Veterinary Biomedical & Clinical Journal

ISSN 2622-1012
Vet Bio Clin J. Vol. 6 No. 1, January 2024 pp: 41-46
DOI: 10.21776/ub.VetBioClinJ.2024.006.01.6

Review Article

Effects of Euthanasia on Animal Research

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Citation :
Maharani, N. P. R., Fadlyah, N. A., Setyaningrum, D. A. W., & Hairunisa, N. (2024). Effects of Euthanasia on Animal Research. *Veterinary Biomedical and Clinical Journal*, 6(1), 41-46. https://doi.org/10.21776/ub.VetBioClinJ.2024.006.01.6

Article history :
Received : 15 February 2024
Accepted : 28 June 2024

Abstract: The use of experimental animals is widely conducted in the medical field, both for research on the processes of disease occurrence (pathogenesis), the causes of disease (etiology), and research on therapy. Therefore, it is important to understand how euthanasia in experimental animals affects the integrity of the sample and its influence on research results. This article was compiled from several literature sources searched from Google Schoolar and PubMed published within the last 10 years, with keywords such as "code of ethics in experimental animals", "methods of euthanasia in experimental animals", "overdose anesthesia methods in animal euthanasia", "barbiturate injection methods in rats", "inhalation methods in animal euthanasia", "biochemical effects of euthanasia", and "histological effects of euthanasia". This paper discusses euthanasia methods, namely overdose anesthesia, inhalation, and injection. This paper aims to discuss how the method of euthanasia and the choice of drugs can impact the biochemical and histological changes in animals after the euthanasia process. Choosing the right euthanasia method is essential, as the species of animals involved and the research goals are considered. Using methods that align with the biological characteristics and behavior of the animals can help ensure the success of the research while also considering ethical aspects and animal welfare.

Keywords: animal research, animal welfare, euthanasia

INTRODUCTION

Euthanasia originates from the Greek language, euthanatos (eu = good, thanatos = death), thus interpreted as a gentle death without suffering (Rompegading & Putra, 2023), representing the depiction of animal death through a humane approach while still considering animal welfare. Some research involving experimental animals necessitates euthanasia before organ sampling, for example, in studies concerning organ function or the collection of large volumes of fresh blood samples (Andersen & Winter, 2019).

In certain research requiring euthanasia, it is important to consider the selection of euthanasia methods to ensure that post-mortem samples obtained remain fresh by minimizing tissue/organ damage (Rompegading & Putra 2023).

There are still knowledge gaps regarding the effects of euthanasia on certain research parameters such as hormone concentrations in animals and whether these hormonal changes can affect experimental results. Knowledge on how environmental factors, such as temperature, light, and noise during the euthanasia process, can influence the biological reactions of the animals still needs to be studied further.

The influence of euthanasia methods on the biological parameters observed in research can have significant effects (Hidayat & Wulandari, 2021), so choosing the appropriate euthanasia method

according to the research objectives is crucial to maintain the integrity of the observed biological parameters in the study. Timing of sample collection immediately after euthanasia and proper sample processing can also minimize the impact of euthanasia methods on the observed biological parameters. The compatibility of the observed biological responses with the research objectives is also crucial in the context of biomedical research, so it can be an important consideration factor in determining the method of euthanasia. The alignment of biological responses with research objectives plays a key role in ensuring the validity and relevance of biomedical research results to ensure that the research makes a significant contribution to scientific understanding and the development of clinical applications (Andersen & Winter, 2019).

In the context of biomedical research, validity and reproducibility are essential to ensure that research findings are reliable and relevant. This also allows other researchers to validate the findings and extend existing scientific understanding. To ensure validity and reproducibility, researchers must pay attention to proper research design, selection of appropriate methods, use of representative samples, accurate statistical analysis, and transparent reporting. In research methods, this includes determining the most appropriate euthanasia method. The selection of different euthanasia methods in a study may affect the reproducibility of the findings (Shomer et al., 2020). Different methods can have different effects on animals, leading to variations in research results. Consideration of pain and suffering experienced by animals during euthanasia may vary depending on the method used. This can affect the physiological and behavioral responses of the animals, potentially affecting the reproducibility of the findings.

METHOD

This literature review was carried out as an overview of the idea that aspects of euthanasia in research with experimental animals can influence the result either in physiologically, biochemically or in other parameters which can then influence the result and validity of a study. We collected 5 original articles obtained from Google Scholar and Pubmed. These articles were published in the last ten years and used various keywords related to euthanasia impacts in animal experimental, including the effects on physiology, biochemistry, and tissue histopathology.

