The Influence of Ginger (*Zingiber officinale Roscoe)* to Protect Cholinesterase Enzyme Activities of Mice that Exposed to Pesticide

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Abstract

Pesticide is a bioactive chemical inggredients that contain toxic material to kill pest organisms. Pesticides that is widely used in Indonesia were organophosphate and carbamate. These pesticides was known as inhibitor for the cholinesterase enzyme. The decreasing activity of cholinesterase enzyme can be solved by eat some food which contain antioxidant especially phytochemicals acid (vitamin C) and some others phytochemicals. To increase cholinesterase enzym level in farmers which exposed with pesticide we need to find a natural inggredients that is cheap, easy to find, easy to be processed, and untoxic. Ginger *(Zingiber officinale Roscoe)* was a plant that have lots of active inggredients and untoxic. The aim of this study was to analyse the influence of ginger extract as antioxidant to the cholinesterase enzym activity of mice that exposed to pesticide. This study was quasi experimental study using post test only control group design. Samples were Swiss Webster male white mice with 20-25 mg of weight, age 3-4 months, healty, and have a normal feces. There were 7 mice in every groups, all samples were 28 mice. Ginger *(Zingiber officinale Roscoe)* extract (0.001 mg/liter and 0.005 mg/liter) was given using feeding tube to every mice that have been exposed by 0.006 mg/liter of pesticide everyday. The results of cholinesterase enzyme test in mice which exposed by pesticide in control groups were 84%. Treatment groups with 0.005 mgr/liter of pesticide exposing, the cholinesterase enzyme level were 26%, treatment groups with 0.005 mg/liter of pesticide exposing and 0,001 mg/liter, their cholinesterase enzyme were 77%. The K3 groups with 0.005 mg/liter of pesticides and 0.005 mg/liter ginger extract have the average level of cholinesterase enzym were 57%. We can conclude that ginger *(Zingiber officinale Roscoe*) extract can influence the cholinesterase enzym due to pesticide expose.

Keywords: *Pesticides, cholinesterase, ginger (Zingiber officinale Roscoe).*

Introduction

Indonesian farmers used to protect their agriculture product from pest using pesticides. Pesticide is an bioactive chemicals which is contain toxic material to kill pests. Pesticide works by inhibit cholinesterase enzyme. The most popular pesticide which is used in Indonesia were organophospat and carbamat. These pesticides were also inhibit cholinesterase enzyme. Liver was an pesticides targeted organ due to its function as a toxin neutralizer, central place of protein, fat, and carbohydrate metabolism, protein plasma and heparin (blood anticoagulant) producer.(1)

The decreasing of cholinesterase enzyme level can be overcome by consume antioxidant especially phytochemicals acid (vitamin C) and others phytochemicals compound.(2) Researches have been found that ginger has some antioxidant such as gingerdiol, gingerol, cafenic acid, camphene, capsaicin, chlorogenic acid, curcumin, delphinidin, eugenol, ferulic acid, gamma terpinene, isoeugenol, melatonin, myrcene, vanilla acid, vanillin dan zingerone. (3)

A study by Anam (2008) prove that the case of farmers which are poisoned by pesticides still exist. All farmers in Batu Mediri, Karang Pule did not use

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personal protective equipment which lead to 90% of them experienced mild poisoning and 10% of them experienced moderate poisoning.(4)

The results of previous research by Rachmaniyah et al. (2017) found that antioxidant in *Psidium guajava L* extract can be used to protect liver cells membrane from cigarette smokes. It can be seen by identify cirrhosis in mice liver's cell after it was inducted by cigarette smokes.(5) A research about the influence of ginger extract to protect pesticide exposing have been not found yet. By these we want to analyse the potential of ginger to increase cholinesterase level for the farmers who are exposed by pesticides.

Method

This research was a quasi experimental study using post test only control group design. This design was chosen due to it was the most simple design to devide subject into two groups or more randomly.

Sample Preparation: Samples were Swiss Webster Mice which was adapted in cages for seven days in Biochemical Laboratory of Airlangga University. There were seven mice in every group therefore this study used 28 mice. Samples were devided into 4 groups randomly. Sampels were devided using simple random sampling way by lottery. All mice must have 20-25 grams of weight, agile in movements, shiny hair, and glowing eyes.

Pesticide Exposing: All mice were exposed to pesticide everyday within a month. Exposing were done using 60 mL syringe with yellow tip. Pesticides fog then were sprayed and transfered to the cages using nebulizer. This process were done until all the pesticide becomes fog completely.

