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Research Article

Effects of Dexamethasone on Locomotor Behavior in Adult Zebrafish Assessed Using AnimApp

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ABSTRACT

Corticosteroids are drugs that contain steroids which are useful for supplementing steroid hormones in the body when needed, suppressing immune function, and reducing inflammation. Undoubtedly, corticosteroids are the most widely prescribed class of drugs worldwide. However, this medication is also known to have side effects in psychiatry, including depression. This study aims to determine the side effects of depression induced by the use of corticosteroid drugs, specifically dexamethasone, through locomotor behavior analysis using an adult zebrafish model (Danio rerio). The drug induction method in this study involved administering dexamethasone 20 µl intraperitoneally, targeting the peritoneum, the central metabolic organ of the Zebrafish. Using a sample of 30 Zebrafish divided into 3 test groups consisting of treatment and control groups. This study uses open-source multiplatform software (animapp) to measure and monitor changes in Zebrafish behavior over a 9-day observation period. (0; 3; 6; dan 9). The results of this study show a significant effect on freezing locomotor behavior after dexamethasone induction in the dexamethasone treatment group of Zebrafish compared to the negative control group (a-sig 0.007) and the dexamethasone treatment group compared to the positive control group (a-sig 0.047). A significant effect was also observed in movement locomotor behavior after dexamethasone induction in the dexamethasone treatment group compared to the positive control group (a-sig 0.002) and the dexamethasone treatment group compared to the negative control group (a-sig 0.045). The significant effects of both locomotor behaviors after dexamethasone induction reflect depressive symptoms characterized by decreased motor activity and increased immobility periods. Therefore, further monitoring of dexamethasone use is necessary, especially for patients suffering from depression, anxiety, and other psychiatric illnesses, to avoid exacerbating their existing depressive symptoms.

Keywords: animapp; corticosteroids; locomotor; side effect of depression; zebrafish

INTRODUCTION

Corticosteroids are synthetic analogs of natural steroid hormones produced by the adrenal cortex, including glucocorticoids and mineralocorticoids. Corticoid drugs, such as glucocorticoids, are generally involved in metabolism and have immunosuppressive, anti-inflammatory, and vasoconstrictive effects (Yamaya et al., 2020). Mineralocorticoids, on the other hand, regulate electrolytes and water balance by affecting ion transport in renal tubule epithelial cells. These drugs have endocrine and non-endocrine indications. Their endocrine role is often in the management of adrenal insufficiency and congenital adrenal hyperplasia. Their non-endocrine role regularly leverages the anti-inflammatory and immunosuppressive effects of corticosteroids (Hodgens & Sharman, 2023; Pitre et al., 2025).

In practice, the term corticosteroid generally refers to glucocorticoid effects. Glucocorticoids are the primary stress hormones that regulate various physiological processes and are essential for life. Corticosteroids are one of the most widely prescribed classes of drugs globally, with an estimated market of over 10 billion USD annually (Hodgens and Sharman, 2023). Citing the British Prevalence Study in 2019, up to 10% of medical or surgical inpatients received

corticosteroids, representing 1-3% of the adult population. A common issue in society related to steroid drugs is the high intensity of people buying, obtaining, and consuming steroid drugs freely (Thibaut, 2019). Previous research by Kumala and Widia (2018) on the evaluation of corticosteroid use in pharmacies found a relatively high percentage of 66.25%, accompanied by a low level of knowledge at 58.25% or a knowledge score of <60%. The relationship between knowledge levels and corticosteroid use was also investigated by Muslikah et al. (2019), who found that out of 100 respondents, at least 90 respondents (90%) purchased corticosteroids at pharmacies without a prescription, and 47 respondents (47%) had low knowledge levels. Long-term and irrational corticosteroid use, such as freely buying, consuming, and abruptly discontinuing long-term drug therapy, can lead to adverse effects that can harm patients (Kumala and Widia, 2018).

