

## Hepatoprotective Effect of Mangosteen Peel Extract on Borax-induced Male Rats

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### Article Info

#### Article history:

Received Jun 25, 2016

Revised Aug 18, 2016

Accepted Aug 26, 2016

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#### Keyword:

Borax

Hepatoprotective

Liver Weight

Mangosteen peel extract

SGOT

SGPT

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### ABSTRACT

The aim of this study was to determine the hepatoprotective effect of mangosteen peel extract (MPE) on borax-induced male rats. Hepatoprotective effect is represented by the variables of liver weight, SGOT (Serum Glutamic oxaloacetic transaminase) and SGPT (Serum Glutamic Pyruvate Transaminase). The true experimental study used randomized separated pretest-posttest control group design. MPE was made with 50% ethanol and 0.5% carboxymethyl cellulose-sodium (CMC-Na) stabilizer solutions. Studied groups included three control groups (positive, negative pretest and posttest) and three experimental groups (MPE dose of 200 mg/kg, 400 mg/kg and 600 mg/kg rats body weight (BW)). Samples consisted 36 male rats. The length of intervention was 16 days by providing borax, and then MPE was started on day 3 (in 3 experimental groups). Results showed that up to 16th day the SGOT tended to decrease ( $\alpha < 0.05$ ), while the SGPT levels was stable, and so was the liver weight. Thus, up to day 16 the MPE was generally able to provide hepatoprotective effects mainly based on the variable SGOT in doses of 200 and 400 mg/kg. It has been proved that liver function of the rats was still relatively normal. This is in relations to the antioxidant potential of the MPE polyphenols.

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## 1. INTRODUCTION

Results of Food Safety Survey by NADF RI 2009 to 1504 Household Food Industries (*Industri Rumah Tangga Pangan, IRTP*) in 18 provinces showed that, compared to others, the majority of non-food grade additives abused was borax (8.80%) [1]. The main danger to health from borax use is that the residues it left behind can cause systemic effects on the body. In addition to its visible influence from outside, such as hardening of the skin and outer skin tissue death, more harmful effects are related to damages of liver, stomach, small intestine, colon, infertility of the testes and ovaries, as well as stimulating the growth of cancer cells. Borax that attack cells, especially in mitochondria, accumulates in the cytoplasm that interfere with cell metabolism. In high doses in the body, borax will cause dizziness, vomiting, diarrhea, abdominal cramps, low blood pressure, anemia, fever and damage to other organs, including brain, and even death [2], [3].

These negative impacts occur because borax is a source of free radicals [4] particularly superoxide radical ( $O_2^*$ ), that is very strong and can damage cells [5]. Borax has chemical properties that is able to bind the hydroxyl group ( $OH^-$ ) on glucose and glycogen, as well as binding  $NAD^+$  coenzyme containing ribose component which are active in energy metabolism pathways the glycolysis [6], [7].

According to the research results, liver is a site where borax is found in high concentrations, in addition to brain and kidneys [8]. The liver is the largest organ where many processes of energy metabolism, lipid, carbohydrate and protein, take place. In this case, the liver is able to detox xenobiotic or toxins that enter the body, and vulnerable to the effects of toxic substances [9], [10].

The finding of borax in high concentrations in the liver will cause a negative impact on liver cells. Studies showed that degeneration and necrosis had already occurred in experimental rats liver histopathology on day 14 after borax administration [11]. Liver damage is always associated with cell necrosis, increased lipid peroxidation and decreased levels of tissue GSH (Glutathione). Other biochemical indicators in blood serum level are the level of SGOT (Serum Glutamic oxaloacetic transaminase), SGPT (Serum Glutamic Pyruvate Transaminase), ALP (Alkaline Phosphatase) and bilirubin [12].

In addition, organ weights and weight loss are indication of organ injury. Changes in organ weights have long been accepted as a sensitive indicator of chemical changes in organs [13], [14].

