Research Article

Subchronic Toxicity of Ethanol Extract of Mangosteen Rind (*Garcinia Mangostana L.*) on Kidney Function in Wistar Rats

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Abstract.

Mangosteen (*Garcinia mangostana L.*) is a fruit that possesses antioxidant, antibacterial, antihistamine, anti-inflammatory, and antidiabetic properties. Before this, an acute toxicity test was conducted on the ethanol extract of mangosteen rind. However, further testing is required, specifically the subchronic toxicity test, to identify any hazardous effects that may not have been found in the previous study. The objective of this study is to evaluate the amount of toxicity based on the mortality rate, alterations in the relative weight of the kidneys, and changes in renal function as shown by levels of urea and creatinine. This study is an experimental investigation that employs the Post-test Only Control Group Design methodology, with a sample of 40 rats. The rats were categorized into two groups: the control group and the treatment group. The treatment group was divided into three subgroups, each receiving a dosage of 250 mg/kg body weight. The doses were 500 milligrams per kilogram and 1000 milligrams per kilogram of body weight, respectively. The control group received simply water and feed. The duration of the treatment was 28 days. Observations were conducted over 28 days, which involved monitoring animal mortality. On the 29th day, a surgical procedure was carried out to assess the comparative weight of the kidneys and collect blood samples for analyzing the levels of urea and creatinine. The findings demonstrated no mortality in the test subjects, although there was an elevation in the relative mass of the kidneys and an increase in urea concentrations. The increase in urea levels was observed in female rats using the Kruskal–Wallis test (P = 0.019). The study was further conducted using the post-hoc Mann–Whitney test. Control female rats exhibited notable disparities when administered dosages of 250 mg/kgBW and 500 mg/kgBW. The repeated administration of an ethanolic extract derived from the peel of the mangosteen fruit had a detrimental impact on the functioning of the kidneys, as evidenced by a rise in urea concentrations.

Keywords: mangosteen, toxicity test, subchronic, kidney

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1. Introduction

The discovery of new drugs were expected to create effective results and reduce side effects [1,2]. The existence of synthetic drugs as a solution has a high price, and the side effects underlie people's mindset to return to traditional medicine because it is easier to treat [3,4]. Disadvantages of traditional medicine include the lack of standardization of raw materials and the lack of research to test their efficacy and safety, so a toxicity test is needed [5].

Indonesia was known as a fertile and prosperous country. There were 40,000 species of medicinal plants identified in the universe, and approximately 30,000 species are estimated to grow in Indonesia. From these data, only 9,000 species were thought to have medicinal properties. About 5% of that amount was used as phytopharmaka, one of which was the mangosteen fruit (Garcinia mangostana L.) [6,7]. Mangosteen has many benefits because it contains α-mangosteen, which was helpful as an antioxidant, antibacterial, antihistamine, anti-inflammatory, and antidiabetic effects [8,9]. The acute toxicity has tested by Hana [10] and the results showed that the ethanol extract of the mangosteen rind was included in the "Practically Non-Toxic" category. Subchronic toxicity test has been carried out by Chayaburakul et al [11]. The subchronic toxicity test of mangosteen extract at doses of 0, 10, 50, 100 mg/kgBW did not find any damage to kidney function [11]. Hence, it is necessary to carry out a subchronic toxicity test to determine the effects that will be caused in the long term [12,13].

One of the organs that are important for drugs and is the main route of elimination of potentially toxic metabolites and foreign substances for the body is the kidney [14,15]. The basic process of elimination occurs in three processes, glomerular filtration, tubular reabsorption, and secretion. This study will examine the effect of subchronic toxicity of mangosteen rind ethanol extract at doses of 250 mg/kgBW, 500 mg/kgBW, and 1000 mg/kgBW on urea and creatinine levels as parameters to assess the presence of toxic effects that appear on the kidneys. National Agency for Drug and Food Control of Indonesia (BPOM) recommended that the experimental animals were white rat rodents (Sprague Dawley or Wistar strains) male and female [12]. The use of male and female rats as experimental animals is due to differences in anatomy, physiology, biochemistry, and behavior, so the bodies of male and female animals will respond to chemicals in different ways [16,17].

2. Material and Methods

2.1. Plant material

Mangosteen was obtained from Tasikmalaya, Indonesia. Mangosteen was identified and characterized at the School of Life Sciences and Technology (SITH), Bandung Institute of Technology-Indonesia. Mangosteen rind criteria for this study were fresh, dry, and purplish-red to dark purple.

2.2. Mangosteen extract preparation

The ethanol extract of mangosteen rind was prepared in the School of Life Sciences and Technology, Bandung Institute of Technology-Indonesia, using the maceration process. First of all, the mangosteen rind is detached and then cleaned and drained. The mangosteen rinds were sliced, then dried in an oven before being processed by a machine to acquire the powder. Mangosteen rind powder was macerated three times using 96% ethanol till submerged for 24 hours. Next, the filtering process is carried out, and the extract is concentrated using a rotary evaporator so that the extract gets concentrated.

