Sodium Cyclamate Effect on Nondisjunction Frequency of *Drosophila melanogaster* Meigen

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ABSTRACT

Sodium cyclamate is one of the artificial sweeetener that have potential health concerns. In this research, a study was conducted to examine the effects of sodium cyclamate administration on nondisjunction frequency of *Drosophila melanogaster*. ♂ homozygous *ebony body* was crossbreeded with ♀ homozygous wild-type fly and were fed with food containing 0% (control group), 5%, 10%, and 15% sodium cyclamate. Based on ANOVA result, F count was 29.578 with the p-value 0.000 < 0.05. Thus, null hypothesis was rejected and research hypothesis states there were significant differences of nondisjunction frequency was accepted. Flies treated with sodium cyclamate had significantly higher nondisjunction than the control flies. Administration of 15% sodium cyclamate caused nondisjunction with the greatest frequency.

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1. INTRODUCTION

Fondness to sweet is inborn to humans, and it dates back to the early civilizations [1]. Honey is the oldest sweetener known to humans and has been an eagerly sought commodity since prehistoric times [2]. Then, sucrose has been the main sweetener added to human diets for decades [3]. But, during world war, there was a scarcity of sugar and this prompted mankind to look into alternate sources of sweeteners [1,4]. This paved the way for artificial sweeteners in the food industry [1]. There are some artificial sweeteners, one of which is sodium cyclamate.

Sodium cyclamate is a white, crystalline, and odorless powder [5]. It is the solid form of the artificial sweetener cyclamate [6]. Although 33 times as sweet as sucrose, it is noncaloric (zero calories) and more stable than other artificial sweeteners [5-7]. Sodium cyclamate made through the sulfonation of cyclohexylamine and it was discovered by Michael Sveda in 1937 [8]. Unfortunately, in the 1970s, the FDA banned cyclamate completely from all food and drug products in the United States because of cyclohexylamine posed some potentially serious health risks [7].

Unlike the United States, the use of sodim cyclamate in Indonesia is still permissible, albeit with certain rules. The use of cyclamate in Indonesia as an artificial sweetener, both types and amounts, are regulated by Peraturan Menteri Kesehatan Republik Indonesia Nomor 722/DepKes/Per/IX/88 and Permenkes RI No 329/Menkes/PER/XII/76. However, this artificial sweetener is very easy to obtain in the market and its usage often does not pay attention to government regulations that have been established. This leads to increased the opportunities for people to consume this harmful substance.

When a person consumes harmful substances, the negative impact is not only obtained by himself, but also his offspring [9,10]. Disability and even infants mortality are some the the effect that can arise from such situation. Some substances can cause disability or death in infatns because the substance can induce nondisjunction [11]. Nondisjunction is the failure of homologous chromosomes to separate properly during meiosis or mitosis [12]. When nondisjunction occurs, during meiosis I or meiosis II, gametes are created that

either contain an extra chromosome or lack a copy of a choromosome [13]. Several syndromes arise from this situation, such as Down Syndrome, Turner syndrome, and Klinefelter syndrome [12-14].

In this study, the effect of sodium cyclamate on nondisjunction frequency was observed. *Drosophila melanogaster* was chosen as a model organism in this study. There are several reasons why this organism was chosen in this study, such as it has a short life cycle and easy to culture in laboratory conditions [15,16]. Moreover, studies of *D. melanogaster* and humans indicate several similarities in nondisjunctional mechanisms in the two species [17].

2. RESEARCH METHOD

2.1. The organsim and environmental conditions

D. melanogaster ebony body strain and wild-type strain from Genetic Laboratory FMIPA UM were used in this study. Flies were cultured in a 200 ml cylindrical glass bottle, with 7 cm diamater and 9 cm height, filled with 30 ml standard food, as described in Fauzi et al [18]. The flies cultures were kept in a research room at Genetic Laboratory FMIPA UM. When there were blackened pupae, the pupae were isolated into plastic tube with 1 cm diameter and 5 cm height. Adult flies that emerge from this plastic tube were using for crossbreeding at treatment stage.

2.2. Crossbreed and treatment stage

♂ homozygous *ebony body* was crossbreeded with ♀ homozygous wild-type fly in glass bottle that the same size as cultured bottle that filled with food containing 0% (control group), 5%, 10%, and 15% sodium cyclamate. Each crossbreed consisting a male and a female, both 2 X 24 hours after hatching from pupae. After 2 x 24 hours crossed, male fly was removed from the bottle. Each treatment as many as three replications.

2.3. Data collection and analysis

The phenotype of first filial generation were observed and calculated. The emergence of *ebony* strain at first filial generation is an indication of nondisjunction occurence. Therefore, the nondisjunction frequency was calculated by calculating the frequency of ebony strain occurence. The frequency data was then transformed using square root transformation. After that, the data were calculated with one way ANOVA test at a significance level of 0.05 if normally distributed assumption were meet. Furthermore, LSD test performed when the ANOVA test result was significant. If normality assumption were not met, the data were calculated using Kruskal-Wallis Test. The statistical analysis of the results were carried out using the IBM SPSS Statistics 22.0 programme.

3. RESULTS AND ANALYSIS

Fig. 1. Is graphs show the average of nondisjunction frequency in control and experimental group. Based on the graph, it can be seen that in the control group (0% sodium cyclamate), filial from nondisjunction product were not found. On the other hand, nondisjunction products were mostly found in 15% sodium cyclamate.