In relations to the occurrence of hepatotoxicity due to borax induction in mice, it is necessary to protect the liver (hepatoprotective) by using plants containing phytochemicals that can serve as antioxidant as well as free radicals scavenger to neutralize free radicals and able to protect coenzyme NAD<sup>+</sup> level.

This study used herbs, mangosteen peel (*Garcinia mangostana* Linn), which contain high polyphenols antioxidants kind, such as xanthenes [15], phenolic acid, flavonoids [16], catechins (including flavonoid), hexadecanoic acid, and oleic acid. The compound xanthenes is an antioxidant found in mangosteen fruit peel. Such compounds have antioxidant activity because of its phenol group [17]. Additionally, mangosteen peel contains flavonoids that is supposedly able to protect NAD<sup>+</sup> level in the cell [18],[19].

The aim of this study was to determine hepatoprotective effect of mangosteen peel extract (*Garcinia mangostana* L) in borax-induced male rats (*Rattus norvegicus*) by measuring the weight of the liver, SGOT and SGPT.

## 2. RESEARCH METHOD

### 2.1. Research type and design

This was a true experimental study, using randomized separated pretest-posttest control group design. There were three types of groups: negative control group (2 sub-groups: pretest (K1) and posttest (K2)), positive control group (K3) and mangosteen treatment group (3 subgroups: group P4, P5 and P6), totally there were 6 groups.

### 2.2. Samples and sample size

Samples used were white experimental rats (*Rattus norvegicus*) of Wistar strain, with inclusion criteria of male, aged 3-4 months, healthy and active. While the exclusion (drop out) criteria were unwilling to eat, ill, stress, inactive, and died before and after obtaining treatment. There were six studied groups (K1, K2, K3, P4, P5 and P6), and each group takes 6 mice, the total sample size was 36 rats.

### 2.3. Herbs and experimental animals

Materials used were dark purple mangosteen fruit from mangosteen plantation in black from Trenggalek district. Harvest Age was 104-110 days after flowering.

The experimental animals were male Wistar strain *Rattus norvegicus*, aged 3-4 months, body weight ranged between 150-250 g, purchased from a rats breeder in Bangil, Pasuruan, under the supervision of the Faculty of Veterinary Medicine, University of Airlangga, Surabaya. Rat feed was pellet produced by PT. Charoen Pokphand Surabaya. Rats' drinking water was gallon water of the brand Aqua. Before treatment, the experimental animals were acclimatized for 16 days during which they were given with standard feed, ad libitum drink, ambient temperature, air humidity between 40-60%.

### 2.4. Chemical substances and instruments

Chemicals for producing mangosteen peel extract includes a sterile distilled water, 50% ethanol, CMC-Na (Carboxymethyl cellulose) for stabilizing a solution of mangosteen peel extract, borax (sodium tetraborate decahydrate), chloroform for rats anesthesia, and 10% formaldehyde buffer to preserve rat organs. As for the phytochemicals (polyphenols and flavonoids) screening of mangosteen peel filtrate we used chloroform-ethyl acetate-formic acid, FeCl<sub>3</sub>, chloroform-methanol, and ammonia vapors. For the analysis of SGOT and SGPT values, the chemical materials were assay buffer, LDH, co-substrate, NADH, dry reagent, dH<sub>2</sub>O and enzyme mix.

For maintenance, treatment and blood sampling, instruments needed were cage (containing 3-4 mice), surgical scissors, scalpel blade, tweezers, gloves, pines, digital scales, organ scale, electronic scales

pocket brand Camry EHA401 models, syringe, stomach sonde, and blood serum. Instruments used to measure the SGPT and SGOT were pipettes, cuvette, syringe, centrifuge, and spectrophotometers.

Tools for making mangosteen peel extract were mill pulverization, glass jars, paper filters, Buchner funnel filter, tubing Erlenmeyer tube, Buchirotary vapor-R-116, Buchi rotary vapor-R-200, Branson ultrasonic 3510, freeze dryer Eyela model of FD-81, Sanyo ultralow Freezer, gram scales, and an analytical balance.