Several examples of common long-term side effects associated with corticosteroid use include bone disorders (typically osteoporosis and fractures), adrenal suppression, hyperglycemia, cardiovascular disease, dyslipidemia, hypertension, immunosuppression, and neuropsychiatric disorders. Neuropsychiatric disorders in corticosteroid therapy can be defined as mania, agitation, mood instability, anxiety, insomnia, catatonia, depersonalization, euphoria, dementia, and finally, depression. Neuropsychiatric side effects arising from long-term corticosteroid therapy, according to Aditya (2016), are related to the release of glucocorticoids (GR), which triggers increased levels of Arginine Vasopressin (AVP) and Corticotrophin-Releasing Hormone (CRH). This activates the sympathetic nervous system and the Hypothalamic-pituitary-adrenal (HPA) axis, which are important biomarkers related to patient mood changes.

Previous research by Marrow et al. (2015) reported that at least 26 patients with retinitis and uveitis, who were initially free of psychiatric illness, developed mania, and 10% experienced depression during corticosteroid therapy. Generally, symptoms appeared within the first 3 days of drug administration and then continued for up to 8 subsequent days, with severe depression frequently reported in long-term and high-dose therapy, while manic symptoms were more often reported in short-term therapy. Generally, mood and cognitive changes caused by corticosteroid therapy are mild and reversible (Marrow et al., 2015). Additionally, research by West and Kennedy (2014) reported an incidence of mental side effects caused by steroid therapy ranging from 2% to 60%. Cases of mental incidence were also reported in a literature review by Lesko et al. (2021), showing that patients receiving corticosteroids at a dose of 40 mg/day experienced acute psychosis, including delusions, paranoia, hallucinations, and insomnia, followed by a percentage of neuropsychiatric effects associated with steroid psychosis including depression (35%), mania (31%), agitation, anxiety, insomnia, delirium (13%), dementia, and even psychosis (14%). A double-blind placebo-controlled experiment on corticosteroid treatment reported that 75% of patients experienced mood and cognitive problems that resolved after steroid therapy was discontinued (Alturaymi et al., 2023).

In initial screening, motor behavior analysis is increasingly used in the detection of neurobehavioral disorders. Adult zebrafish have emerged as a simple and efficient model for studying locomotion, defined as the movement of fish from one place to another using various methods (Zug, 2022). Based on the background above, the researcher is interested in investigating the depressive side effects related to the drug mechanism of corticosteroids using a zebrafish model. For this behavioral analysis, manual analysis cannot be used, thus requiring a specialized tool to detect fish movement. An example is the open-source Animapp application, which offers advantages such as lower cost and the ability to depict fish movement by analyzing frame-by-frame.

METHODS

Equipment

In this study, the tools used include tanks or aquariums with clear thinwall containers measuring 17.5 x 12.2 x 6.8 cm, an OHAUS analytical balance, a surgery table, loops, fish nets, a thermometer, a 1 cc syringe, a 10-cc syringe, a 0.5 cc insulin syringe, an Infinix 40 Pro mobile phone camera, AnimApp software, and SPSS 26 software. The materials used in the research include 0.9% NaCl and dexamethasone 5 mg/1ml, alarm pheromone, artemia fish feed, and ice cubes.

Preparation of Test Animals

The study received ethical approval under No: 017-KEP-UB-2024. Before the experiment, five-month-old zebrafish underwent a one-week acclimatization period, adhering to ethical standards set by the Canadian Council on Animal Care (2020). Zebra fish were obtained from the Faculty of Fisheries and Marine Science, Brawijaya University. Regarding the maintenance of zebrafish before the experimental study was conducted. Acclimatization was carried out for 7 days to ensure the zebrafish's health and adjustment to the new habitat. Acclimatization is carried out in a thin wall tank containing 1 tank with 1 fish and 800 mL of water, with a light cycle of 14 hours of light and 10 hours of darkness (14:10) (Demin et al., 2021). With regular feeding provided twice a day. The acclimatization process aims for the test animals to adapt to the new environment (Muchdar et al., 2020).

The test animals were fasted for 1 day before the test was conducted. This was done to ensure the test animals were in the same condition and to reduce the influence of the food consumed on the absorption of the administered drug (Deswati et al., 2020). The groups in the study consist of 3 test groups: (1) positive control test group, exposed to alarm pheromones; (2) negative control test group, treated with saline solution (NaCl); (3) dexamethasone treatment test group. Each test group consists of 10 research subjects. Thus, the total number of research subjects or research samples consists of 30 samples.

