



EFFECT OF ADMINISTRATION OF Sodium CYCLATE ($C_6H_{12}NNaO_3S$) ON THE NUMBER OF POLYMORFONUCLEAR CELLS (PMN) IN RATS (*Rattus norvegicus*)

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Abstract

Cyclamate is one of the artificial sweeteners that is still often consumed by the public as a food or beverage additive. In some countries, cyclamate is prohibited for consumption because it is carcinogenic. This study was conducted to determine the effect of sodium cyclamate on the number of polymorphonuclear cells (PMN).

The type of research used was a laboratory experimental. The sample used was a male rat totaled of 25 which were divided into 5 treatment groups which was without sodium cyclamate, administered sodium cyclamate at a dose of 4.5 mg / 200 g BW, administered sodium cyclamate at a dose of 9.5 mg / 200 g BW, administered sodium cyclamate at a dose of 14.5 mg / 200 g BW, and administered sodium cyclamate at a dose of 19, 5 mg / 200 g BW.

The research finding revealed the statistical significancy for $p < 0.05$ indicated that there was an effect of sodium cyclamate to the number of polymorphonuclear cells. The usage of sodium cyclamate is known to generate oxidative stress, which results in cell damage and leukocytosis.

Keywords: Sodium Cyclamate, Polymorphonuclear Cell Count, Hematological Characteristic.

Abstrak

Pangan adalah sesuatu yang berasal dari berbagai sumber, termasuk bahan tambahan pangan. Siklamat merupakan salah satu pemanis buatan yang masih sering dikonsumsi masyarakat sebagai bahan tambahan makanan atau minuman. Pada beberapa negara, siklamat dilarang untuk dikonsumsi karena mempunyai sifat yang karsinogenik. Penelitian ini dilakukan untuk mengetahui pengaruh pemberian natrium siklamat terhadap jumlah sel polimorfonuklear (PMN).

Jenis penelitian yang digunakan adalah eksperimental laboratorium. Sampel yang digunakan adalah tikus jantan berjumlah 25 ekor yang dibagi menjadi 5 kelompok perlakuan yaitu tanpa natrium siklamat, diberi natrium siklamat dengan dosis 4,5 mg/200 g BB, diberi natrium siklamat dengan dosis 9,5 mg/200 g BB, diberi natrium siklamat dengan dosis 14,5 mg/200 g BB, dan diberi natrium siklamat dengan dosis 19,5 mg/200 g BB.

Berdasarkan hasil uji statistik, didapatkan nilai signifikan ($p < 0,05$) sehingga dapat dikatakan bahwa ada pengaruh pemberian natrium siklamat terhadap jumlah sel polimorfonuklear (PMN). Kesimpulannya yaitu ada pengaruh pemberian natrium siklamat terhadap jumlah sel polimorfonuklear (PMN). Pemberian natrium siklamat diketahui memiliki efek stres oksidatif yang akan menyebabkan kerusakan sel dan leukositosis.

Kata Kunci: Natrium Siklamat, Hitung Sel Polimorfonuklear, Karakteristik Hematologi.



1. INTRODUCTION

Food is anything that comes from biological sources of agricultural, plantation, forestry, fishery, animal husbandry, water and water products, both processed and unprocessed, which is intended as food or drink for human consumption, including food additives, food raw materials, and other materials used in the process of preparing, processing, and making food or beverages (BPOM RI, 2014).

Rapid technological advances are able to influence developments in various fields such as the food processing industry, it cannot be denied that the use of these food additives is very diverse from preservatives, sweeteners, dyes, and others (Sukmawati et al., 2015). According to the Decree of the Minister of Health of the Republic of Indonesia No.722/Menkes/Per/IX/88, artificial sweeteners are food additives that produce excessive sweetness, sharpen the sweetness of food, and are suitable for consumption by diabetics and dieters because artificial sweeteners do not contain calories.

One example of an artificial sweetener that is still often used in Indonesia is cyclamate. Cyclamate was chosen as an artificial sweetener that is often used because of its low price and the resulting sweet taste is 30 times sweeter than sucrose (Rasyid et al, 2011). However, in some countries such as America, Canada, and England, cyclamate can no longer be used as a food additive because its degradation product (cyclohexylamine) is carcinogenic (Setiawati et al, 2013).