### **2.5. Mangosteen peel extract and doses**

The making of mangosteen peel extract (MPE) used maceration and sonication methods, with a polar solvent of 50% ethanol. As much as 500 mg mangosteen peel powder, whose the outer peel had been removed, was mixed with 2 L of 50% ethanol until it became homogeneous, deposited for 24 hours (maceration process), sonicated for 15 minutes and filtered. It was repeated 5 times to form the filtrate. Then it was evaporated with rotavapour (45°C) to form a viscous extract. Furthermore, it was freeze dried for 24 hours, which eventually the extract was formed in the form of dry powder. The yield was 15% formed. The dose applied was 200 mg/kg, 400 mg/kg and 600 mg/kg rat BW.

### **2.6. Borax solution and doses**

Borax used has a chemical term sodium tetraborate decahydrate, as a white powder in the solvent of distilled water, mixed until homogeneous. Borax dose applied was approximately 261.1 mg/kg of rat body weight/day.

### **2.7. Preparations for treatment**

Preparation MPE given with sonde to the mice are was MPE suspension in CMC-Na (Carboxy Methyl Cellulose-Sodium) 0.5%.

### **2.8. Procedure**

Before the intervention, pretest negative control mice (K1) was not given anything. Posttest negative control (K2) was given only with 0.5% CMC-Na solution, starting on day 1 to 16. The positive control (K3) was given only with borax solution to day 16. Treatment in the three groups of rats with borax (from day 1 to day 16) was followed with the provision of MPE starting on day 3 to 16 using a stomach sonde in 3 doses of 200 mg/kg bw (P4), 400 mg/kg bw (P5) and 600 mg/kg bw (P6), 1-2 ml per rat. Pretest of day 0 and posttest of day 17 was done by scaling liver weight, and measuring SGPT and SGOT levels in their blood serum.

### **2.9. SGOT and SGPT test methods**

SGOT examination procedure, directly by measuring AST (aspartate transaminase) activity based on the amount of oxaloacetate produced by AST. In this test, oxaloacetate and NADH were converted into malate and NAD by malate dehydrogenase enzyme. Decreased levels of NADH at 340 nm absorbance was proportional and in accordance with the AST activity. Working stages were three: stage of using 96 wells plate, stage of using cuvettes, and stage of measurement.

SGPT examination procedure, indirectly by measuring the activity of ALT (Alanine transaminase) based on the amount of pyruvate produced by ALT. In this test, pyruvate and NADH were converted into lactate and NAD by the enzyme lactate dehydrogenase (LDH). Decreased levels of NADH at 340 nm absorbance was proportional to the ALT activity. Working stages were three: stage of using 96 plate wells, stage of using cuvettes, and stages of measurement.

### **2.10. Statistical analysis**

Data analysis was performed using IBM SPSS statistics 21 for windows as follows: a) analysis with descriptive statistics, b) test for normality of data distribution with the Kolmogorov-Smirnov test, c) homogeneity test by Levene's test, d) comparative test, between groups before and after being treated with one way ANOVA (Analysis of Variance). on  $\alpha=0.05$ , and e) a further test with post-hoc multiple-comparison test with Tukey test [20], f) if the data were not homogeneous or not normally distributed, then tested with non-parametric comparative Kruskal Wallis test, g) test further use is non-parametric Mann Whitney test.

## **3. RESULTS AND ANALYSIS**

### **3.1. Liver weight**

Results of measurements of the weight average of the liver in each study group are presented in Table 1. We can see below this.

Table 1. Weight Average of the LIVER in each Studied Groups

No.	Studied groups	Weight Average of the liver ( $\bar{x} \pm$ standard deviation) gr
1	K1	7.05 $\pm$ 0.25
2	K2	6.59 $\pm$ 0.74
3	K3	7.35 $\pm$ 0.60
4	P4	6.60 $\pm$ 0.85
5	P5	7.21 $\pm$ 0.69
6	P6	7.26 $\pm$ 1.32

The significance level ( $\alpha$ ) of Kruskal Wallis comparative test is 0.435 ( $\alpha > 0.05$ ).