Before the induction of dexamethasone depression in Zebrafish intraperitoneally. It is necessary to perform the anesthesia stage first to eliminate pain when the test animal is injected. The anesthesia guidelines for Zebrafish follow the anesthesia procedures approved by (*Appendix a: Anesthetic Agents for Use in Fish*, n.d.). The anesthesia method used is the Hypotermia method. This method is used because it is classified as short-term anesthesia. Anesthesia was performed by cooling the container containing water and ice, maintaining a stable temperature of below 10°C (Ackerman et al., 2005). Monitor the behavior of the injected Zebrafish when the research sample does not react when held.

Dexamethasone induction in fish is performed using an insulin syringe with a dose of 20 µl intraperitoneally. Induction of intraperitoneal injection in zebrafish is not recommended with large doses and should not be repeated (Kalueff & Cachat, 2011). Therefore, in this study, the induction of dexamethasone was only performed once post-anesthesia of the fish. After induction, the Zebrafish are placed in a recovery tank for 5-10 minutes depending on the individual Zebrafish's response to the anesthetic effects. Generally, the effects of anesthesia wear off completely within 10 minutes, and the subjects are ready to be observed.

Observation and Analysis of Zebrafish Behavior

The locomotor observation of Zebrafish with behavioral parameters such as freezing and movement was conducted over 9 days, with observation times on the sample induction day and every 3 days thereafter (0, 3, 6, dan 9). The position of the recording device is placed directly above the observation tank. Observation was conducted by recording the behavior of Zebrafish for 3 minutes. Raw data collection related to Zebrafish behavior was conducted with the help of behavior analysis software using Animapp. It is a platform for analyzing the movement of objects in videos. It operates on the principle of analysis by extracting position information from each frame of the analyzed video. Objects that can be used range from flies to mice. The AnimApp application will not run if it does not use the command prompt from the Python system, specifically Anaconda (Rao et al., 2019). Animapp recognizes the most common video formats (mp4, mkv, avi) at a resolution of 640x480 or higher. But before being analyzed, the video format needs to be adjusted first. The video will not be processed if the threshold for video format settings for analysis is not adjusted (Rao et al., 2019). Behavioral movement analysis is conducted by running the command "Animapp" on the Anaconda prompt. Detected objects are marked with a yellow circle as shown in Figure 1. The output from the data analysis is presented in the form of an Excel spreadsheet with 4 columns consisting of: (1) Column A (horizontal movement of the test animal or x-axis); (2) Column B (vertical movement of the animal or y-axis); (3) Column C (frames); (4) Column D (distance of test animal movement).

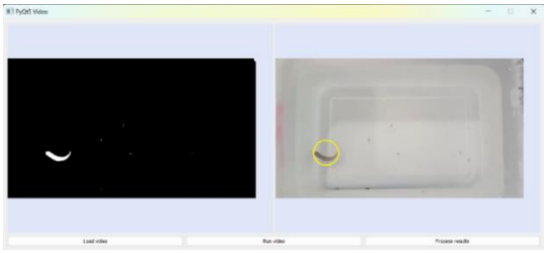

Figure 1. Video Analysis in Animapp

RESULTS AND DISCUSSION

One of the parameters for measuring anxiety in Zebrafish is freezing behavior. The influence of freezing behavior in each group shows that there are significant differences in the dexamethasone-positive control group (a-sig 0.047) and the dexamethasone-negative control group (a-sig 0.007). It is observed that the dexamethasone treatment group experiences more freezing compared to both control groups. This is related to the increase in glucocorticoid hormones, which leads to a decrease in the activity of the prefrontal cortex (PFC) and hippocampus, disrupting the feedback of the hypothalamic-pituitary-adrenal (HPA) axis.

