THE ROLE OF PUMELO FRUIT JUICE (CITRUS MAXIMA VAR NAMBANGAN), VITAMIN C AND LYCOPENE SUPLEMENTATION LIVE FUNGTION DISTURBANCE OF OCHARATOXIN-EXPOSURED MICE (MUS MUSCULUS)

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ABSTRACT

The aim of this study was to know the potency of pumelo fruit juice (citrus maxima var nambangan), vitamin C, lycopene, and combination of vitamin C and lycopene, suplementation as antioxydant compound on hepatotoxicity of ochratoxin (OTA)-induced mice (Mus musculus). Thirty five *male mice (Mus musculus) strain of balb/c, with age between two and three month divided randomly into seven groups of treatment (n=5), including K0, K1, K2, PI, P2, P3, P4, respectively for control group that was feed only with Olive Oil adjuvant (K0), CMCNa (K1), ochratoxin A (K2), pumela fruit juice (citrus maxima var nambangan) dose of 0,5 ml/30 gram BW/day (P1), vitamin C dose of 5,85μg/30 gram BW/day (P2), lycopene dose of 0,1025 μg/30 gram BW/day (P3), and combination of vitamin C and lycopene (P4). The experiment was conducted for three weeks periode, which is K0, K1, K2 groups were subjected with compound of treatment for a week period strated at second week, whereas P1, P2, P3, P4 groups were subjected with compound of treatment for two weeks period and subjected with OTA for a week strated at the third week The data collected were analyzed with Kruskal Wallis test and the result showed there was a significant different ($p<0.05$) between groups of treatment. This study had been proved that effectivity of pumelo fruit juice (citrus maxima var nambangan) supplementation on inhibit hepatotoxicity was similiar compared with vitamin C, lycopene, and combination of vitamin C and lycopene, throughtout these potency on inhibiting incresing of SGOT and SGPT blood serum on mice that exposed with OTA. Supplementation of Pummelo fruit juice (Citrus maxima var nambangan), vitamin C, lycopene and combination of vitamin C and lycopene have been proved decreasing, serum level of SGPT and SGOT on ochratoxin treated mice. This research also proved there was no significant different ($p>0.05$) the biologic potency of Pummelo fruit juice compare with vitamin C, lycopene and combination of lycopene and vitamin C on inhibited free radical reactivity due to ocrhatoxin exposure on mice.*

Keywords : Pumelo Fruit (Citrus Maxima Var Nambangan), Ochratoxin, Liver Functions

INTRODUCTION

Okratoksin (OTA) is a mycotoxin types which is produced by mold. Mycotoxins are commonly found as contaminants in foodstuffs and processed foods, such as on grains such as corn, wheat, rice, beans, soybeans, coffee, cocoa, spices, wine and fruits (Papachristou and Markaki, 2004).

In developed countries, regulations that arrange the amount of okratoksin contaminants in food materials has been implemented and closely monitored.

Currently okratoksin metabolism inside mammalian body are not recognizing completely. Two of the organs that are known to have an important role in the biotransformation okratoksin, also affected by the negative impact in cases of poisoning by okratoksin are kidney and liver. It was reported that, in the case of poisoning by okratoksin, accumulation of the mycotoxin compounds was highest in blood, kidney and liver, (Ringot ***et. al.,*** 2006).

Okratoksin metabolized in the liver produced reactive metabolites. These changes is being catalyze by P-450 enzymes to oxidize okraloksio through hidroksilasi substrate chain reaction. This chain reaction will produce O_2•- (superoxide) and H_2O_2. O_2• which is very dangerous when they are concurrent with H_2O_2 because it can be formed hidioksil radical ($OH^{\bullet}$). Besides, $OH^{\bullet}$ can be reacted with metals transition such as Fe^{2+} and Cu^{2+} through the Fenton reaction (Halliwell and Gutteridge, 2007).

Lipid peroxidation in membranes cell will lead to the increasing inside membrane permeability, which is resulting passive mitochondrial swelling that, will worsen the damage of cell (Hayati, 2011). The damage of mmitochondrial membrane may resulting the decreasing in ATP production that leads to disruption of the cell membrane permeability and Na-K pump, that result to the accumulation of water in cells and organelles (hydropic degeneration). The retrogressive cells process that is reversible may turn into irreversible and triggers the death of cell in necrosis, if there is increasing accumulation of intracellular calcium ions, which enter the cell permeability through disruption of membrane cell (Gavin, 2007).

