury. The negative control (K–) displayed only late, minimal edema as a secondary postmortem effect. Inflammatory cell infiltration was most prominent in P1, beginning within one hour and spreading widely, reflecting a vital reaction to seawater aspiration. P2 and K– showed slower, localized infiltration, linked mainly to autolysis, while K+ showed strong infiltration due to trauma. Hemorrhage followed a similar pattern: P1 demonstrated diffuse bleeding from vascular rupture caused by intrapulmonary pressure, P2 showed lighter multifocal hemorrhage, and K+ presented significant hemorrhage that could mimic drowning artifacts. No hemorrhage occurred in K–, confirming that bleeding requires external triggers. Overall, the combination of diffuse edema, strong inflammatory infiltration, and hemorrhage in P1 indicates active drowning with vital physiological responses.

The microscopic view of the lung can be observed in:

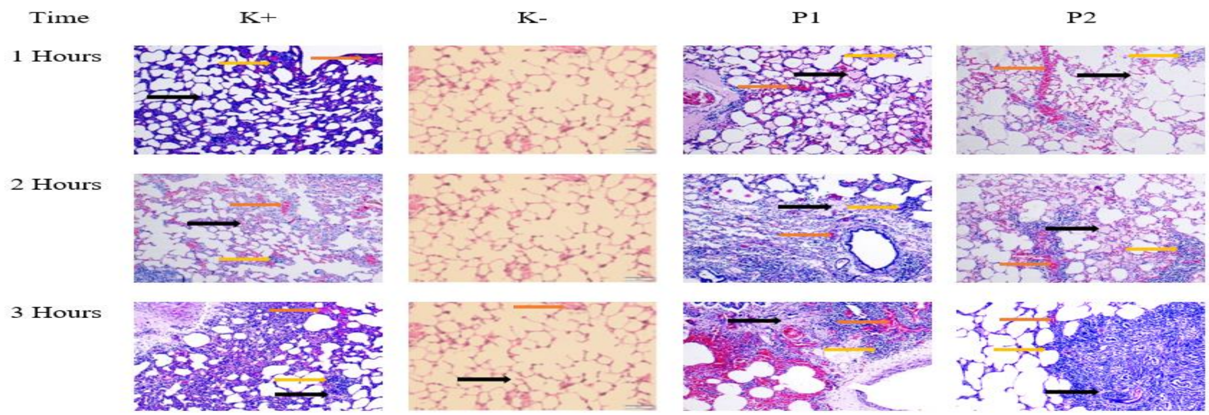

Figure 1 Microscopic view of the lung tissue in 4 treatment groups with a postmortem interval of 1 to 3 hours. Magnification 10x, HE staining.

Kidney Histological Changes

The kidney analysis focused on glomerular congestion and tubular necrosis. In P1, glomerular congestion was detected early and became widespread, resulting from hypoxia and venous stasis caused by forced drowning and the hypertonic effect of seawater. P2 also developed congestion but with slower progression and lower severity, reflecting a passive postmortem process. K+ exhibited progressive congestion due to trauma-related circulatory disturbance, while K– showed only mild, delayed congestion consistent with natural autolysis. Tubular necrosis appeared earliest and most severely in P1, characterized by epithelial cell detachment and ischemic injury driven by systemic hypoxia and osmotic imbalance. In P2, necrosis was slower and less intense, mainly linked to autolysis. K+ and K– showed only mild, late necrosis, associated with decomposition rather than hypoxic injury. These findings highlight that forced drowning produces the most severe and distinct renal changes, while passive drowning and controls exhibit weaker, secondary alterations. Thus, glomerular congestion and tubular necrosis may serve as supportive forensic indicators when combined with lung histology.

The microscopic view of kidney can be observed in:

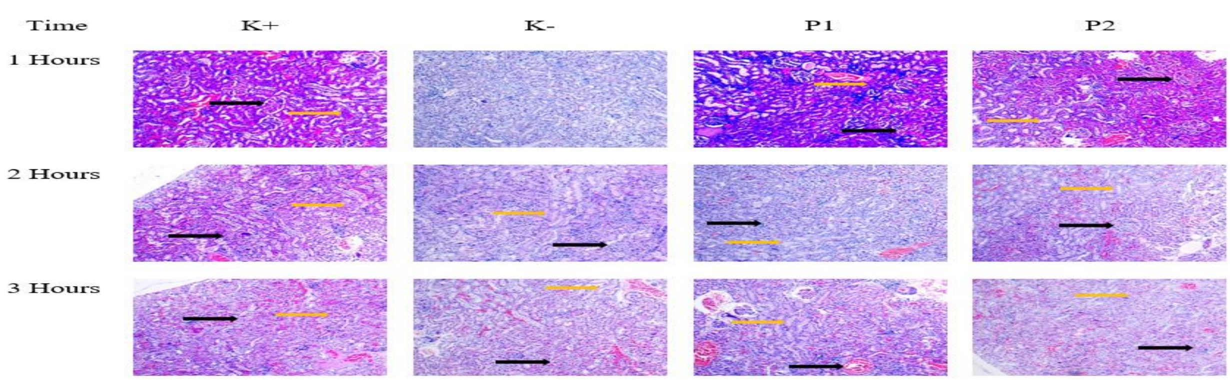

Figure 2. Microscopic view of kidney tissue in 4 treatment groups with a postmortem interval of 1 to 3 hours. Magnification 10x. HE staining. Black arrows indicate glomerular congestion, and yellow arrows indicate tubular necrosis.

CONCLUSSION

The histological examination revealed that in the lungs, rats subjected to forced drowning exhibited rapid and severe pathological changes such as alveolar edema, vascular hemorrhage, and inflammatory cell infiltration progressing significantly within 1 to 3 hours postmortem. In contrast, rats that drowned naturally showed slower and milder alterations within the same interval. Similarly, kidney histology demonstrated that the forced drowning group developed glomerular congestion and tubular necrosis earlier and more severely, with congestion scores of 2, 3, and 3 and necrosis scores of 2, 3, and 3 at 1, 2, and 3 hours postmortem, respectively. Meanwhile, the natural drowning group displayed later onset and comparatively milder changes.

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