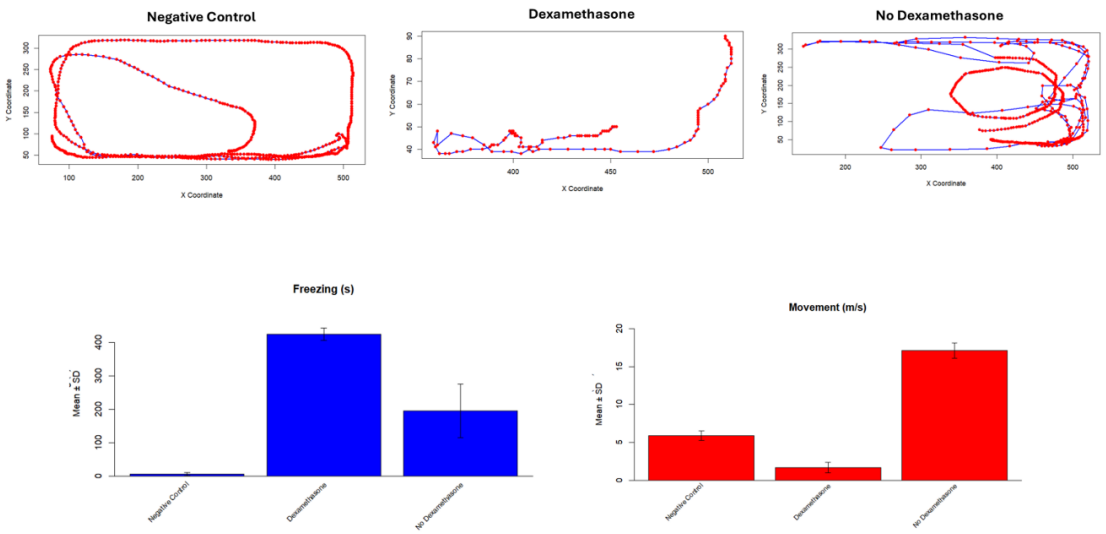

Figure 2. Post-Hoc Freezing Test Result for Zebrafish Movement on Day 0

This results in a sustained increase in cortisol, causing the core symptoms of depression such as anhedonia or demotivated behavior (Cooper et al., 2018; Skórzewska, 2021). Significant differences were observed between the dexamethasone treatment group and the negative control group (a-sig 0.045) and between the dexamethasone treatment group and the positive control group (a-sig 0.002). In the dexamethasone treatment group, there was less movement behavior compared to the negative control group and the positive control group. This is related to the aspects of depression or depressive symptoms in terms of motivation and motor behavior. Generally, patients experiencing depression exhibit symptoms from the motivational aspect such as (1) feelings of lack of motivation or difficulty in starting activities; (2) decreased level of social participation or interest in social activities. Changes in motor behavior due to depressive symptoms include moving or speaking less than usual (Al Aziz, 2020; Hindradjat et al., 2021).

Testing the side effects of dexamethasone in terms of freezing behavior showed significant differences on day 0 and day 9 (a-sig 0.005). This means that the side effects of dexamethasone are "reversible" or can return to their original state. Generally, mood and cognitive changes caused by corticosteroid therapy are mild and reversible (Morrow et al., 2015). There was no significant difference on day 0 and day 9 in the positive control group (a-sig 0.139) where the side effects of depression induced by alarm pheromone could not return to their original condition. This is because the administration of alarm pheromone is a strong fear trigger in zebrafish (Egan et al., 2009). In the negative control group, there was no significant difference observed on day 0 and day 9 (a-sig 0.386) triggered by several factors that could cause stress in zebrafish. The factors that could stress zebrafish include poor water quality, inadequate environmental conditions, and the absence of supportive substrates for hiding (Council, 2020). In this study, it was found that there was a difference in movement behavior in zebrafish treated with dexamethasone on days 0 and 9 (a-sig 0.005), indicating that the side effect of depression caused by dexamethasone induction in this group was reversible or could return to its original state. No significant difference was observed on days 0 and 9 in the positive control group (a-sig 0.075), where the side effect of depression induced by alarm pheromone could not return to its original condition. This is because the administration of alarm pheromone is a strong fear trigger for zebrafish (Egan et al., 2009). The lack of difference in the negative control group (a-sig 0.075) is triggered by several factors that can cause stress in fish.

