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Effect of Formaldehyde Inhalation on Leukocyte Counts and Malondialdehyde Levels in Wistar Rats (*Rattus norvegicus*)

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Abstract: Formaldehyde is a chemical compound that is often found in everyday life, such as in the chemical industry to research laboratories. Formaldehyde has toxic properties that can harm humans in long term exposure. The genotoxic and cytotoxic properties of formaldehyde can cause oxidative stress. This study assesses the number of leukocytes and levels of malondialdehyde in Wistar rats induced by 40 ppm formaldehyde. This study used 12 Wistar rats taken at random and put into 2 groups, namely the control and treatment groups. The treatment group was given formaldehyde inhalation at a concentration of 40 ppm for 16 weeks. At the 16th week, rat blood was taken retroorbital to calculate the number of leukocytes and levels of malondialdehyde. The calculation results were then tested statistically using IBM SPSS Statistics 25 software. The results showed that there was no significant difference ($p>0.05$) between the number of leukocytes in the control group and the treatment group. This study also showed that there was a significant difference ($p<0.05$) between malondialdehyde levels in the control group and the treatment group. In conclusion, there was no effect of formaldehyde induction with a concentration of 40 ppm on the number of leukocytes and there was an effect of formaldehyde induction with a concentration of 40 ppm on malondialdehyde levels in Wistar rats.

Keywords: Formaldehyde, formalin, leukocyte, malondialdehyde, wistar rats.

Pendahuluan

Formaldehyde is a simple aldehyde compound that is synthesized by the catalytic oxidation of methanol. The nature of this compound is a pungent odor, colorless, and toxic. Formaldehyde is widely used in many commercial products, such as plastics, wood products, medicines, textiles, and cosmetics (Kang et al., 2021). Formaldehyde exposure can be through many routes, such as oral, dermal, and inhalation. Inhalation is the most common route of exposure in the environment. Exposure to formaldehyde in the environment is more common indoors than outdoors (Kang et al., 2021; Pullen Fedinick et al., 2021). The fact that formaldehyde is easily found in everyday life causes many people to be constantly exposed to formaldehyde, both in small and large amounts.

IARC classifies formaldehyde as a compound that is carcinogenic to humans. According to epidemiological evidence from IARC, there is strong evidence that formaldehyde causes nasopharyngeal cancer. However, there is not enough epidemiological evidence to prove an association between formaldehyde and leukemia (Protano et al.,

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2022). Leukemia is a disorder caused by excessive leukocyte production in the bone marrow. Normally, a number of leukocytes are produced according to the needs needed by the body. But in leukemia, leukocytes are produced abnormally and thus become inefficient (Sahlol et al., 2020).

Formaldehyde is also known to induce an increase in intracellular reactive oxygen species (ROS), which can induce oxidative stress. Oxidative stress is an imbalance between oxidant compounds and antioxidants that causes oxidative damage. Oxidative damage can occur at the biomolecular level, such as proteins, lipids and nucleic acids (Ghelli et al., 2021). An example of oxidative damage to lipids is lipid peroxidation. Malondialdehyde is an example of a secondary product malondialdehyde (Ayala et al., 2014).

Research by Wultsch et al. (2015), showed an increase in malondialdehyde in carpenters. The levels of malondialdehyde in the exposed group were significantly higher than those in the control group and exceeded those found in the healthy control group. Experimental animal studies were performed by Susilawati et al. (2022) also showed that the plasma malondialdehyde concentration in the treatment group was significantly increased compared with the control group. This study administered formaldehyde by inhalation at a concentration of 20 ppm for 16 weeks to Wistar rats. Therefore, the aim of this study was to assess the effect of formaldehyde exposure on white blood cell count and malondialdehyde concentration in Wistar rats.

Material and Method

This study used 12 Wistar rats divided into 2 groups, that is the control group and the treatment group. The treatment group was given 10% formaldehyde inhalation at a concentration of 40 ppm for 16 weeks. Calculation of the number of leukocytes was carried out with a hematology analyzer. Measurement of malondialdehyde levels using spectrophotometry with the Thiobarbituric Acid Reactive Substances (TBARS). Statistical analysis for leukocyte counts used the Mann-Whitney U Test, while for malondialdehyde levels used the Independent-Sample T Test. The calculated results were then tested statistically using IBM SPSS Statistics 25 software.