Based on the BPOM RI survey report when conducting laboratory sampling and testing of School Food Snacks (PJAS) taken from 866 Elementary Schools or Madrasah Ibtidaiyah spread across 30 cities in Indonesia, during 2011 4,808 samples were taken and 1,705 (35.46 %) samples did not meet the requirements (TMS) of food safety and quality. From the results of testing parameters for food additives from 3,925 samples of PJAS products, it was also found that 421 (10.73%) samples contained cyclamate that exceeded the required limit (Jamil et al., 2017).

Aisyah et al (2003) explained that giving cyclamate has an effect that causes anemia which is characterized by a decrease in hemoglobin (Hb) levels and red blood cell damage resulting in abnormal accumulation of white blood cells (leukocytes). Damage that occurs in the neutrophil lobe tends to cause leukocytosis, indicating an abnormality that occurs, namely inflammation.

Based on the above background, the researcher wanted to conduct a study on the effect of giving sodium cyclamate ($C_6H_{12}NNaO_3S$) on the number of polymorphonuclear cells (PMN) in rats (*Rattus norvegicus*).

2. RESEARCH METHODS

The research was carried out in January - June 2018 at the Infectious Unit for Development and Experimental Animal Research, Faculty of Veterinary Medicine, Airlangga University, Surabaya for the isolation and treatment of experimental animals and at the Hematology Laboratory of the Health Polytechnic



of the Ministry of Health Surabaya for polymorphonuclear examination. The type of research used in this research is an experimental laboratory with a *posttest only control group design*. Sample and The materials used in this study were male Wistar rats (*Rattus norvegicus*) 2-3 months old with an average weight of 200 grams obtained from the Infectious Unit for Research and Development of Experimental Animals, Faculty of Veterinary Medicine, Airlangga University, Surabaya and artificial sweetener sodium cyclamate obtained from stores. foodstuffs.

In this study, 25 experimental animals were used and then grouped into 5 groups, namely the negative control group, treatment 1 (given pellets and artificial sweetener in the form of sodium cyclamate orally at a dose of 4.5 mg/200 g as much as 2 mL for 10 minutes). days), treatment 2 (feeding pellets and artificial sweetener in the form of sodium cyclamate orally at a dose of 9.5 mg/200 g as much as 2 mL for 10 days), treatment 3 (feeding pellets and artificial sweetener in the form of sodium cyclamate orally at a dose of 14.5 mg/200 g as much as 2 mL for 10 days), and treatment 4 (feeding pellets and artificial sweetener in the form of sodium cyclamate orally at a dose of 19.5 mg/200 g as much as 2 mL for 10 days) then examination of polymorphonuclear cell (PMN) type count in the form of a blood smear (diffcount).

The calculation of statistical tests in this study used the normality test which was used to determine whether the data obtained were normally distributed or not and the homogeneity test (*Levene's Test*) was

used to determine whether the data obtained were homogeneous or not. If the data is normally distributed and homogeneous, then proceed with the *One-Way Anova* then followed by the *Post Hoc (Tukey) test*, if one or both of them are not normally distributed or are not homogeneous, then it is continued with the *Kruskal-Wallis* then continued with *Mann-Whitney*.

3. RESULTS AND DISCUSSION

In this study, 25 experimental rats were grouped into 5 groups, namely the negative control group, treatment 1, treatment 2, treatment 3, and treatment 4. The number of polymorphonuclear cells (PMN) obtained from examination with the test material in the form of rat blood was taken through the heart. . Rat blood examination was carried out at the Hematology Laboratory of the Health Polytechnic of the Ministry of Health Surabaya and the following results were obtained:

Table 1. Results of Calculation of the Number of Polymorphonuclear Cells in the

Negative Control Group counted in 100 leukocytes:

| Replicatio n | Negative Control | | |
|-----------------|------------------|------|---|
| | N | E | B |
| 1 | 28% | 3% | - |
| 2 | 25 % | 4% | - |
| 3 | 30% | 3% | - |
| 4 | 28% | 1% | - |
| 5 | 24% | 2% | - |
| (Σ) | 27% | 2.6% | - |



| Replication | Treatment | | |
|-------------|-----------|------|---|
| | N | E | B |
| 1 | 27% | 2% | - |
| 2 | 28% | 1% | - |
| 3 | 30 % | 3% | - |
| 4 | 26% | 2% | - |
| 5 | 28% | 3% | - |
| (Σ) | 27.8% | 2.2% | - |

