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The Relationship Between The Total Count of Leukocyte Types and Formahldehyde Induction in Wistar Rats

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Abstract: Formaldehyde is a chemical compound with a high level of toxicity. In the industrial sector, formaldehyde is commonly found in cosmetic products, resins, adhesives, plastics, and wood products. Therefore, the objective of this research is to determine the effect of formaldehyde induction with concentrations of 20 ppm, 30 ppm, and 40 ppm on the total count of leukocyte types. This study is a qualitative research with a true experimental design employing a post-test only control group design. The study used male Wistar rats (Rattus norvegicus) totaling 24 individuals, which were divided into 4 groups: a control group and 3 treatment groups. Each group consisted of 6 rats induced with formaldehyde at concentrations of 20 ppm, 30 ppm, and 40 ppm. The Wistar rats will be terminated, and retro-orbital blood sampling will be conducted to count the various types of leukocytes in order to determine whether there are differences in leukocyte type counts among rats induced with formaldehyde. Data analysis will employ the Kruskal-Wallis test. The results of this study showed that there was a statistically significant difference ($p < 0.05$) between formaldehyde and the total count of leukocyte types. In this study, it was found that there is a decrease in the total count of leukocyte types in the formaldehyde-exposed groups at 40 ppm, 30 ppm, and 20 ppm when compared to the control group.

Keywords: Formaldehyde, leukocytes, total count of leukocyte types, toxicity, wistar rats.

Introduction

Formaldehyde is a chemical compound that classified as aldehyde. Formaldehyde is a product of the oxidative reaction of methanol to carbon dioxide (CO2) and water (H2O), which is colorless and highly toxic (Kang et al., 2021). Formaldehyde commonly used for industrial sector such as cosmetics, resins, adhesives, plastics, and wood products. Formaldehyde was chosen because of it's low cost and high purity (Gerberich *et al*, 2013). In the medical field, formaldehyde can be used as a preservative for corpses and also as medicine (Kang et al., 2021).

World Health Organization (WHO) recommends a limit for the use of formaldehyde in closed rooms, namely 0.1 mg/m3 or 0.08 ppm due to the high level of formaldehyde toxicity (Kang et al., 2021). Exposure to formaldehyde for a prolonged period can be highly dangerous. Due to high toxicity level of formaldehyde it can cause irritation to the eyes, nose, throat and lungs, other risks that can occur are loss of the ability to smell, increasing the possibility of damage to the lining of the upper respiratory tract, coughing, and wheezing (Asare-Donkor et al., 2020).

The International Agency for Research on Cancer (IARC) raised the classification of formaldehyde which was originally in group 2A (probably carcinogenic to human) to group 1 (carcinogenic to human) in 2012 (Nielsen *et al*, 2013). The toxic effects caused by formaldehyde can increase the number of B cells and decrease the number of T cells (CD3), T-cytotoxicsuppressor cells (CD8), with no increase or decrease in the number of T-helper-inducer cells (CD4) (Jia et al., 2014). Leukocytes, commonly known as white blood cells, are cells that function as the body's primary defense system against foreign pathogens that enter the body (Martínez-Castro *et al*, 2014). Leukocytes can be classified into 5 types: basophils, neutrophils, eosinophils, lymphocytes, and monocytes. These types of leukocytes have different functions. Basophils play a role in non-specific immune responses against pathogens and other organisms that can cause disease. Neutrophils, the most abundant white blood cells in the blood, play a major role in fighting off pathogens such as bacteria or viruses.

Eosinophils play an important role in responding to parasitic infections and can trigger allergic reactions. There are 2 types of lymphocytes, namely B lymphocytes and T lymphocytes. B lymphocytes are involved in the humoral immune response, while T lymphocytes play a role in recognizing certain pathogens and killing them. Monocytes are white blood cells whose role is to clean up dead cells in the body (Hall & Guyton, 2014). Based on the background mentioned, the role of white blood cells in the body's defense and the potential negative effects of formaldehyde, this study focuses on the impact of formaldehyde induction on the differential leukocyte count in Wistar rats. This research is expected to provide insights into the exposure limits of formaldehyde.

Material and Method

In this study we used 24 adults Wistar rats (Rattus norvegicus) with weight range 150-200 gram. They were kept on the same environmental condition. Wistar rats will be randomly divided into 4 groups, namely the control group and 3 treatment groups. Treatment group 1 will be induced with formaldehyde at a dose of 20 ppm, treatment group 2 will be induced with formaldehyde at a dose of 30 ppm, and treatment group 3 will be induced with formaldehyde at a dose of 40 ppm.