COMMONLY USED EUTHANASIA METHODS

The methods used in euthanizing experimental animals are designed to ensure a quick and humane death with minimal suffering. There are several common methods used during euthanasia, and the species being studied also influences the type of method we will use. Anesthesia overdose, this method involves the use of an anesthetic drug at a very high dose, resulting in painless death. The drugs often used in this method are typically barbiturates which can cause central nervous system depression, resulting in the loss of consciousness followed by death (Thomas & Lerche, 2016). The application of this method is commonly used for larger animals such as dogs, cats, and horses. Because death in this method occurs very quickly and does not cause stress to the animal, it does not cause significant changes in the animal's tissues. This is done through intravenous or intraperitoneal injection. However, this method should only be performed by a veterinarian or trained and licensed professional (Leary & Johnson, 2020).

The inhalation method involves the use of an inhalant to induce painless death. The most commonly used inhalant is carbon dioxide (CO_2), while other permissible inhalants include

halothane, enflurane, methoxyflurane, and nitrous oxide. This method is usually used for small animals such as rats, hamsters, and rabbits. These animals will be placed in a closed room where the inhalation concentration is gradually increased. This method is effective for euthanizing large numbers of animals simultaneously and it causes minimal stress on rats, reducing the risk of neurotransmitter changes in the brain. However, it still needs to be done carefully by administering the inhalation dose accurately to avoid animal suffering due to hypoxia (Leary & Johnson, 2020).

And finally, the method of injection, also known as direct drug injection. This method is carried out in stages, starting with the injection of a sedative or anesthetic drug intraperitoneally until the animal is unconscious, followed by an intracardiac injection (directly into the heart) or through intravenous injection using high-dose barbiturate drugs as previously described (Thomas & Lerche, 2016). The injection method can cause changes in tissues and organs due to the pain from the injection process, which is felt by the rats (experimental animals), resulting in stress. This could potentially affect changes in neurotransmitters in the rat's brain. The selection of drug agents should also be considered because some drug agents used can cause hypoxia, such as Cobalt Chloride. However, this method is often used for animals of various sizes. This method also has a rapid effect when administered intravenously (Leary & Johnson, 2020). The use of euthanasia by injection is recognized as the fastest and most reliable method for euthanasia. This method is designed to minimize pain and suffering in animals, but there needs to be safety awareness in its use.

The choice of euthanasia method must be carefully considered, prioritizing animal welfare and personnel safety. An understanding of the biochemical and histological changes in animals after the application of euthanasia methods can help in research and the development of better practices in treating animals undergoing such conditions (Thomas & Lerche, 2016).

IMPACT OF EUTHANASIA ON RESEARCH PARAMETERS

Minimizing fear and discomfort in experimental animals can be done by minimizing physical contact in addition to implementing ethical compliance in addition to maintaining the accuracy and validity of the study (Wahyuwardani et al., 2020). Excessive fear will increase cortisol hormone concentrations due to uncomfortable situations, which can have an impact on the research parameters.

Biochemical and histological changes can occur in experimental animals and can be observed through biochemical analysis and histological examination. Biochemical analysis involves the study of the body's chemical components, such as enzymes, proteins, and metabolites. Meanwhile, histological examination involves observing the structure and composition of body tissues under a microscope. These changes can occur in response to various conditions, including disease, trauma, or the use of certain interventional methods such as euthanasia. Understanding these biochemical and histological changes can provide crucial insights into understanding animal health conditions, scientific research, and veterinary medical practice (Hidayat & Wulandari, 2021).

Physical methods such as blunt force trauma or decapitation can cause biochemical and histological changes in animals. However, there is no specific information about these changes. It is possible that the changes that occur may be influenced by stress, for example if the animals witness the euthanasia process of other animals, or euthanasia processes that do not cause immediate death, resulting in stress that affects the neuroendocrine conditions and can further affect the biochemical and histological structure of tissues/organs. A study showed that the levels of a powerful lipid mediator called prostanoid, which is involved in cardiovascular physiology and blood pressure, can

be affected by the euthanasia process. This could be due to the formation of post-mortem artifacts such as prostanoids in the brain, bone marrow, and kidneys, or the de novo formation of prostanoids due to external stimuli during animal euthanasia, such as prolonged hypercapnia or ischemia, especially impacting prostanoids in the spleen (Kratz et al., 2022).

Biochemical and histological examinations are also necessary to understand the impact of various euthanasia methods, but the statement emphasizes the need to carry out these methods correctly and humanely. The use of plastic cones to restrain animals can help reduce the pressure from handling and minimize the risk of injury to personnel (Hidayat & Wulandari, 2021).

Biomedical research and medical diagnostics require accurate analysis results to make precise diagnoses and design effective treatment methods. Ensuring sample integrity helps avoid or minimize experimental biases that may arise from changes or damage to the samples. This supports the internal validity of the research and confidence in experimental results. In therapeutic research and drug development, an accurate understanding of biological responses to specific substances is crucial. Intact and integrity-preserved samples support more accurate identification of research targets.