Ginger Extract: Ginger Extract were given to the mice by feed tube everyday. This extract were powder, it must be dissolved in warm water first. We use 0.005 mg ginger extract powder for 1000 mL of warm water. Sugar was added as it was needed to give taste. This dosage were given to the mice only 1 mL per day within 30 days. The dosage were chosen based on Donatus et al. (1987) in Anam (2015) about Cholinesterase Enzyme level in Farmer's blood that exposed by pesticide after they take *Curcuma zanthorrhiza*.(4)

Cholinesterase Enzyme Level Test: This test were done using tintometer test kit. All these tests were done in Biochemical Laboratory of Airlangga University. The results of cholinesterase enzyme level will be categorized below:

1. Normal Poisoning: > 75%-100%
2. Mild Poisoning: > 50%-75%
3. Moderate Poisoning: > 25%-50%
4. Heavy Poisoning: 0%-25%

Cholinesterase Enzyme test has 4 stages, it was:

a. Reagent Test: Indicator and substrate solution were tested with control blood (blood from a person who did not exposed to pesticide or pesticide free).

b. Blood taking: A tube with indicator solution were prepared for control and blood samples. The mice were anesthetized and killed. 1 mL of blood were taken from heart vein using sterile syringe. 0.01 mL of blood samples were put in the tube to be homogenized.

c. Substrate Solution Adding: All tube in seconds stage would be added by 0.5 mL of substrate solution started from control tube, in this time we noted the time as zero. Substrate then was added to every tubes with a minutes interval from zero.

d. Color comparison and results: All tubes will form some colors from green to yellow. These color then compared to the comparator in the tintometer test kit. Every color will be read as percentage of cholinesterase enzyme.

Findings: The results of cholinesterase enzyme level in mice in every groups can be seen in table below.

Table 1. Cholinesterase enzyme concentration in mice groups

Number	Cholinesterase enzyme level (%)			
	K_0	K_1	K_2	K_3
1	90	30	80	50
2	90	30	80	60
3	80	20	80	60
4	80	20	70	60
5	80	20	70	60
6	80	30	80	60
7	90	30	80	50
Average	84	26	77	57

The table shows that the average level of cholinesterase enzyme of K0 (Control Groups) was

84%, this results was categorized as normal poisoning (>75%-100%). The average level of K1 groups (exposed by 0.005 mgr/liter of pesticide without ginger extract treatment) was 26%, this results was categorized as heavy poisoning. The average level of cholinesterase enzyme of K2 groups (exposed by 0.005 mg/liter of pesticide with 0.001 mg/liter of ginger extract treatment within a month) was 77%, this results was normal. The last groups K3 (exposed by 0.005 mg/liter of pesticide with 0.005 mg/liter of ginger extract treatment within a month) have 57% of average level of cholinesterase enzyme, this results was categorized as moderate poisoning.

The difference of Cholinesterase enzym level in every groups can be seen below.