Table 1. The Comparison of the number of leukocyte types between the control group and the treatment group

Hitung Jenis Leukosit	Kelompok	n	Median	Rerata	SD	(minimum-maksimum)	Range
Neutrofil	Kontrol	6	4193,50	4608,33	1342.771	3556 - 7098	3542
	Formaldehida 20 ppm	6	2011,50	2073,83	1317,333	800 - 4507	3707
	Formaldehida 30 ppm	6	900,50	1255,33	1059,459	612 - 3400	2788
	Formaldehida 40 ppm	6	285,50	312,83	119,672	189 - 500	311
Eosinofil	Kontrol	6	550,50	515,00	195,092	267 - 750	483
	Formaldehida 20 ppm	6	98,50	208,50	266,979	87 - 753	666
	Formaldehida 30 ppm	6	85,00	83,67	13,692	62 - 100	38
	Formaldehida 40 ppm	6	25,50	36,00	30,430	4 - 87	83
Basofil	Kontrol	6	8,00	7,67	1,862	5 - 10	5
	Formaldehida 20 ppm	6	3,00	3,67	2,422	1 - 7	6
	Formaldehida 30 ppm	6	3,00	4,50	3,674	1 - 10	9
	Formaldehida 40 ppm	6	0,50	0,83	0,983	0 - 2	2
Limfosit	Kontrol	6	79,50	79,33	5,354	72 - 86	14
	Formaldehida 20 ppm	6	58,00	51,33	17,614	25 - 67	42
	Formaldehida 30 ppm	6	35,50	37,33	11,448	20 - 52	32
	Formaldehida 40 ppm	6	11,00	14.50	8,894	8 - 32	24
Monosit	Kontrol	6	671,00	586,00	198,920	200 - 736	536
	Formaldehida 20 ppm	6	758,50	769,83	78,216	663 - 867	204
	Formaldehida 30 ppm	6	164,50	204,17	173,737	55 - 541	486
	Formaldehida 40 ppm	6	89,00	156,00	158,910	32 - 459	427

*Sign. level at P < 0.05

Wistar rats will be induce by 10% formaldehyde consentration in 16 weeks. Formaldehyde will be injected into cotton placed inside the cage, which will then be covered with plastic to allow formaldehyde vapor to move horizontally. Subsequently, the formaldehyde vapor will be inhaled by the Wistar rats. Wistar rats will be exposed to formaldehyde vapor for 6 hours each day.After 16 weeks, Wistar rats will be terminated, and blood will be collected through retro-orbital sampling. Once the samples are gathered, blood smears will be prepared.

Subsequently, the count of different types of leukocytes will be conducted by observing the blood smear preparations using a microscope.

Result and Discussion

Result

In Table 1, it was found that there was a significant decrease in various leukocyte types, including neutrophils, eosinophils, basophils, lymphocytes, and monocytes. The most significant decrease was observed in the treatment group induced with a formaldehyde concentration of 40 ppm.

Discussion

In the research conducted using Wistar rats (Rattus norvegicus), a total of 24 rats were divided into 4 groups, including 1 control group (healthy) and 3 treatment groups induced with formaldehyde at different concentrations. Each group consisted of 6 randomly selected Wistar rats. The type of cage used was a cage type registered in HAKI by Dr. Anak Agung Ayu Niti Wedayani, M.Sc with registration number 000473934 in 2023. The Wistar rats were exposed to formaldehyde for 16 weeks each day, with exposure concentrations of 20 ppm, 30 ppm, and 40 ppm administered through formaldehyde-injected cotton. Each day, the Wistar rats were exposed for 6 hours. After 16 weeks, the Wistar rats were terminated, and blood was collected through retro-orbital sampling.

The formaldehyde solution used in this research was a 10% formaldehyde solution with formaldehyde concentrations of 20 ppm, 30 ppm, and 40 ppm. In a study conducted by Al-Sarraj *et al.* (2013) on rabbits exposed to inhalation of a 10% formaldehyde solution with a concentration of 12 ppm, it showed a significant decrease in the number of lymphocytes and eosinophils, but no significant decrease in the number of basophils and monocytes (Al-Sarraj et al., 2013). In the research conducted by Al-Sarraj et al., (2013), it was found that there was a significant increase in eosinophils and lymphocytes with a significant decrease in neutrophils among the 3 treatment groups compared to the control group. However, in the group of rats receiving treatment, there was no significant change in basophils and monocytes when compared to the control group (Al-Sarraj et al., 2013).

