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The Effect of Beetroot (*Beta vulgaris* L.) Juice on Cholinesterase Activity In Farmers Exposed to Organophosphate Pesticides

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Abstract

A decrease in cholinesterase (chE) enzyme activity is an indicator of pesticide poisoning, especially organophosphate pesticides. The-chE activity reduction will result in an increase in the amount of acetylcholine and will bound to muscarinic and nicotinic receptors in the central and peripheral nervous system which can lead to the onset of Alzheimer's disease. However, antioxidant products can slow the progression of Alzheimer's disease by protecting neurons from oxidative stress. Red Beetroot (*Beta vulgaris* L.) contains betalains and phenolic compounds which act as an antioxidants that are capable of preventing such illness. This study aims to prove the concept that antioxidant compounds in red beetroot juice can increase the chE activity in subjects exposed to organophosphate pesticides. This research is an observational study with an experimental approach. This research used 25 farmers who were exposed to organophosphate pesticides as respondents. They were given 500 ml of beetroot juice twice a day for 2 consecutive months. Cholinesterase levels were measured before and after consuming red beet juice. Measurement of cholinesterase levels was carried out using a kinetic photometric test. This method was developed based on recommendations from the German Society of Clinical Chemistry (DGKC). The results showed that the average of chE (U/L) Level Before Treatment was 8.102 and 8.380 after treatment with a p value 0,62. It can be concluded that there was an increase in the level of cholinesterase but it was not statistically significant. This may be caused by a different response to activity of cholinesterase after consuming beetroot juice in each subjects, age difference, smoking habits, and personal protective equipment.

Keywords

Beetroot, cholinesterase, organophophate, pesticide

INTRODUCTION

Pesticides are poisons intentionally made by humans to kill plant-disturbing organisms and disease-spreading insects (1). The high usage of pesticides are detrimental to health, particularly for the workers who contact with pesticides and the impacted population. The health risk of pesticides exposure in the workers may due to improper handling of that poisoning substance. Besides, the population are also vulnerable to pesticides poisoning due to the contamination of such substance that enter the food chain, either because of ingestion and inhalation of pesticides residue or direct contact of pesticides through the skin.

Every day, a thousands of farmers working on agriculture land are poisoned by pesticides. it is estimated that millions of people involved in agriculture sector suffer from pesticide poisoning every year. Based on data from the World Health Organization (WHO) and the United Nations Environment Program (UNEP), 1-5 million cases of poisoning occur in people working in the agricultural sector. In addition, the population around the agricultural sites are very at risk of being exposed to pesticides either through air, soil or contaminated water. Moreover, organophosphate pesticides are the most toxic insecticides that is usually used in the consumer goods so that the population will also be impacted. Pesticides exposure in a small amount can cause death, but it takes more than a few mg to cause death in adults. Organophosphate will inhibit the action of pseudokholinesterase in plasma and cholinesterase in red blood cells and in synapses. Based on previous research, exposure to pesticides can cause cancer, Alzheimer's disease and even birth defects. Pesticides also have the potential to damage the nervous system, reproductive system, and endocrine system (2). In a Nutrients Journal have reported that betalains has high antioxidant and anti-inflammatory abilities in vitro and in vivo tests. This has sparked interest in researchers to examine the role of beets in the field of clinical pathology in several diseases characterized by oxidative stress and chronic inflammation such as liver disease, arthritis and even cancer (3).

Red Beetroot (*Beta vulgaris* L.) is preferred as a rich source of betacyanin, including a group of fruits that have reddish to purple betalains pigments consisting mainly of betanin and isobetanins. Betalains and phenolic compounds present in red beetroot have been reported to increase resistance in low density lipoprotein (LDL) to oxidize and to prevent cancer and cardiovascular disease by reducing the oxidative effects of free radicals on lipids (4). Free radicals or reactive oxygen species (ROS) play many important roles in physiology and pathological processes. Oxidative stress is a biological phenomenon that results from a biochemical imbalance

between the formation and cleansing / buffering of free radicals (5).

Workers in the agrochemicals and biological fertilizers company are the most often exposed to pesticides, especially organophosphate pesticides. Based on the results of the situation analysis, many complaints arose from the operators who indicated that there were symptoms of poisoning. While the temporary solution that has been done is to move positions in other parts for a while and there is no way to minimize the symptoms of poisoning that arise. Whereas, from the results of previous studies, (5) explained that natural antioxidant products can slow the progression of Alzheimer's disease by protecting neurons from oxidative stress. Over the past few years, oxidative stress has been described as a co-lethal factor in cases of poisoning caused by organophosphate pesticides. Many studies emphasize the role oxidative stress in cases of organophosphate poisoning. Previous studies have shown that the administration of date palm extract is able to restore atrazine poisoning, a herbicides that cause oxidative damage to sperm parameters, reduce testicular antioxidant enzymes, GSH, SOD, CAT, and GPx levels in mice (6). Another studies found the presence of oxidative stress and inhibition of acetylcholinesterase on farmers exposed to organophosphate pesticides (7). Antioxidants have been suggested as organophosphate antidotes. Meanwhile, Nurulain et al. did not find that giving glutathione (GSH) and NAC do not provide a survival effect on acute exposure induced paraoxon (a very dangerous type of OP pesticide) in Wistar mice. This shows that antioxidants have no role in the toxicity of acute phase II pesticides (8).