Basically, free radicals such as, $O2^{\bullet-}$, H_2O_2 , and $OH^{\bullet}$ can be formed in the normal metabolic processes of the body. However, the body antioxidant enzymes, such as catalase, superoxide dismutase (SOD), glutathione and other antioxidant compounds that derived from food ingredients, can mitigate this. Such as vitamin C, vitamin E, and selenium, Induction of free radicals persistent and increasing of free radicals amount that come from outside the body may disturb the balance of antioxidant defenses, which will result the oxidative stress.

The addition of antioxidant supplements that come from outside of the body is one of the effective ways in reducing oxidative stress. One of the foods that contain lots of antioxidant is pomelo fruit that is contain vitamin C and lycopene.

Pomelo fruit (*Citrus maxima*) has been shown containing vitamin C and lycopene that has antioxidant effects. Lycopene is known to work as an antioxidant by capturing free radicals (Scavenger antioxidant) and break the chain of peroxidation that triggered by free radicals (chain breaking antioxidant). Meanwhile vitamin C acts as an antioxidant by capturing free radicals. It was reported that provision of lycopene for 3,025 μg/100 g and 38mg of vitamin C/l00g that is contained in tomatoes may lower SGPT and SGOT of the liver on experimental mice that induced by CCl_4 (Wahyono, 2006).

The content of vitamin C antioxidant and lycopene in pomelo juice are quite high. The flesh of the pomelo fruit (*Citrus maxima*) is containing 43 mg of vitamin C and 350 μg lycopene for every 100 grams of fruit flesh (Maulida and Zulcarnean, 2010).

Research on the effects on the kidneys that poisoning by okratoksin have been conducted, however, the impact of okratoksin toward the liver damage (hepafotoksisites) is still limited. As a tropical country, with abundant sources of biodiversity, this study aims to determine the potential of pomelo fruit (*Citrus maxima var Nambangan*) as a local variety, also as an antioxidant to prevent cells dysfunction that caused by exposure to hepatocytes okratoksin.

Problem Formulation

Based on the background of the problem, the problem formulation can be made as follows:

1. Is the provision of pomelo juice (*Citrus maxima var Nambangan*), vitamin C and lycopene may decrease SGOT levels of mice blood serum that caused by exposure of okratoksin?
2. Is the provision of pomelo juice (*Citrus maxima var Nambangan*), vitamin C and lycopene may decrease SGPT levels of mice blood serum that caused by exposure of okratoksin?

Objectives of the Research

1. General Purpose

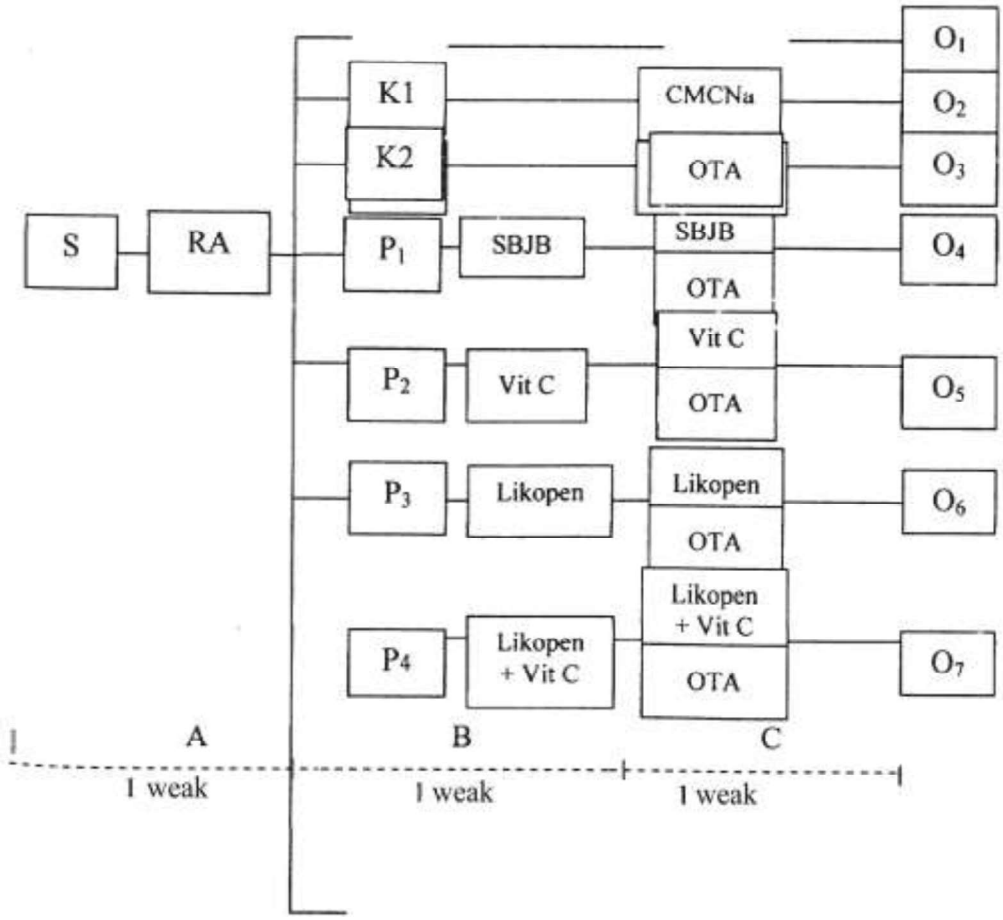
   Determine the effect of pomelo juice (*Citrus maxima var Nambangan*), vitamin C and lycopene in preventing the liver damage that caused by exposure of okratoksin.