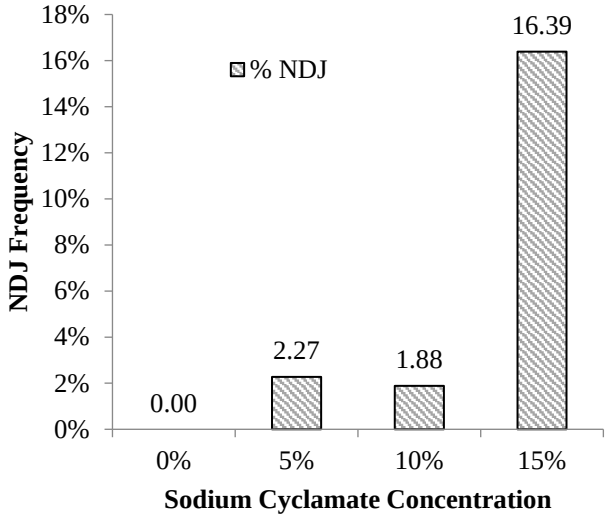

Figure 1. The average of nondisjunction frequency (% NDJ) in control and experimental group

The summary of ANOVA test on the nondisjunction frequency can be seen in Table 1, while the results of LSD test with level of sig. 0.05 can be seen in Fig. 2. Based on ANOVA result, F count was 29.578 with the p-value 0.000 < 0.05. Thus, null hypothesis was rejected and research hypothesis states there were significant differences of nondisjunction frequency was accepted. Based on LSD test result, it can be seen that sodium cyclamate treatment affecting nondisjunction frequency in fruit fly. All experimental group had significantly higher nondisjunction than the control group. Then, the 5% and 10% treatments had nondisjunction frequencies that did not differ significantly. On the other hand, the 15% treatment had a significantly higher nondisjunction frequency than the other groups.

Table 1. Summary of ANOVA Test on Nondisjunction Frequency of *D. melanogaster*

Source	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	26.336	3	8.779	29.578	.000
Within Groups	2.374	8	.297		
Total	28.710	11			

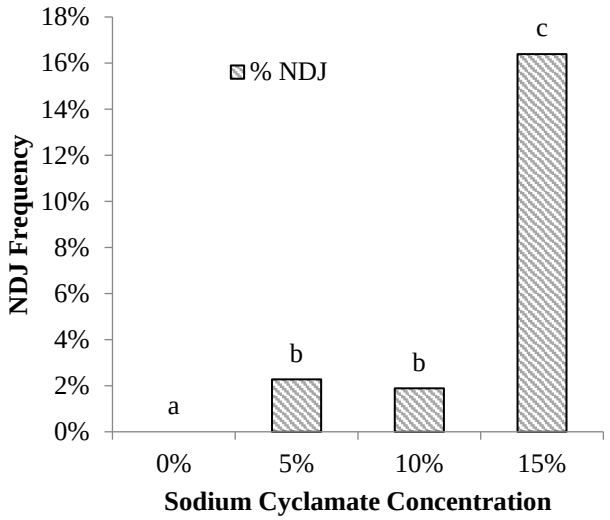

Figure 2. Summary of LSD test on Nondisjunction Frequency (% NDJ) of *D. melanogaster.*
Note: bar with the sam alphabet notation are not significantly different at P ≤ 0.05

Nondisjunction is the failure of homologous chromosomes to separate properly during meiosis or mitosis [12]. In this research, nondisjunction during oogenesis was the focus of the study. Normally, all filial is heterozygous wild-type strain if homozygous *ebony body* is crossbreeded with homozygous wild-type fly. The genotype of homozygous wild-type strain is +/+, so this fly will produce gametes that 100% carying + allele [19]. On the other hand, homozygous wild-type strain is e/e, so this fly will produce gametes that 100% carying e allele. Thus, when the two strains are crossbreeded, formed filial that have +/e genotype [19]. But, if nondisjunction phenomenon occurs during gametogenesis in wild-type strain, it will form two kind of gametes that carries two + alleles at once or gametes that do not carry + allele at all. So, if a gamete that does not carry + allele at all is fertilized with a gamete carrying e allele, it will produce ebony strain that have e/0 genotype. The result of this study that showing the increasing the frequency of *ebony* strain occurrence on high concentration sodium cyclamate treatment indicating this substance interferes the meiotic division process.

When a normal disjunction occurs in meiotic division, homologous chromosomes or chromatids separate to opposite poles [20]. This process involves many genes [21,22]. Mutations in these genes will affect normal disjunction during meiosis, such as through malfunction of the spindle apparatus or through errors that occur in kinetochore with the other meiotic apparatus [21,23,24]. Related to that, indeed, some chemical mutagenes can induce nondisjunction in those way [23,24].

However, based on various reports, sodium cyclamate is not indicated as a mutagen [25-27]. So, the results in this study that show sodium cyclamate affected nondisjunction is not due to this substance attack on genes controling meiotic division. Furthermore, until now, no studies have reported that sodium cyclamate directly interfere spindle formation. Responding to the results of this study, the study from molecular aspects that try to reveal the mechanism of sodium cyclamate in influencing nondisjunction needs to be done in subsequent research.

4. CONCLUSION

The results of this study indicate sodium cyclamate can increase nondisjunction frequency in *D. melanogaster*, espeically during oogenesis. Studies using reciprocal crossbreed, studies using more replication and treatment levels, and studies using different model organisms need to be done to validate the findings of this study. Studies from the molecular aspect also needs to be done to reveal the involvement of sodium cyclamate in nondisjunction events.

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