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SUBCHRONIC TOXICITY EFFECT OF MANGOSTEEN RIND ETHANOL EXTRACT (*Garcinia mangostana* L.) ON LIVER DAMAGE IN WISTAR RATS

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ABSTRACT— Mangosteen (*Garcinia mangostana* L.) has antioxidant, antibacterial, antitumor, anticancer, and antiproliferative functions. Previously, an acute toxicity test of the ethanolic extract of mangosteen rind had been carried out, so further testing was needed, namely the subchronic toxicity test, to determine the toxic effects that were not detected in previous studies. This study aims to assess the degree of toxicity of changes in liver function considered from the levels of SGPT and SGOT. This research is an experimental study using the Posttest Only Control Group Design method using 40 individuals. Rats were divided into the control group and treatment group. The treatment group was divided into three groups: a dose group of 250 mg/kg BW, 500 mg/kg BW, and 1000 mg/kg BW. The control group was only given drinking water and feed. Dosing is provided for 28 days. Observations were made for 28 days, and on the 29th day, blood was taken to check the SGPT and SGOT. The results showed an increase in SGPT levels in male rats at a dose of 250 mg/kg BW and SGOT levels in female rats at 250 mg/kg BW but did not cause subchronic toxicity effects on the liver damage. There was no significant difference between male and female SGPT levels using the Kruskal Wallis test ($p = 0.240$ and $p = 0.152$). The SGOT level of male rats using the Kruskal Wallis test was found to be $p = 0.061$, and the SGOT level of female rats using the One Way ANOVA test was obtained $p = 0.101$.

KEYWORDS: mangosteen, toxicity test, subchronic, liver

1. INTRODUCTION

Indonesian people have long used herbal plants as alternative medicines because they are easy to find and because the prices were relatively affordable, especially for people with lower middle incomes [1]. A plant that is widely used and consumed as a supplement and traditional medicine is the mangosteen fruit (*Garcinia mangostana* L.). Not only the fruit, but the skin of the mangosteen fruit has also been used to treat canker sores, gout, and dysentery because the xanthone compounds in it have antibacterial activity [2]. In addition, mangosteen rind is also believed to be able to overcome infection, inflammation, and heal wounds. Xanthones are bioactive compounds in mangosteen rind that are thought to have antidiabetic effects that have potential effects in lowering fasting blood glucose and increasing plasma insulin in people with diabetes mellitus [3], [4].

Extract of mangosteen rind is now widely circulated as a dietary supplement as well as alternative medicine. The content of the active substances xanthones and anthocyanins in mangosteen rind extract is known to have very high antioxidant activity which is needed to fight free radicals and maintain health and endurance

[3], [5], [6]. One of the conditions for the proper use of traditional medicine is the appropriate dose of therapy. This was because the active substance in supplements or traditional medicines exceeds the allowed dose, it might trigger adverse effects or even be toxic to the body [7]. Although the various benefits of mangosteen rind extract have been widely known, the toxic effects are not known for certain [8]. To determine the toxicity of a material that will be used as a drug, it is necessary to conduct a study using Lethal Doses 50 (LD50) as a parameter [9].

The toxicity test consists of three stages: a toxicity test on an acute scale, a toxicity test on a subchronic scale, and a toxicity test on a chronic scale. Subchronic toxicity test is a toxicity test aimed at finding the toxic effect of substances that have previously been tested in acute toxicity tests by giving one dose for a period of 28 to 90 days. A chronic toxicity test is a toxicity test carried out for 12 months. From the research that will be carried out, it is hoped that it can provide information covering the effects of physiology, hematology, and biochemistry [8].

The toxic effect of a substance can be seen in the liver because the liver functions as a detoxifier for substances in the body and plays a role in metabolic processes [12], [13]. Liver is the largest organ and gland in the body which is located in the abdominal cavity and is susceptible to toxic compounds. The cause could be due to the administration of excessive doses of drugs over a very long period. Consumption of drugs in excessive doses can cause the release of hepatotoxins so these compounds can damage liver cells (hepatocytes). so that the levels become increased in the blood serum.

2. MATERIALS AND METHODS

2.1 Plant Material

This study used the mangosteen rind as the object of research. Mangosteen rind extract was obtained from the School of Biological Science and Technology, Bandung Institute of Technology, West Java. For an extract from the skin itself, it is obtained from one of the areas in the city of Tasikmalaya.

2.2 Mangosteen extract preparation

The mangosteen rind that has been separated was weighed and cleaned first, then sliced thinly and stored in a hydrator oven basket for a 12-hour drying process. Next, the mangosteen rind is ground by a machine until it becomes powder. The mangosteen rind powder was put in a vessel and then poured with 96% ethanol as a solvent until it was submerged. The vessel was tightly closed to protect from light and left for 1 day while stirring repeatedly. The macerate was placed in a porcelain dish, while the remaining pulp was added to taste more liquid. Maceration was carried out 3 times. As the final stage of the extraction process, the filtrate was combined and evaporated in a water bath to obtain a thick extract.