2. The Specific Objectives
   a. Explaining the effect of pomelo juice (*Citrus maxima var Nambangan*), vitamin C and lycopene on levels of SGOT mice blood serum that caused by exposure of okratoksin.
   b. Explaining the effect of pomelo juice (*Citrus maxima var Nambangan*), vitamin C and lycopene on SGPT levels of mice blood serum caused by exposure of okratoksin.

MATERIALS AND RESEARCH METHOD

Type of Research

Type of research, which was conduct, is experimental laboratory research, which was using the design of Rondomized post test only control group design. Samples and treatments cultivated under controlled conditions and measured, therefore the treatment effect will be more reliable.



Description :

A	: The first week of research (animal adaptation)
B	: The second week of research
C	: The third week of research
CMCNa	: Sodium Carboxymethyl Cellulose
KO	: Control with the provision of adjuvant Olive Oil
K1	: Control with the provision of adjuvant CMCNa
K2	: Control with the provision of okratoksin A
0	: Observation
OTA	: Okratoksin

P1	: Treatment 1 (SBJB + Okratoksin)
P2	: Treatment 2 (Vitamin C + Okratoksin)
P3	: Treatment 3 (Lycopene + Okratoksin)
P4	: Treatment 4 (Vitamin C + Lycopene + Okratoksin)
RA	: Random Allocation
S	: Sample
SBJB	: Pomelo Juice
Vit C	: Vitamin C

Experiment Unit, Sample Size and Randomization

1. Experiment Unit

Experimental units that were used in this study are male mice (*Mus musculus*) adult strain BALB/C, with ages between two to three months and weight between 20 to 30 grams. Experimental animals obtained from the animal care unit of Central Veterinarian Pharmacy (PUSVETMA) Surabaya.

2. Randomization

Thirty-five (35) male mice were caught, aged between two to three months being adapted for one week in advance before being used as the study sample. In the adaptation period, all mice were get main food and standard maintenance until the age and weight are eligible for use in this research. The whole set of mice as animal model. Following that, 35 mice were divided into seven groups: which are three groups of control and four groups of treatment as seen in research design.

Variables Classification and Operational Definition of Variables

1. Classification of Variables

a. Independent variables, which are:
   - Pomelo Juice *(Citrus maxima var nambangan)*
   - Vitamin C
   - Lycopene
   - Combination of Vitamin C and Lycopene

b. Controlled variables, which are:
   - The age of animal experiments
   - The gender of animal experiments
   - The body weight of experimental animals
   - The dosage and the way of treatment provision
   - The dosage of Okratoksin A

c. The dependent variables are:
- The levels of SGPT and SGOT blood serum.

Materials Research

Materials research which is needed and used in this study are:

1. Standardized Pomelo Juice

The materials that is needed are standardized Pomelo juice (*citrus maxima var nambangari*) which is containing vitamin C at 416.50 μg / ml and 7.60 for lycopene μg/ml.