Notes:

- K1 = pretest negative control group, not receiving anything on day 0  
 K2 = posttest negative control, receiving only a solution of 0.5% CMC-Na from day 1 to day 16  
 K3 = positive control group, receiving only borax dose of 261.1 mg/kg bw/day from day 1 to day 16  
 P4 = treatment group, receiving borax of 261,1mg/kg bw/day from day 1 to day 16, and receiving mangosteen peel extract of 200 mg/kg/day starting on day 3 to day 16  
 P5 = treatment group, receiving borax of 261,1mg/kg bw/day from day 1 to day 16, and receiving mangosteen peel extract dose of 400 mg/kg bw/day starting on day 3 to day 16.  
 P6 = treatment group receiving borax of 261,1mg/kg bw/day from day 1 to day 16, and receiving mangosteen peel extract dose of 600 mg/kg/day starting on day 3 to day 16.

Complete results of liver weight measurements are presented in Figure 1 below this.

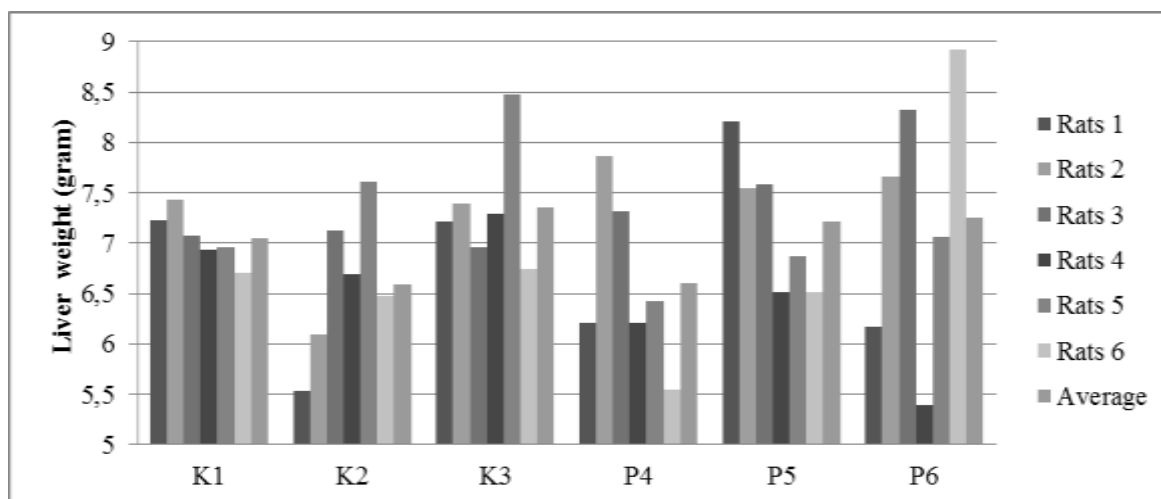


Figure 1. Results of liver weight measurements and the average in rats

Descriptively the average weight of the liver was the highest in group K3 as shown in Figure 1. Results of statistical tests revealed normally distributed data ( $\alpha > 0.05$ ), but not homogeneous ( $\alpha=0.035$ ). Results of non parametric Kruskal Wallis test revealed  $\alpha=0.435$  ( $\alpha > 0.05$ ), indicating no significant difference between one group and the others. Thus, the organ weight did not increase.

Boric acid causes a decrease in the concentration of metabolites such as glucose, glycogen and lactate, due to the formation of complexes between boron and hydroxyl compounds. In addition, boric acid causes damage to some parts of mitochondria. Lack of ATP metabolites and breakdown of mitochondrial metabolism is harmful to the survival and function of cells, especially tissue that depends highly on energy, such as skeletal muscle [21]. Borax has chemical properties that is able to bind hydroxyl group (OH<sup>-</sup>) on glucose and glycogen, as well as binding coenzyme NAD<sup>+</sup> containing ribose component which are active in energy metabolism pathways, the glycolysis [6],[7].