Table 1. Results of Dexamethasone Induction on Zebrafish (Freezing Parameter)

Behavior / Treatment	*No Freezing*	*Freezing*	Total	*a-sig*	OR
Dexametason	3452	2548	6000		
No Dexametason	1502	4498	6000	0,000*	4,00
Total	4954	7046	12.000		

* : significance (<0,05) ; OR : Odd Ratio

Table 2. Results of Dexamethasone Induction on Zebrafish (Movement Parameter)

Behavior / Treatment	*No Movement*	*Movement*	Total	*a-sig*	OR
Dexametason	4661	1339	6000		
No Dexametason	2534	3466	6000	0,000*	4,7
Total	7195	4805	12.000		

* : significance (<0,05) ; OR: Odd Ratio

In this study, to determine the relationship between dexamethasone administration and the occurrence of freezing and movement, a Chi-square test was conducted. Raw data were obtained through the analysis of 600 frames of behavior from 10 fish in each group, resulting in 12,000 frames analyzed for each occurrence of freezing and movement behavior. It was found in the results presented in Table 1 that there was a difference in the number of freezing episodes between the group given dexamethasone and the group not given dexamethasone. The number of freezing episodes was more frequently observed in the group given dexamethasone compared to the group not given dexamethasone. Based on the output table results related to the correlation between dexamethasone induction and freezing events, it is known that a-sig (0.000) indicates a significant result, thus it can be interpreted that there is a relationship between dexamethasone induction and freezing events.

The increase in freezing episodes in this case is related to anxiety, which is a clinical symptom of depression. Whereas when the Zebrafish response experiences anxiety, it will exhibit more freezing behavior compared to moving behavior. Whereas when the Zebrafish response experiences anxiety, it will exhibit more freezing behavior compared to moving behavior. The anxiety response in Zebrafish is mediated by the hypothalamus-pituitary-interrenal (HPI) axis, which is functionally and homologously similar to the hypothalamus-pituitary-adrenal (HPA) axis found in human neuroanatomy (Demin et al., 2021).

It is known that in the calculation of the Odds Ratio (OR) value, the freezing behavior parameter showed an Odds value of 4.00, which means that the administration of dexamethasone fourfold can increase the incidence of freezing. This indicates that the risk of freezing is four times higher in the group treated with dexamethasone compared to the group that did not receive any intervention or the negative control group that was only given a saline solution of Sodium Chloride, which had no effect on the test animals. As shown in Table 2, dexamethasone induction significantly impacts movement behavior. We observed a distinct decrease in movement episodes in the dexamethasone-treated group compared to the control group. There is a difference in the number of movements between the group given dexamethasone induction and the group not given dexamethasone. Where the movement behavior is more commonly found in the group that was not given dexamethasone compared to the group that was induced with dexamethasone. Based on the output table results related to the correlation between dexamethasone administration and movement behavior (a-sig 0.000), it can be interpreted that there is a relationship between dexamethasone induction and movement behavior. The decrease in movement episodes in this case is related to anxiety, which is a clinical symptom of depression. The Odds Ratio (OR) for movement events was calculated to be 4.7. This indicates that dexamethasone administration reduces the likelihood of movement by 4.7 times. Consequently, the risk of movement events is 4.7 times lower in the dexamethasone-treated group compared to the control group.

CONCLUSION

This research provides partial support for the theoretical understanding of depression as a side effect of corticosteroid therapy, particularly when using dexamethasone. The study found evidence of depression-like side effects induced by dexamethasone in zebrafish models, characterized by altered behaviors. Importantly, these observed side effects were reversible. The findings indicate a clear association between dexamethasone induction and these behavioral changes, suggesting a significant impact on the well-being of the zebrafish. Therefore, further monitoring of dexamethasone use is necessary, especially for patients suffering from depression, anxiety, and other psychiatric illnesses, to avoid exacerbating their existing depressive symptoms

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CONFLICT OF INTEREST

All authors declared that there was no conflict of interest.

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