2. Materials for the manufacture of animal models

Materials research which is needed in this study were male mice, chaff, standard food, drinking water, HCL, ether, alcohol 70%, and formalin.

3. Materials for laboratory tests

Materials that are required for the various examinations in this study are the materials for SGOT and SGPT examination Materials that are required for examination SGOT, SGPT is blood serum, SGPT reagent, SGOT, alcohol, disposable syringe.

Decision Procedure and Data Collection

Data that included as the result of research are collected in the form of primary data. To ensure the reliability and validity, research was conducted in standard laboratory and has a complete equipment and adequate experience in the manufacture of animal model and management of animal experiments. This research was conducted at the Faculty of Pharmacy, Airlangga University Surabaya.

Data Analysis

To the data obtained, conducted statistical analysis using SPSS. 18. The method, which was used to determine the normality of distribution of variables, is using normality test. Further testing of data was using ANOVA test when it is obtained the normal distribution of data variables, while the distribution obtained the un-normal variables, the study will use Kruskal Wallis test.

1. Liver tissue extraction and blood samples

Necropsy action was conduct after the animal experiments is being anesthetic using ether to decreased the level of consciousness, then a necropsy is conducted and then conducted the liver excision. Liver that had been excised and processed further were used for the examination of MDA with TBA method.

2. Treatments of samples

a. Examination of SGOT and SGPT levels on mice blood serum was examine by the method.

RESULTS AND DISCUSSION

RESULTS OF RESEARCH

1. Pomelo Juice (*Citrus maxima var Nambangan*) Vitamin C and Lycopene Concerning SGOT levels of Mice Blood Serum (*Mas musculus*) which is Exposed by Okratoksin A

Results of statistical analysis with Kruskal Wallis test the effect of pomelo juice (Citrus maxima var Nambangan), vitamin C, lycopene, and a combination of

vitamin C and lycopene, to SGOT levels of mice blood serum which is exposed by okratoksin A, shows that there is a very real difference (p <0.001) among treatments. The average and standard deviation of serum SGOT levels in all treatments can be seen in Table 1.

Table 1 Levels of SGOT blood serum in all treatments

Treatment	Average ± Standard Deviation IU/L
K_0 *(Olive Oil)*	$71.60^{a} \pm 12.58$
K_1 (CMCNa)	$122.80^{ab} \pm 33.43$
K_2 (Okratoksin A)	$594.00^{c} \pm 207.27$
P_1 (SBJB)	$130.80^{b} \pm 13.92$
P_2 (Vitamin C)	$117.60^{b} \pm 30.00$
P_3(Lycopene)	$132.80^{b} \pm 33.00$
P_4 (Vitamin C + Lycopene)	$182.20^{b} \pm 100.20$

Different superscripts in the same column indicate significant differences at a confidence level $\alpha = 0.05$ ($P < 0.05$)

In Table 1 it is known that, the provision of pomelo juice (PI) was significantly shown (P <0.05) can decreased SGOT levels in mice which is exposed by okratoksin. Effectiveness of Pomelo juice (PI) in lowering SGOT levels of mice serum which is exposed by okratoksin A in this study, was not significantly different (p> 0.05) with the provision of vitamin C (P2), lycopene (P3), and the combination of vitamin C and lycopene (P4).
SGOT levels of mice blood serum in the group that given pomelo juice (Citrus var Nambangan), lycopene, vitamin C, and the combination of vitamin C and lycopene, in succession was 130.80 ± 13.92 IU / L, 117.60 ± 30 IU / L, 132.80 ± 33 IU / L, and 182.20 ± 100.20 IU / L where significant (p <0.05) proved to be lower when compared to the group who were given only okratoksin A (K2) where the blood serum of SGOT levels reached 594.00 ± 207.27 IU / L. Meanwhile, SGOT blood serum of mice in the group that were given pomelo juice (Citrus maxima var Nambangan), vitamin C, lycopene, and the combination of vitamin C and lycopene, significantly (p <0.05) higher when compared with the negative control group who received adjuvant Olive Oil (K0), but not significantly different (p> 0.05) when compared with the negative control group who received adjuvant CMCNa (Kl).