Impaired energy metabolism that causes a decrease in ATP synthesis, which occurs as in ischemic disorder, will cause Na<sup>+</sup> and Ca<sup>2+</sup> pump experience a shortage of energy and causes more Na<sup>+</sup>, Ca<sup>2+</sup> and H<sub>2</sub>O flow into the cell (influx), while more K<sup>+</sup> to flow out of the cell (efflux). Consequently, swelling occurs from endoplasmic reticulum to the cell. Such cellular swelling is the first manifestation of virtually all forms of cell injury. When it affects the cells in the organ, it will cause paleness, increased turgor, and increased organ weights [22].

The increase in organ weight is one indicator of cell injury due to the use of chemicals. Analysis of organ weights in toxicology studies is an important final point to identify potential harmful effects of chemicals [23]. Descriptively, organ weight of the positive control group, after 16 days of intervention, had the highest increase, although it was not different significantly from the other groups. However, the results showed that rats' liver that had been induced with borax for 16 days did not show significant increase in weight after being given with mangosteen peel extract. It was suspected there was the role of mangosteen peel extract polyphenols in protecting the organ from cellular swelling that can lead to an increase in weight of the liver. Moreover, mangosteen peel contains flavonoids that is allegedly able to protect  $\text{NAD}^+$  level in the cell [18], [19], so as to prevent ATP production decline which also have an impact on cellular swelling. The lowest (the best) average organ weight was that in group receiving MPE of 200 mg/kg.

### 3.2. SGOT

Results of measurements of the SGOT average of the liver in each study group are presented in Table 2. And results of post hoc test comparing difference between groups are presented in Table 3. We can see all below this.

Table 2. The SGOT Average in Rats Serum in each Studied Groups

No.	Studies groups	SGOT Average ( $\bar{x} \pm$ standard deviation) U/L
1	K1	91.00 $\pm$ 9.65
2	K2	105.33 $\pm$ 22.68
3	K3	125.00 $\pm$ 14.85
4	P4	95.67 $\pm$ 9.58
5	P5	87.00 $\pm$ 12.63
6	P6	117.33 $\pm$ 13.63

The significance level ( $\alpha$ ) of comparative *Anova* test between all groups is 0.00 ( $\alpha < 0.05$ ).

Table 3. Results of post hoc "Tukey" test comparing difference between groups

No	Between Groups	Significance ( $\alpha$ count)	Correlation
1	K1 – K2	0.536	Not significantly different
2	K1 – K3	0.040	Significantly different
3	K1 – P4	0.993	Not significantly different
4	K1 – P5	0.997	Not significantly different
5	K1 – P6	0.040	Significantly different
6	K2 – K3	0.208	Not significantly different
7	K2 – P4	0.855	Not significantly different
8	K2 – P5	0.273	Not significantly different
9	K2 – P6	0.708	Not significantly different
10	K3 – P4	0.017	Significantly different
11	K3 – P5	0.001	Significantly different
12	K3 – P6	0.940	Not significantly different
13	P4 – P5	0.903	Not significantly different
14	P4 – P6	0.133	Not significantly different
15	P5 – P6	0.013	Significantly different

Notes:

a)  $\alpha < 0.05$ =significant difference

b)  $\alpha > 0.05$ =not significant difference

The complete results of SGOT measurements are presented in Figure 2. Based on the results of the study, blood serum levels of AST in all study groups were significantly different ( $\alpha=0.00$  or  $\alpha < 0.05$ ). Thus, there was a significant hepatoprotective effect of MPE on the decline in blood serum levels of AST mice that have been induced with borax for 16 days. The highest average level (125.00  $\pm$  14.85 U/L) was still in positive control group (receiving only borax for 16 days). AST level was the lowest (the best) in the provision of MPE of 400 mg/kg bw (87.00  $\pm$  12.63) U/L. Functionally, rat's liver function was still relatively normal, because the normal SGOT value in male rats is 60-300 U/L, and female 80-250 U/L [24].