| Replication | Treatment | | |
|-------------|-----------|------|---|
| | N | E | B |
| 1 | 34% | 1% | - |
| 2 | 32% | 1% | - |
| 3 | 31% | 3% | - |
| 4 | 33 % | 4% | - |
| 5 | 29% | 2% | - |
| (Σ) | 31.8% | 2.2% | - |

| replication | Treatment | | |
|-------------|-----------|------|---|
| | N | E | B |
| 1 | 32% | 2% | - |
| 2 | 33% | 3% | - |
| 3 | 37% | 1% | - |
| 4 | 34% | 2% | - |
| 5 | 35 % | 4% | - |
| (Σ) | 35.2% | 2.4% | - |

| Replication | Treatment | | |
|-------------|-----------|------|---|
| | N | E | B |
| 1 | 39% | 2% | - |
| 2 | 40% | 3% | - |
| 3 | 38% | 1% | - |
| 4 | 37% | 4% | - |
| 5 | 38% | 4% | - |
| (Σ) | 38.4% | 2.8% | - |

Information:
 N : Neutrophils
 E : Eosinophils
 B : Basophils

statistical test data analysis *One-Way Anova*, a significant value ($p < 0.05$) was obtained for neutrophils, namely there was an effect of sodium cyclamate on the number of

polymorph cells. on the nucleus (PMN) in Wistar rats, while for eosinophils a significant value ($p > 0.05$) was obtained for eosinophils, namely there was no effect of sodium cyclamate on the number of polymorphonuclear cells (PMN) in Wistar rats.

From the results of data analysis, it can be seen that in the negative control group (without giving sodium cyclamate, given 2 mL of distilled water for 10 days then blood samples were taken surgically on day 11) for the number of polymorphonuclear cells (PMN) the results were 27% for neutrophils and for eosinophils of 2.6% where the average is still in the normal range between 12-38% (neutrophils) and 1-4% (eosinophils) (Aiba et al., 2016).

The significant increase in leukocytes is caused by the body's defense reaction against the entry of foreign objects. Increase and decrease in the number of leukocytes can occur due to physiological or pathological influences. Leukocytosis that occurs due to physiological factors can be caused by muscle activity, fear stimulation, and emotional disturbances, while the influence of pathology can be caused by pathological processes in response to disease attacks (Astawan et al., 2012).

Sodium cyclamate has a metabolic product that is toxic to the body, namely cyclohexylamine, where this compound is a carcinogenic compound. Accumulation of sodium cyclamate can cause free radicals in rats, causing an imbalance and causing cell damage. The accumulation of sodium cyclamate causes oxidative stress so that essential fatty acids in the plasma membrane are lost and this



disrupts membrane permeability. As a result, free radicals from artificial sweeteners can easily enter cells and affect organelles in cells, then these free radicals can damage lysosomes, cell nuclei and so on, causing mutagenesis (Dewi, 2010).

Based on the increase in the number of polymorphonuclear cells in neutrophils after administration of artificial sweetener in the form of sodium cyclamate at a dose of 19.5 mg / 200 g (treatment group 4) compared to negative control, it shows that sodium cyclamate contains harmful compounds that can cause the formation of free radicals, where the free radicals are This can lead to an antioxidant imbalance that can cause cell damage in the body or infection, thereby increasing the permeability of blood vessels so that leukocytes migrate to the site of injury or infection and infection stimulates the release of adrenal hormones which can affect the circulation of leukocytes resulting in leukocytosis. The increase in the number of neutrophils is a result of the immune mechanism that works in response to infection in the body. This is related to the main function of neutrophils, namely the destruction of foreign materials through the process of phagocytosis, namely chemotaxis where cells will migrate towards particles, attachment of particles to cells, ingestion of particles by cells, and destruction of particles by lysozyme enzymes in phagocytosing (Utami et al., 2013). An increase also occurred in eosinophils by looking at the average results in each treatment, but there was no significant increase because the average results were still within the normal threshold. Eosinophils are known to have the

main function of neutralizing the presence of toxic materials so that they are present in large quantities in certain places, especially during infection (Lokapirnasari and Yulianto, 2014).

4. CONCLUSIONS AND RECOMMENDATIONS

Based on the results of the research and discussion conducted, it can be concluded that there is an effect of giving sodium cyclamate on the number of polymorphonuclear cells (PMN). Suggestions for future researchers are to conduct research on how to inhibit cell damage due to consuming artificial sweeteners for a long period of time and conduct further research on other food additives.

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