2. The effect of Pomelo Juice (Citrus maxima var Nambangan) Vitamin C and Lycopene Concerning SGOT levels of Mice (Mus musculvs) Blood Serum, which was Exposed by Okratoksin A

Results of statistical analysis with Kruskal Wallis test the effect of pomelo juice (Citrus maxima var Nambangan), vitamin C, lycopene, and the combination of

vitamin C and lycopene, concerning the SGOT levels of mice blood serum which was exposed by okratoksin, showed that there were highly significant differences ($p < 0.001$) among treatments. The average and standard deviation of serum SGPT levels in all treatments can be seen in Table 2.

Table 2 Levels SGPT blood serum in all treatment

Treatment	Average ± Deviation Standard IU/L
K_0 *(Olive Oil)*	$35.20^{a} \pm 4.55$
K_1 (CMCNa)	$48.80^{b} \pm 17.15$
K_2 (Okratoksin A)	$574.20^{c} \pm 225.53$
P_1 (SBJB)	$41.80^{b} \pm 6.42$
P_2 (Vitamin C)	$41.60^{ab} \pm 10.90$
P_3(Lycopene)	$42.40^{ab} \pm 8.90$
P_4 (Vitamin C + Lycopene)	$5L20^{ab} \pm 20.47$

Different superscripts in the same column indicate significant differences at a confidence level $\alpha = 0.05$ ($P < 0.05$)

In Table 2 it is known that the provision of pomelo juice (PI) was shown to significantly ($P < 0.05$) lower SGPT levels in mice that exposed by okratoksin A. The effectiveness of pomelo juice (PI) in lowering serum SGOT levels of mice which is exposed by okratoksin A in this study, was not significantly different ($p > 0.05$) with the provision of lycopene (P3) VIT C (P_2) and the combination of vitamin C and lycopene (P4).

Blood serum SGPT levels in the group of mice given pomelo juice (Citrus var Nambangan), vitamin C, lycopene, and the combination of vitamin C and lycopene, are respectively in succession 6:42 ± 41.80 IU / L, 41.60 ± 10.90 IU / L, 42.40 ± 8.90 IU / L, and 51.20 ± 20.47 IU / L which was significantly ($p < 0.05$) proved to be lower when compared to the group who were given only okratoksin A (K2) where the blood serum SGPT levels reached 574.20 ± 225.53 IU / L. Meanwhile, blood serum SGPT in the group of mice given pomelo juice (*Citrus maxima var nambangan*), was significantly ($p < 0.05$) higher when compared with the control group, lowering levels of SGOT and SGPT, as well as increasing the amount of SOD in hepatocytes cells of white rat which were exposed to CCl_4.

Elevated levels of SGOT and SGPT blood serum of mice is the consequence of damage of membrane lipid structure of hepatocytes via the peroxidation, which is caused by okratoksin A (OTA). Membrane lipid peroxidation in liver cells as well as disruption of protein synthesis during the exposure of okratoksin A (OTA), may lead to the death of cell and tumor progression (Dalle et al, 2006; Gagliano et al, 2006; Bouaziz et al., 2008; Jiang et alt 2012). The damage to the structure of the liver membrane may lead to the release of liver enzymes in the cell transaminase. There are two enzymes that act as biocatalyst in transamination reactions, which are: 1) the enzyme Aspartate Aminotransferase (AST) or Serum

Glutamic Oxaloacetic Transaminase (SGOT) and 2) the enzyme Alanine Amino trans/erase (ALT) or Serum Glutamic Pyruvic Transaminase (SGPT). The release of these enzymes from the hepatocytes resulted increasing amounts of it in serum (Boyer et al, 2006).

The results of this study proved that the provison of pomelo juice (*Citrus maxima var Nambangan*), vitamin C, lycopene and the combination of vitamin C and lycopene can prevent necrosis of hepatocytes cells that caused by exposure of okratoksin A (OTA), which was obtain a reduction in the levels of SGOT and enzyme, as well as SGPT in blood serum of mice.

CONCLUSION

Based on these results, it can be concluded that:

1. Provision of pomelo juice (*Citrus maxima var Nambangan*), vitamin C and lycopene may decrease blood serum SGOT levels of mice caused by exposure of okratoksin.
2. Provision of pomelo juice (*Citrus maxima var Nambangan*), vitamin C and lycopene may decrease serum SGPT levels of mice caused by exposure of okratoksin.

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