The enzyme aminotransferase (formerly transaminase) is most often used as a specific indicator of hepato-cellular necrosis. The enzyme aspartate aminotransferase (AST, formerly serum glutamic oksaloacetate transaminase or SGOT) and alanine aminotransferase (ALT, formerly serum glutamic pyruvic transaminase or SGPT) catalyzes the transfer of amino group of aspartic acid and alanine respectively to the keto group of ketoglutaric acid. ALT is mainly localized in the liver but AST is present in various tissues such as the heart, skeletal muscles, kidney, brain and liver [25],[26].

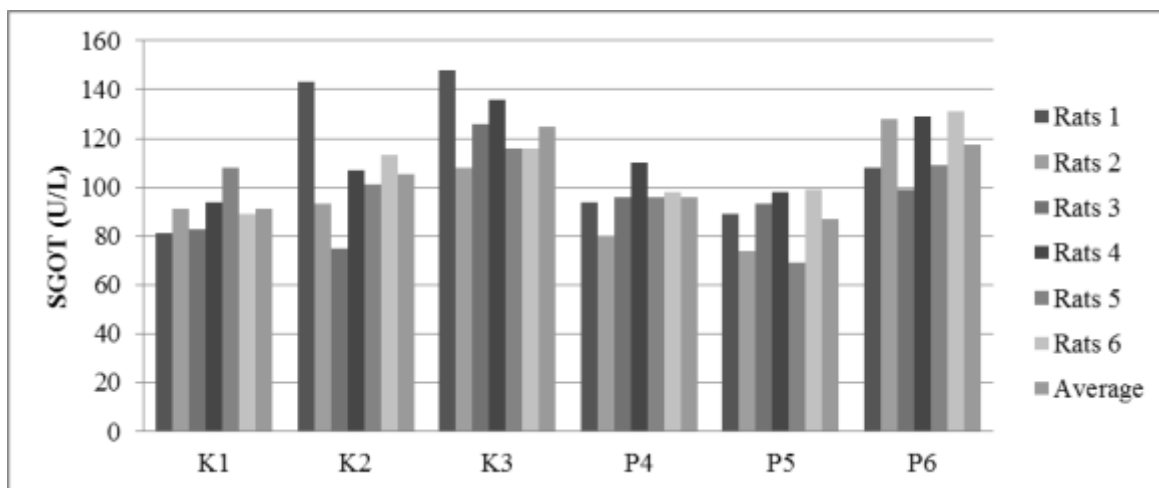


Figure 2. Results of SGOT measurement and the average in rats serum

The results of a study by Octavia (2012) [11], that examined the effect of borax on histopathological profile of the liver in white rats (*Rattus norvegicus*), using experiment up to day 14 of intervention, showed that borax does not cause congestion, but caused degenerative changes and necrosis in liver cells. And Astiningrum (2011) [27] studied about the effect of borax on liver damage swiss Webster strain of male mice, in which borax was administered in doses of 150, 300, 450, and 600 mg/kg/day for 27 days, evidently in dose of 600 mg/kg/day was found to increase SGOT (AST) and SGOT (ALT) levels in mice liver.

AST is present in both mitochondria and cytosol of hepatocytes. ALT is localized to the cytosol [26],[28]. In cytosolic and mitochondria, AST was a true isoenzyme and immunologically distinct [29]. Approximately 80% of AST activity in human liver is contributed by mitochondrial isoenzyme, whereas most AST activities circulating in normal individuals are derived from cytosolic isoenzymes [25],[30].