2.3 Experimental Animal

The experimental animal was *Rattus norvegicus*, a Wistar strain which is obtained from the Veterinary Laboratory of the Faculty of Medicine, Padjadjaran University, West Java-Indonesia. Their weight ± 200 gram with an age range of 6-8 weeks. The number of experimental animals needed was 40, consisting of 20 females and 20 males. Before undergoing treatment, animals were acclimatized for 1 week at a temperature of 22°C ± 30°C, with a relative humidity of 30-70% and a cycle of 12 hours of light and dark. Mice were fed and watered ad libitum. During acclimatization, rats were randomly selected to enter the experimental or control groups. All of these research procedures have followed the instructions from the care and use of laboratory animals and have been accepted by the research ethics committee for animal care from the Faculty of Medicine, Padjadjaran University, West Java-Indonesia, withs number:

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2.4 Sub Chronic Toxicity Study

Rats were divided into 4 groups with 5 males and 5 females each. The negative control group was only given water and pellets, while the P1-P3 groups were the treatment group which was given water, pellets, and ethanol extract of mangosteen rind at a dose of 250 mg/kg BW (P1), 500 mg/kg BW (P2), and 1000 mg/kg. Kg BW (P3). The test preparation was given orally with repeated doses every day for 28 days [8].

All rats that were still alive on day 28 were anesthetized with ether for blood collection. Measurement of SGPT and SGOT levels as biomarkers of liver damage was carried out by taking 3-5 mL of blood from the jugular vein using a sterile injection.

2.5 Biochemical Analysis

Blood samples taken from the jugular vein were then put into a vacutainer tube equipped with a serum separator and centrifuged for 10-15 minutes at 3000 rpm to obtain blood serum [13].

A total of 100 µL of blood serum and 1000 µL of SGPT/SGOT reagent were mixed in a cuvette and then incubated for 5 minutes at 37°C. The absorbance size was measured 3 times in 1 minute intervals with a spectrophotometer at a wavelength of 340 nm. The measured absorbance was calculated by the formula ΔAbs/minute ×1746 for IU/L SGOT, and ΔAbs/minute ×1768 for IU/L SGPT. Where ΔAbs/minute is the average change in absorbance per minute and Iu is units per liter of serum. The two formulas are used to determine the levels of SGPT and SGOT. The SGPT/SGOT levels were then averaged and compared for each treatment group.

2.6 Statistical Analysis

The data were described with mean ± standard deviation (SD) and statistically analyzed using Kruskal Wallis, then continued with the Mann Whitney Test when the data showed a significant difference ($p < 0.05$) in Kruskal Wallis.

3. RESULT

3.1 SGPT Serum

According to Table 1, it can be seen that the highest average SGPT level in male rats was in a group that received a dose of 250 mg/kg BW and the lowest average SGPT level was in the P3 group which was 78.4 UI/L. Meanwhile, in female rats, the highest average SGPT level was in the control group and the lowest average SGPT level was in dose 1000 mg/kg BW.

Table 1 Average levels of SGPT after administration of ethanol extract of mangosteen rind

Group	Average ALT levels ± SD (mg/dL)				*p*
	Control	250 mg/kgBB	500 mg/kgBB	1000 mg/kgBB	
Male	129.20±73.85	145.80±71.85	114.40±20.95	78,40±45,58	0.240
Female	154.40±42.07	139,80±101.11	109.60±62.36	84.40±41.81	0.152

Note: *)Kruskal Wallis ; $p < 0.05$

The results of the Kruskal-Wallis test on male and female rats showed that the administration of ethanol extract of mangosteen rind at a dose of 250 mg/kg BW, 500 mg/kg BW, and 1000 mg/kg BW respectively, for 28 days did not show a significant change on the average level of serum (p = 0.240 for male rats, p = 0.152 for female rats). SGPT levels presented in Table 1 are all within the normal range (63-175 UI/L).

In this study, group 1000 mg/kg BW had the lowest average for SGPT. This was different from the research of [16] regarding the subchronic toxicity test of the ethanol extract of bitter melon pulp on liver function and histopathological changes of Wistar strain rats. In this study, it was stated that the higher the dose given, the higher the results of SGPT on examination which could mean toxic to the liver.

3.2 SGOT Serum

Table 2 showed that the highest average SGOT level in male rats was found in the control group (145.8 UI/L) and the lowest average SGOT level was found in the 1000 mg/kg BW group (186.40 UI/L). Meanwhile, in female rats, the highest average SGOT level was in 250 mg/kg BW (279 UI/L) and the lowest average SGOT level was in the 1000 mg/kg BW group (182.20 UI/L).