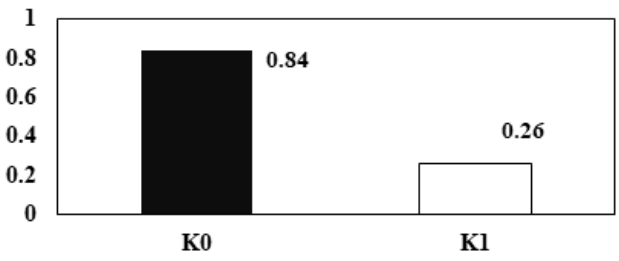

Figure 1. The Difference of K0 Groups and K1 Groups

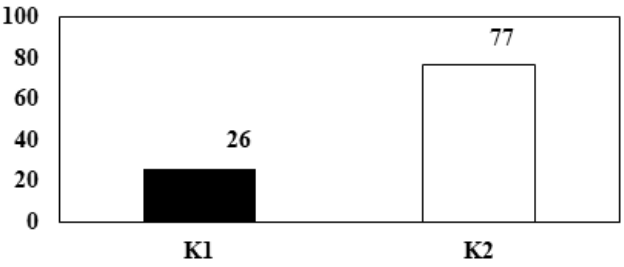

Figure 2. The Difference of Cholinesterase Enzym Level of K1 and K2 Group

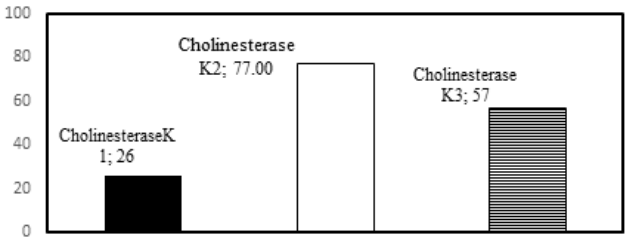

Figure 3. The Difference of Cholinesterase Enzym Level of K1, K2, and K3 Group

Discussion

The cholinesterase enzyme level in Ko group (control group) and K1 group (exposed by 0.005 mg/liter of pesticide without ginger extract treatment) was different. The lowest level of cholinesterase enzyme in K0 group was 80% while the highest was 90%. All these results in K0 group was categorized normal. While in K1 group, the average level of cholinesterase enzyme was 26%. This result categorized as moderate poisoning.

This results showed that the exposing of pesticide can affect cholinesterase enzyme level. The decreasing of cholinesterase enzyme level can be indicated that organophosphate compound was entering the body by inhalation. It distributes in the blood and inhibit the cholinesterase enzyme activity. This condition can be normal after 2 weeks. Organophospate was anticholinesterase. It was irreversible to inhibit enzyme by aging mechanism due to dealkalation from dialkylphosphorylated enzyme intermediates.

The exposing of pesticide make the cholinesterase enzyme activity in central and autonomous nerve system decrease. In a long time period this will make massive stimulate in cholinergig nerve and central nerve system due to acetilcholin stimulating which usually function to continue nerve stimulation to the receptors of muscle cells and glands. The accumulation of acetilcholin stimulate the sympathetic and parasympathetic central nervous system, so that muscle contraction increases.

The comparation of K1 group (exposed by 0.005 mg/liter of pesticide without ginger extract treatment) result and K2 group (exposed by 0.005 mg/liter of pesticide with 0.001 mg/liter of ginger extract treatment within a month) result showed that K1 has a lower cholinesterase enzyme level than K2. It means that there was an influence after 0.001 mg/liter ginger extract treatment within a month to the cholinesterase enzyme level. All the results in K2 group was normal. So that ginger extract can fix cholinesterase enzyme activity in blood. This happen due to phytochemicals acid (C vitamin) and phytochemical compound. Researches (Fugio, et al.) have been found that ginger has some antioxidant such as gingerdiol, gingerol, cafenic acid, camphene, capsaicin, chlorogenic acid, curcumin, delphinidin, eugenol, ferulic acid, gamma terpinene, isoeugenol, melatonin, myrcene, vanilla acid, vanillin dan zingerone.[6]

K1 group (exposed by 0.005 mg/liter of pesticide without ginger extract treatment) the average level of

cholinesterase enzyme was 26% and K3 group which was exposed by 0.005 mg/liter of pesticide with 0.005 mg/liter of ginger extract treatment within a month, their cholinesterase was increase to 57%.

Cholinesterase enzyme level in K1 group which was exposed to pesticides decreasing to 26% (moderate poisoning), while in K2 group which was exposed by 0.005 mg/liter of pesticide with 0.001 mg/liter of ginger extract treatment within a month their cholinesterase enzyme level was increasing to 77% and becomes normal. However when we increase the dosage of ginger to 0.005 mg/liter, their cholinesterase enzyme level was decreasing to 57% or becomes mild poisoning.

Based on Aisyah (2002), tocopherol in ginger extract will inhibit malondialdehyde (MDA) formation in mice so that ginger can help to fix cholinesterase enzyme activity in mice only in small dosage (0.001 mg/liter). This happen due to in higher dosage ginger extract can be toxic and kill the mice.[7]

Conclusion

1. Ginger *(Zingiber officinale Roscoe)* extract can influence cholinesterase enzyme due to pesticide exposing in mice. The exposing of 0.005 mg/liter orghanophospat pesticides influence cholinesterase enzyme level to 26%.
2. The consumtion of 0.001 mg/liter of ginger extract in mice influence cholinesterase enzyme level to 77%.
3. The consumtion of 0.005 mg/liter of ginger extract in mice influence cholinesterase enzyme level to 57%.

Additional Informations

Ethical Clearance: taken from Ethics Committe of Health Polytechnic of Surabaya on April 23, 2018.

Source of Funding: Health Polytechnic of Surabaya

Conflict of Interest: No

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