ALT cytosol is associated with pyruvate use in glycolysis. ALT mitochondrial is involved in the conversion of alanine to pyruvate in gluconeogenesis, and AST plays an important role in transport across the mitochondrial membrane [31],[32]. Hepatocellular damage due to any disorder allows plasma membrane leakage, so that intracellular enzymes such as ALT or AST flow into the bloodstream. Due to some hepatotoxicants, the increase of liver aminotransferase synthesis also becomes a source of elevated levels of serum enzyme in hepatocellular injury [33]. Increased levels of serum AST occurs in mitochondria after an extensive tissue necrosis. Therefore, mitochondrial AST determination is recommended in myocardial infarction cases and is also elevated in chronic liver disease [34].

Necrosis of the liver tissue occurs due to oxidative stress. Oxidative stress occurs due to excessive generation of free radicals and/or lack of antioxidants (eg. a substance called glutathione, vitamin A and E) to bind the radicals. Oxidative stress occurs in conjunction with the ATP depletion and reduced glutathione levels in the cells [35].

The tendency of AST decrease in blood serum in mice is thought to result from polyphenols contribution of mangosteen peel extract as antioxidants or scavenger of free radicals that prevent oxidative stress in mice liver, which will result in liver necrosis.

Polyphenol is a group of secondary metabolites involved in the cleanup of H<sub>2</sub>O<sub>2</sub> in plant cells. In this case the polyphenolic compound has a high antioxidant potential, providing protection against cancer by inhibiting oxidative damage, which is known as a potential cause of mutations. Free radicals cause oxidative damage to lipids, proteins, and nucleic acids [36]. Polyphenol antioxidant properties are the main feature of the capacity of scavenging free radicals [37]-[39].

### 3.3. SGPT

Results of measurements of the SGPT average of the liver in each study group are presented in Table 4.

Table 4. The SGPT Average in Rats Serum in Each Groups

No.	Treatment Groups	SGPT Average ( $\bar{x} \pm$ standard deviation) U/L
1	K1	56.67 $\pm$ 5.20
2	K2	47.83 $\pm$ 8.66
3	K3	58.50 $\pm$ 11.47
4	P4	49.33 $\pm$ 6.68
5	P5	50.50 $\pm$ 5.43
6	P6	56.83 $\pm$ 6.05

The significance level ( $\alpha$ ) of Kruskal Wallis comparative test is 0.189 ( $\alpha > 0.05$ ).

The complete results of SGPT measurement are presented in Figure 3.