Result and Discussion

Univariate analysis

Univariate analysis was performed on the variable number of leukocytes and levels of malondialdehyde which consisted of the control and treatment groups. The results of univariate analysis of leukocyte count and malondialdehyde levels can be seen in table 1 and table 2.

Table 1. Univariate Analysis of Leukocyte Count

Group	N	Mean ± SD
Control group	6	8.833,33 ± 5.639,385
Treatment group	6	9.633,33 ± 4.305,655

Table 2. Univariate Analysis of Malondialdehyde Levels

Group	N	Mean ± SD
Control group	6	0,220133 ± 0,0000816
Treatment group	6	0,377583 ± 0,1479498

Bivariate analysis

The results of the bivariate analysis of leukocyte counts (table 3) showed no significant difference between the control and treatment groups ($p>0.05$), while the results of the bivariate analysis of malondialdehyde levels (table 4) showed a significant difference between the control group and the treatment group ($p<0.05$).

Table 3. Bivariate Analysis of Leukocyte Counts

Group	n	Mean ± SD	*p*
Control group	6	8.833,33 ± 5.639,385	0,522
Treatment group	6	9.633,33 ± 4.305,655	

Table 4. Bivariate Analysis of Malondialdehyde Levels

Group	n	Mean ± SD	*p*
Control group	6	0,220133 ± 0,0000816	0,048
Treatment group	6	0,377583 ± 0,1479498	

Discussion

According to the study's findings, there was no discernible difference between the leukocyte counts of the treatment group and the control group ($p>0.05$). These results are consistent with the study by Al-Sarraj and Al-Habity (2013) which showed no significant difference in leukocyte counts between the treatment group II and the control group using rabbits as experimental animals. Group II in the study was exposed to 12 ppm formaldehyde for 4 months.

The results of this study also showed that the leukocyte counts in the treatment group increased compared to the control group. This is because formaldehyde is irritating so that it can trigger inflammation (Li et al., 2017). According to earlier trial data, the rise in leukocyte counts in the treatment group was not statistically significant. This may be because, according to a study by Zhang et al. (2010), the colony-forming unit-granulocyte-macrophage (CFU-GM) count decreased in the formaldehyde-exposed group, which in turn inhibited the proliferation of myeloid progenitor cells. Leukocyte numbers may drop as a result of the suppression of myeloid progenitor cell growth. The results of this investigation showed that the control group and the treatment group had different levels of malondialdehyde.

Additionally, the findings of this study are in line with a study by Susilawati et al., (2022), which revealed a significant difference between the mean levels of malondialdehyde in the treatment group (which received 20 ppm of formaldehyde) and the control group. Descriptively, this study also showed that the treatment group had a higher mean score than the control group. This is also supported by study by Uluçam and Bakar (2016), which discovered that formaldehyde-treated rats had higher plasma malondialdehyde levels than the control group. The rise was statistically significant ($p <0.05$). Research by Mohammed et al., (2021) also showed that there was an increase in malondialdehyde levels in rat serum due to formaldehyde induction.

Inflammation and ROS have a relationship with each other. Formaldehyde which is irritative can cause inflammation (Asare-Donkor et al., 2020). Under inflammatory conditions, oxidative stress generated by polymorphonuclear leukocytes (PMNs) leads to the migration of inflammatory cells. Migration of inflammatory cells not only contributes to the elimination of pathogens and foreign particles, but also tissue damage (Mittal et al., 2014).

The level of malondialdehyde produced is influenced by lipid peroxidation, which is impacted by an increase in ROS. On the other hand, large amounts of ROS in biocomponents cause cellular damage that results in inflammation by damaging DNA, carbohydrates, proteins, and lipids. Pro-inflammatory indicators and ROS can work together directly. Pro-inflammatory indicators are created in proportion to the amount of ROS produced. The activation of NF-κB by ROS can result in the transcriptional activation of genes related to inflammation, as demonstrated by a wealth of experimental data (Ranneh et al., 2017). By enhancing intracellular ROS pathways, formaldehyde can also have a direct impact on the rise in malondialdehyde levels (Tesfaye et al., 2021).

Conclusion

According to the result, there was no difference in the typical number of leukocytes between the control group and the treatment group when Wistar rats were exposed to 40 ppm formaldehyde inhalation for 16 weeks. The treatment group, which was exposed to 40 ppm of formaldehyde for 16 weeks, had higher mean levels of malondialdehyde than the control group.

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