Several studies have shown that the administration of antioxidant activity compounds can minimize pesticide poisoning. Thus the researchers want to examine whether red beetroot juice (*Beta vulgaris* L.) that is proven to have antioxidant activity is able to minimize pesticide poisoning through increased cholinesterase activity.

MATERIALS AND METHODS

The equipments that used in this research were Terumo volume 3 ml syringe, EDTA vacutainer tube, torniquet, cotton, Centrifuge Brand: TOMY, Type: MX-105, GENESYS ™ 10S UV-Vis spectrophotometer - Thermo Fisher Scientific, Thermometer, Dropper, Dropper measure, ice box, ice pack. Meanwhile, the materials used were 96% alcohol, distilled water, deionized aquades, phosphate buffer pH 7.4, thiol 5.5'-dithio-bis (2-nitro benzoic acid) {DTNB} 0.33 mM, acetylcholine 5 mM.

Red beetroot juice was made by mixing 150 mg of red beetroot in 1 L of water. Afterward, it was blended using a blender (servings per person), divided into 2 servings

to drink twice a day (500 ml/serving), at the morning and at the evening (9). They consume this juice every day for 2 months.

Blood sampling was done by damming with tourniquet. Alcoholic cotton was applied in a circular manner as an antiseptic and was allowed to dry. The syringe needle was inserted in positions 30-45°C until a sign of blood comes out of the needle then the syringe was drawn to the limit of 2 with a blood volume of 2 mL. The needle was taken from the vein and the blood was inserted into the vacuum tube containing the EDTA and was homogenized (10).

Separation of Erythrocytes and Plasma was done by inserting the blood that has been taken into the vacuum container and it was centrifuged using Centrifuge Brand: TOMY, Type: MX-105 for 10 minutes at 4000 rpm. Erythrocytes that have been separated from plasma were taken and placed in test tubes (11).

Measurement of chE was done by taking erythrocytes that have been separated from plasma and it was then added with deionized water to the same as the initial volume of blood. Afterwards, it was diluted 60 times using a buffer (phosphate buffer 0.1 M, pH = 7.4), and it was frozen for hemolysis. The mixture was thawed again and diluted with buffer 9 times. After that, it was added with thiol reagent 5.5'-dithio-bis (2-nitro benzoic acid) {DTNB} (DTNB concentration 0.33 mM) and was allowed to stand for 10 minutes. Then the mixture was added to the ATCh substrate (ATCh concentration of 1.0 mM) and was read with absorbance of 412 nm using a UV-Vis GENESYS ™ 10S spectrophotometer - Thermo Fisher Scientific by using a blank containing erythrocyte hemolysis in a buffer (12).

This research is an observational study with an experimental approach since the data was taken through laboratory tests. Additionally, the participant was interviewed by using a questionnaire which ask data on age, length of work and symptoms felt. The population and sample in this study were male farmers in the Mojokerto region who were exposed to organophosphate pesticides. The inclusion criteria were 30 years old and above, and has been working for at least 1 year.

The data was analyzed by a paired t-test using SPSS 16 software. Statistical tests were performed at 95% confidence level and a significant difference if $p < 0.05$. The participants were given an informed consent and the number of Ethical Clearance Certificate is 091/KE/VII/2019.

RESULTS

The study population (respondents) were farmers exposed to organophosphate pesticides. All of the respondents were male as many as 25 people with different working periods as shown in table 1. Respondents were exposed to organophosphate pesticides

daily because they work as farmers on an agricultural land.

The parameters of the examination carried out were the levels of cholinesterase. Cholinesterase levels were measured before and after administration of red beet juice. The results of the examination of cholinesterase levels can be seen in table 1. The normal range of cholinesterase levels ranged from 4,620 - 11,500 U / L. The higher levels of cholinesterase the better because cholinesterase has a function to convert acetylcholine into acetate and choline that can prevent the accumulation of acetylcholine. Conversely, if the level of cholinesterase is lower than 4,620 U / L, it will show the symptoms of poisoning due to the accumulation of acetylcholine.