Referring to the results obtained from this research, the higher the concentration of formaldehyde used, the greater the effect. In this study, the comparison of leukocyte count values based on Table 1 shows a significant decrease, especially in the group of rats exposed to formaldehyde at a concentration of 40 ppm. In rats exposed to formaldehyde with the highest concentration of 40 ppm, there was a decrease in the count of leukocyte types (neutrophils, eosinophils, basophils, lymphocytes, and monocytes) compared to the control group. In the groups of rats exposed to formaldehyde at 20 ppm and 30 ppm, a significant decrease in neutrophils and monocytes was found, while there was no significant decrease in eosinophils, basophils, and lymphocytes. These results are slightly different from the findings of the study conducted by Al-Sarraj et al., (2013).

The results of this research show that the toxicity level of formaldehyde at high concentrations has an impact on the leukocyte count, with a higher exposure concentration resulting in a greater effect. A significant decrease in the count of neutrophils, eosinophils, basophils, lymphocytes, and monocytes was found in the research subjects when compared to the control group. This decrease in leukocyte count is supported by research conducted by H.-W. Kuo *et al.* (1996) on formaldehyde inhalation, which found a decrease in the level of WBC (white blood cell) (Kuo et al., 1997). In addition to the study conducted by H.-W. Kuo et al., (1996), the results of this research are also supported by a study conducted by Lyapina *et al.* (2003), where the study was conducted on 29 workers exposed to formaldehyde through the application of carbamide-formaldehyde adhesive in the industrial field.

The study stated that there was a significant decrease in the number of leukocytes in the research subjects (Lyapina et al., 2004). In the study conducted by van der Laan *et al.* (2022), which used formaldehyde exposure at concentrations greater than 10 ppm, it was found that there was no significant change in the relationship between the control group and the treatment group exposed to low-concentration formaldehyde (p=0.59). However, when comparing the control group, the treatment group exposed to high-concentration formaldehyde, and the treatment group exposed to low-concentration formaldehyde, a significant difference was observed (p=0.59). The study reported significant regression results regarding leukocyte reduction, and these findings represent a large population (van der Laan et al., 2022).

Factors influencing the decrease in the count of leukocyte types can be attributed to chromosomal damage caused by formaldehyde exposure through inhalation, leading to abnormalities and micronuclei in lymphocytes formed in the bone marrow (Ye et al., 2005)This cell damage occurs due to the release of Reactive Oxygen Species (ROS), which increases. Elevated ROS levels trigger oxidative stress, resulting in changes in DNA, protein, and lipid structures (Ramos et al., 2017). During the course of this research, the health of the Wistar rats was well-maintained, and they were provided with food and water before and after formaldehyde induction. During formaldehyde induction, there was a change in the behavior of Wistar rats, which went from being calm to exhibiting aggressiveness, as evidenced by their constant movement around the cage.

This change in behavior occurred because when Wistar rats were exposed to formaldehyde concentration for 6 hours, and the subsequent inhalation of formaldehyde could irritate the upper respiratory tract and eyes, leading to an increase in ROS, which could trigger oxidative stress. This indicates that rats experience stress when exposed to formaldehyde concentrations, and stress can be a factor that affects leukocyte production. This happens when ROS production increases (Pizzino et al., 2017). Furthermore, in the study conducted by van der Laan *et al.* (2022), it was stated that the effects of formaldehyde itself activate oxidative stress, which subsequently leads to a decrease in the production of leukocytes.

This decrease occurs due to damage to DNA, leading to the formation of a DNA methylation-based biomarker of leukocyte telomere length (DNAmTL). DNAmTL is a genetic factor sensitive to chemical exposure, and its function is to serve as a biomarker for assessing telomere length. Shortening of telomeres on leukocyte chromosomes occurs. Telomere shortening results in a period of senescence, a state in which cells cease to replicate. Therefore, this becomes one of the factors causing a reduction in the number of leukocytes (van der Laan et al., 2022). Moreover, formaldehyde induction can also lead to the inhibition of proliferation activity (cell division) from myeloid stem cells into other types of cells (lymphocytes, macrophages, granulocytes, megakaryocytes, and erythrocytes), a process that requires the assistance of granulocyte-macrophage progenitors (CFU-GM). The effect of formaldehyde exposure will inhibit the formation of CFU-GM, leading to a decrease in the formation of leukocytes (Zhang et al., 2010)

Conclusion

In this study, there were significant changes in the count of leukocyte types, especially in the treatment group that was induced with formaldehyde at a concentration of 40 ppm. These changes occurred due to the effect of formaldehyde during inhalation, leading to an increase in Reactive Oxygen Species (ROS) and resulting in oxidative stress, which can trigger damage to bone marrow cells, disrupting the production of leukocytes.

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