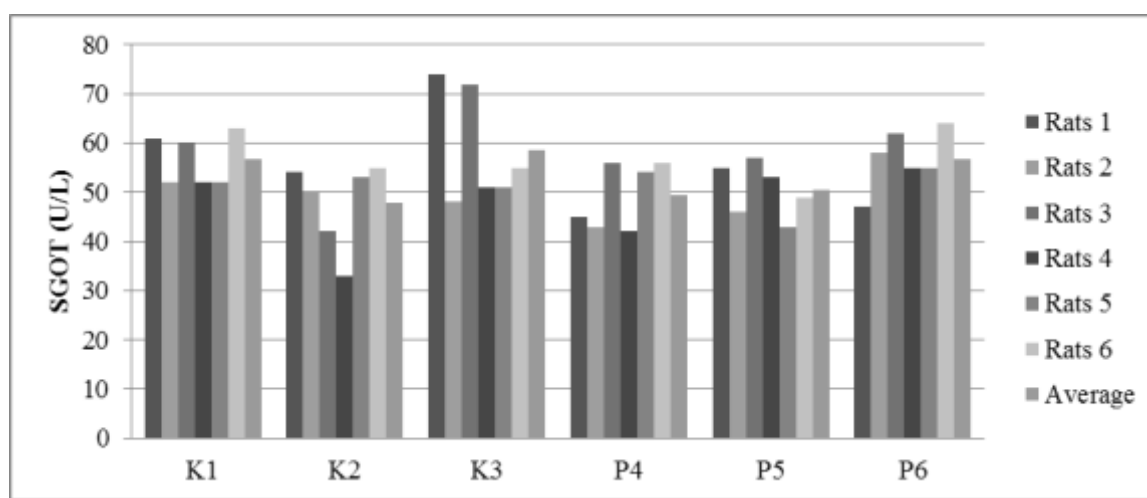


Figure 3. Results of SGPT measurement and the average in rats serum

Based on the research results, ALT level in blood serum of all groups did not differ significantly ( $\alpha > 0.05$ ). This indicates that rats liver that had been induced with borax for 16 days did not have elevated ALT level significantly after being given with mangosteen peel extract. However, the highest mean ( $58.50 \pm 11.47$  U/L) remained in positive control group (receiving only borax for 16 days). The lowest (the best) ALT level was in the provision of mangosteen peel extract of 200 mg/kg BW. Functionally, liver function of the rats was still relatively normal since the threshold value of normal SGPT in male rats is 25-55 U/L, while in females rats 25-50 U/L [24].

SGPT (ALT or alanine aminotransferase) is an enzyme that catalyzes the transfer of amino groups to form oxaloacetate liver metabolite [40]. This enzyme consists of 496 amino acids, encoded by genes located along the arm of chromosome 8 [41],[42]. Thus, in the case of hepatocellular injury or death, the release of ALT from damaged liver cells is increasing by measuring ALT activity in blood serum. ALT, cytosolic enzyme, is found in highest concentrations in the liver and more specifically to the liver [43]. In addition to the liver, it is also found in kidney, and, in smaller amounts, in skeletal muscle cells [44].

The tendency of stability and slightly decreased ALT level in rats blood serum is suspected to result from the role of MPE polyphenols as antioxidants or free radicals scavenger that prevent oxidative stress in rats liver, which will may result liver necrosis.

Aside from liver disease and damage, ALT serum activity can be influenced by a number of factors unrelated to liver necrosis [44]. Therefore, the variability of ALT levels is allegedly influenced by many factors that could not be predicted in this study.

#### 4. CONCLUSION AND SUGGESTION

Results revealed that up to day 16 SGOT was decreasing ( $\alpha < 0.05$ ), while SGPT levels was stable ( $\alpha > 0.05$ ), so was the weight of the liver ( $\alpha > 0.05$ ). The highest average weight of the organ, SGOT and SGPT was in positive control group. In treatment group, the lowest was in group of 200 mg/kg (for organ

weights and SGPT) and a group of 400 mg/kg (in SGOT). Thus, until day 16 MPE was generally able to provide hepatoprotective effects, mainly based on SGOT variable in doses 200 and 400 mg/kg. It has been proved that liver function of the experimental rats was still relatively normal. This happens because there is the role of polyphenols MPE in protecting from cellular swelling that could lead to an increase in liver weight, and there is the role of polyphenols as a protective of NAD<sup>+</sup> and antioxidant levels, which may protect from rising the levels of SGOT and SGPT due to necrosis from oxidative stress after borax induction.

The use of mangosteen peel extract should be improved as antioxidant supplements. In addition, further studies should be carried out on the protective effects of mangosteen peel extract on organs other than the liver and using other non-food grade additives.

#### ACKNOWLEDGEMENTS

I would like to express my deep gratitude to Prof. R. Bambang Wirjatmadi, dr, M.S., M.CN., Ph.D, Sp.GK, Prof. Mangestuti Agil, M.S., Apt., and Dr. Merryana Adriani, S.KM., M.Kes, my research supervisors, for their patient guidance, enthusiastic encouragement and useful critiques of this research work. I would also like to thank to Prof. I Ketut Sudiana, Drs., M.Si. for his advice and assistance in keeping my progress on schedule. My grateful thanks are also extended to Mrs. Evi Arfianti, S. KM., M. Kes., Mr. Sujarwo, Mr. Heri and Mrs Endah for their help in doing at the laboratory pharmacy and biochemical. Finally, I wish to thank my husband, my parents, daughter, sister, brother for their support and encouragement throughout my study.

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