Some euthanasia route choices such as intravenous or intraperitoneal injection methods are considered to be the most acceptable euthanasia routes as they can provide effective distribution of euthanasia drugs to achieve the desired effect (Leary & Johnson, 2020; Thomas & Lerche, 2016). The addition of local anesthetics such as lidocaine or bupivacaine to the administration of pentobarbital can help reduce abdominal movements after euthanasia in laboratory rats.

However, using anesthesia before euthanasia can impact cytokine secretion in experimental animals. A brief anesthetic with isoflurane right before euthanasia by decapitation may have the least dampening effect on the measured cytokines. Conversely, prolonged anesthesia with isoflurane during the burn procedure does have a dampening effect. When performing longer procedures, ketamine-xylazine with buprenorphine seems to provide a better balance to avoid significant exaggeration or dampening of inflammation compared to isoflurane or pentobarbital. Also, a specific increase in the IL-6 response when buprenorphine was used in combination with isoflurane or pentobarbital during the burn procedure was observed, which may require further investigation (Al-Mousawi et al., 2010).

A comparison study in euthanasia method was made between CO_2 and cervical dislocation in rats to conduct metabolomics studies of the retina and retinal pigment epithelium (RPE) to understand retinal metabolism and degenerative diseases. CO_2 exposure for 5 minutes decreases ATP and GTP while increasing glucose and amino acid metabolism in the RPE/choroid. It's recommended to avoid CO2 in euthanasia, isolate quickly in cold HBSS, and store postmortem eyes in a culture medium at 4°C. Harvest the retina within 1 hour and RPE/choroid within 8 hours postmortem for stable metabolites (Zhu et al., 2018).

Thiamylal sodium (TM) is a derivative of barbituric acid. It is used as an alternative to phenobarbital sodium and secobarbital sodium for euthanizing mice. TM is effective at doses of 200 mg/kg or higher, but it has the potential to cause kidney and liver damage. Therefore, the choice of euthanasia drug depends on the objectives and research methods.

In research and diagnostics, compliance with standards and regulations is essential. Having integrity-preserved samples is evidence that the experimentation adheres to ethical guidelines and established practices. In the development of disease models, preserving sample integrity supports the formation of more realistic and relevant models, so that research results can be applied more widely

because validity and accuracy are maintained. The search results primarily discuss various methods of euthanasia and considerations for their use (Watanabe et al., 2024),

Laboratory rodents are commonly used for metabolomics studies involving tissues due to their similarity to human metabolism and ease of handling. However, the potential impact of anesthesia or euthanasia on metabolism during tissue sampling is often overlooked. A study by Overmyer et al. (2015) examined the effects of different anesthesia and euthanasia methods on collecting skeletal muscle, liver, heart, adipose, and serum from C57BL/6J mice. The study found that there were significant differences in metabolite levels based on the collection method. Samples taken after euthanasia showed increased levels of glucose 6-phosphate and other glycolytic intermediates in skeletal muscle, and several nucleotide and purine degradation metabolites in the heart and liver. Adipose tissue was less affected, but lactate and succinate levels were higher in euthanized animals. Ketamine showed greater variability compared to isoflurane and pentobarbital as pre-euthanasia methods. Based on these findings, it is recommended that for metabolomics studies, rodent tissue should be collected under anesthesia only, not euthanasia, to ensure optimal results (Overmyer et al., 2015).

The use of nitrogen anoxia and non-barbiturate anesthetic methods could be considered as appropriate alternatives for euthanizing piglets in transcriptomic research. This is because no significant differential expression in the protein-coding genes was observed between these euthanasia methods. However, it was found that small nuclear RNAs (snRNAs) involved in eukaryotic spliceosomal machinery were significantly higher in pituitary samples collected using nitrogen anoxia (log2fold change ≥ 2.0, and adjusted p-value ≤ 0.1). As snRNAs play an important role in pre-mRNA splicing and can subsequently affect gene expression, it's important to consider euthanasia as a potential confounding factor in research findings (Chakkingal et al., 2023).

CONCLUSION

The impact of euthanasia on research involving experimental animals can have a significant effect on the validity and reproducibility of a study. The use of euthanasia methods that are tailored to the research objectives and the biological and behavioral characteristics of the experimental animals can help ensure the success of the research, therefore we cannot ignore nor neglect the potency of euthanasia as confounding. Minimizing suffering is an application of the principle of animal welfare, which certainly plays a role in maintaining the validity of the research by ensuring that experimental results are not affected by uncontrolled stress or suffering. Clarity and transparency in reporting euthanasia methods are also important in order to help other researchers accurately replicate experiments.

ACKNOWLEDGEMENT

We would like to thank the Faculty of Medicine Universitas Trisakti for supporting this article publication. All of the authors confirm their contribution to the paper preparation. The authors did not receive support from any organization for the submitted work and declare no conflict of interest.

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