Table 1. Results of Cholinesterase Level Before and After Treatment

Patient Code	Age (years old)	chE (U/L) Level Before Treatment	chE (U/L) Level After Treatment	Normal Value of chE (U/L)	*p* Value
1	50	11.340	9.405	4.620 – 11.500	0.620
2	46	3.967	10.479		
3	62	5.670	6.436		
4	54	5.666	6.830		
5	54	9.371	8.276		
6	50	8.759	7.759		
7	53	7.302	9.915		
8	57	9.775	5.770		
9	63	8.718	4.547		
10	50	8.441	7.357		
11	60	7.446	9.047		
12	41	6.655	9.342		
13	38	4.351	7.380		
14	63	11.284	9.685		
15	60	9.927	9.611		
16	60	7.133	9.954		
17	64	6.673	8.013		
18	32	8.198	7.995		
19	64	11.086	6.007		
20	52	6.840	10.999		
21	65	9.410	10.035		
22	35	10.583	7.428		
23	60	6.542	8.723		
24	61	6.121	7.989		
25	50	11.287	10.516		
Average		8.102	8.380		

**U/L = Unit per Liters*

The statistical analysis of the effect of red beet juice consumption on cholinesterase levels can be seen in table 1. The results show that consumption of red beet juice on cholinesterase levels was not statistically significant with a P value 0.620. It means that consumption beetroot juice was not able to increase cholinesterase levels significantly.

DISCUSSION

Farmers are one of the subjects who are vulnerable to pesticides. In a plantation area in Mojokerto, east Java, the farmers use organophosphate pesticide. Organophosphate pesticides are the most toxic pesticides than the other groups. This pesticide is able to inhibit the performance of the cholinesterase enzyme. The enzyme normally hydrolyzes acetylcholine into acetate and cholin. When the enzyme is inhibited, it causes the amount of acetylcholine to increase and binds to the muscarinic and nicotinic receptors of the central and peripheral nervous system. Therefore, this causes symptoms of poisoning that affect all parts of the body. There are some people who have shown the indications of poisoning symptoms marked by a decrease level of the enzyme cholinesterase, characterized by complaints such as dizziness and nausea. It was also found in the farmers working on agricultural land in Mojokerto. However, symptoms of organophosphate poisoning vary greatly. Each symptom is very dependent on the presence of persistent acetylcholine or depression stimulation followed by central or peripheral nerve stimulation. Early symptoms such as salivation, lacrimation, urination and diarrhea (SLUD) occur in acute organophosphate poisoning due to stimulation of muscarinic receptors so that the acetyl kholin content in the blood increases in the eyes and smooth muscle (13).

Organophosphate is a type of pesticide that is most widely used in agriculture. Cholinesterase levels are known as biomarkers in patients exposed to organophosphate pesticides. In the results of the study in table 1 showed that some cholinesterase levels are still in the normal range, but there are some others that have chE levels close to the lower limit (symptoms of poisoning), as in patient coded by number 2 and number 13. Based on the results of statistical calculations obtained, the p value was 0.62 which indicates that consumption of red beet juice did not significantly influence the levels of cholinesterase. It may be caused by a different habits and personal protective equipment. It can be seen in patient coded by number 2, he was experienced a very significant increase in cholinesterase levels with an increase of 6512 chE (U/L). Furthermore, the patient coded by number 11 increase in cholinesterase levels by 2613 chE (U/L), the patient coded by the number 12

increase in cholinesterase levels by 2687 chE (U/L), the patient coded by the number 13 with an increase 3029 chE (U/L). Lastly, the patient coded by the number 20 increase in cholinesterase levels by 4159 chE (U/L). From this data, there was an increase in the level of cholinesterase but it was not statistically significant.

One study suggested that the nitrate content was not the only compound contained in red beetroot that were proposed to have beneficial effects on health and disease. Beetroot is a rich source of phytochemical compounds including ascorbic acid, carotenoids, phenolic acids and flavonoids. Beets are also one of the few vegetables that contains a group of highly bioactive pigments known as betalains. Members of the betalains family are categorized as red-purple betacyanin pigments or yellow-orange betaxanthin pigments. Based on other studies, it mentioned that phenolic compounds can react with residues of active amino acids found on the active side of ChE through hydrogen bonds, hydrophobic interactions and π-π interactions. The results show that hydroxyl groups in the structure of phenolic compounds are believed to increase cholinesterase activity (14). This is the underlying mechanism why antioxidant in beetroot can increase cholinesterase level. This supports the results of this study on several patient codes that indicate an increase in cholinesterase activity.

However, there are several other factors that can influence the level of cholinesterase, including nutritional status, health conditions, age, sex, ambient temperature, smoking habits, and habits of using personal protective equipment (PPE) (15). In the results of this study, it was note that each respondent has a different response after consuming red beet juice on cholinesterase activity. This can be due to the influence of age, smoking habits and habits of using PPE that are different from each respondent. Unfortunately, this research did not examine the effect of age, smoking habits and habits on cholinesterase levels, but it could be that these factors are the cause of not being statistically significant results. This research is preliminary data that can be used as a basis for further research to determine the efficacy of red beet juice in increasing levels of cholinesterase by involving age and habits.

CONCLUSIONS

Based on the results of the study concluded that there was an increase in the level of cholinesterase but it was not statistically significant. The increasing of cholinesterase levels can be caused by antioxidant properties of red beetroot juice (*Beta vulgaris* L.) so it can be minimize the pesticide poisoning through increased cholinesterase activity. In further studies it is recommended to increase the dose, duration

of treatment and involve factors of age and habits.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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