Table 2 Average levels of SGOT after administration of ethanol extract of mangosteen rind

Group	The average level of SGOT ± SD (mg/dL)				*p*
	Control	250 mg/kgBB	500 mg/kgBB	1000 mg/kgBB	
Male	266.60±57.09	264.40±80.58	194.00±58.21	186.40±48.09	0.061
Female	258.00±40.04	279.00±98.66	182.20±37.12	202.40±65.00	0.101

Note: *) Kruskal-Wallis, **) One Way ANOVA; p < 0.05

The results of the Kruskal-Wallis test for the male group showed that after administering the ethanol extract of mangosteen rind at a dose of 250 mg/kg BW, 500 mg/kg BW, and 1000 mg/kg BW respectively for 28 days did not show a significant change in the average SGOT level of rat blood serum (p= 0.061). Likewise, with the One Way ANOVA test for a group of female rats, it was found that the administration of ethanol extract of mangosteen rind at a dose of 250 mg/kg BW, 500 mg/kg BW and 1000 mg/kg BW for 28 days did not give a significant change on the average SGOT level (p= 0.101).

The SGOT levels (relative to the control group) were found to be following the results of previous studies which stated that the administration of ethanol extract of mangosteen rind in gradual doses did not only cause a hepatotoxic response in rats [14]. It should be noted that high levels of SGOT (> 143 UI/ L) in Table 4.2 might be caused by metabolic factors of the experimental animals used, this is evidenced by the SGOT level in the control group which was more than 143 UI/L in both male and female rats. The presence of SGOT which normally diffuses in vital organs and blood vessels might also be one of the factors that influence the measurement results [12], [15]. This measures SGOT alone in the toxicity test is not sufficient to state the degree of liver damage. SGPT levels which are the main indicators of liver damage also need to be reviewed simultaneously [15]. In this study, the results of SGOT levels were also the lowest, even at a dose of 1000 mg/kg BW, which was different from the results of who said that the higher the dose, the better. is toxic to the liver [16].

4. DISCUSSION

The liver is an organ that has an important role in the metabolic process of the ethanol extract of

mangosteen rind, so giving the extract can enter the enterohepatic cycle and detoxify the liver. Detoxification can occur through two mechanisms, namely Kupffer cell apoptosis and cytochrome P450 biochemical reactions. The initial stage of the biochemical reaction is through large amounts of P450 enzymes on cytochrome P450 which are present in hepatocyte cells. This enzyme converts foreign compounds and other toxins into inactive. Detoxification based on biochemical reactions is divided into phase I (hydroxylation, oxidation, and reactions through CYP450) and phase II (esterification). These metabolites are then secreted into the gall bladder and eliminated via the gastrointestinal tract.

Giving the extract in certain doses can have a good effect on the liver. This matter-based on in vitro research, mangosteen rind extract has a high antioxidant content due to the presence of xanthone compounds. Based on the results of research by in Indonesia, the highest xanthone content was found in the mangosteen rind, which was 107.76 mg per 100 g of the rind and the xanthone content in the mangosteen rind was around 70-75% [17]. Xanthones are needed by the body, one of the benefits is to prevent oxidative stress by Reactive Oxygen Species (ROS) which can damage cell components [15].

Mangosteen rind contains polyhydroxyxanthone, which is a derivative of mangostin, and mangosteen, its functions as antioxidant, antibacterial, antitumor, anticancer, and antiproliferative [17]. In addition to xanthones, mangosteen rind also contains other vitamins and minerals such as Vitamin B1, B2, B5, B6, and Vitamin C [19]. There was also what was done by in Thailand regarding the chronic toxicity of mangosteen rind extract, stating that rats receiving mangosteen rind extract at doses of 500 and 1000 mg/kg BW/day had levels of SGPT (63-175 UI/L) and SGOT (74-143 UI/L) that increased above normal levels. The potential for sub-chronic toxicity of extracts and formulations containing mangosteen rind on levels of SGPT and SGOT is still under-appreciated, so the toxicity data still needs to be reviewed [19].

5. CONCLUSIONS

Administering ethanol extract of mangosteen rind at a dose of 250 mg/kg BW, 500 mg/kg BW, and 1000 mg/kg BW, did not cause subchronic toxicity effects on liver damage when viewed after examining the levels of SGPT and SGOT levels in rats. There was an increase in SGPT levels in male rats after administering the mangosteen rind ethanol extract at a dose of 250 mg/kg BW, as well as in female rats who also experienced an increase in SGOT levels after administering the mangosteen rind ethanol extract at the same dose of 250 mg/kg BW., but not to the point of causing toxic effects.

6. ACKNOWLEDGEMENT

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