2.3. Experimental animals

Subchronic toxicity test investigation employed standard male and female Wistar rats with criteria of 6-8 weeks of age, weighing 150-200 g and acclimatization for seven days before treatment. Acclimatization was carried out at room temperature with 12 hours light and 12 hours of dark cycle. animals were given water and food available at any time (ad libitum), and animals were randomized into control and experimental groups. All experimental protocols were in line with the Guide for the Care and Use of Laboratory Animals and approved by the local animal care committee, The Health Research Ethic Committee of Faculty of Medicine Universitas Padjajaran Bandung-Indonesia, with ethical approval # 686/UN6.KEP/EC/2021.

2.4. Subchronic toxicity test

Determination of dosage following the guidelines of the National Agency for Drug and Food Control of Indonesia (BPOM) and the Organization for Economic Co-operation and Development (OECD). The procedure of animal care begins with acclimation. The experimental animals are separated into 4 groups, each consisting of 10 rats consisting of 5 rats with female sex and 5 rats with the male sex. The control group was just given water and feed, while the treatment group was the group that was given water, feed, and a dose of mangosteen rind ethanol extract in a varied amount of 250 mg/kgBW, 500 mg/kgBW, and 1000 mg/kgBW, respectively. The test preparation was given orally with multiple dosages per day. Observations were taken for 28 days, for the number of rat deaths. On the 29th day, Animals were sacrificed using the cervical luxation procedure, which was previously anesthetized using ketamine, urea and creatinine levels were studied, the relative organ weight of the kidneys was estimated.

2.5. Biochemical analysis

The principle utilized in testing urea is the Enzymatic ultraviolet (UV) test, the Urease–GLDH method. Urea nitrogen was oxidized using urease and Glutamate dehydrogenase (GLDH) enzymes, and absorbance changes were recorded using a spectrophotometer at a wavelength of 340 nm at 37°C. The test process involves 10 μL of test serum to be reacted with 1000 μL of test reagent for urea nitrogen testing in a 5 mL test tube, homogenized with the help of a vortex. Absorbance was measured with a spectrophotometer at 37°C at precisely 30 seconds at a wavelength of 340 nm, and then absorbance was measured again at precisely 60 seconds.Creatinine examination uses the kinetic test without deproteinization Jaffe technique. Creatinine interacted with alkaline picrate to generate a red hue and was measured using a spectrophotometer at a wavelength of 492 nm, temperature 37°C. The examination process requires 50 μL of test serum to be reacted with 1000 μL of test reagent for creatinine examination in a 5 mL test tube, homogenized with the help of a vortex. Absorbance was measured by spectrophotometer at 37°C after 60 seconds at a wavelength of 492 nm; absorbance was measured again after 120 seconds.

2.6. Relative organ weight

The relative organ weight (ROW) of each animal was calculated as follows:

$$ROW = \frac{Organ\ weight\ (g)\ x\ 100}{body\ weight\ of\ the\ animal\ on\ sacrifice\ day\ (g)}$$

3. Results and Discussion

3.1. Effect of ethanol extract of mangosteen rind on death of experimental animals

The data showed no fatalities of test animals during the observation duration. No deaths suggested that the ethanol extract of mangosteen rind with doses of 250 mg/kgBW, 500 mg/kgBW, and 1000 mg/kgBW was not hazardous. Research conducted by Hana verified that the acute toxicity test with a maximum dose of 5000 mg/kg BW had an LD50 value in the non-toxic category [10,12].

Effect of Ethanol Extract of Mangosteen Rind on Relative Organ WeightRelative organ weight is a means to detect if an exposed organ is injured or not. In toxicity testing, relative organ weight assessment shows good sensitivity in predicting toxicity and correlates with histological abnormalities [18,19].

TABLE 1: Relative organ weight test in male and female rats.

Groups	Average relative organ weight ± SD (mg)				p
	Control	250mg/kgBW	500mg/kgBW	1000mg/kgBW	
Male	0.442±0.099	0.447±0.045	0.542±0.038	0.533±0.050	0.072*
Female	0.505±0.050	0.593±0.047	0.546±0.064	0.542±0.065	0.114*

*tested using one way Anova

There were no significant difference (p>0.05) on relative organ weights in experimental animals which fed ethanol extract of mangosteen rind at doses of 250 mg/kg BW, 500 mg/kg BW, and 1000 mg/kg BW. Kidneys are the principal route of the metabolism of potentially hazardous metabolites and foreign substances. Many medicines and phytochemicals can have a harmful effect on the kidneys by damaging the activity of mitochondria, blocking tubular transit, raising oxidative stress, or the generation of free radicals. Renal reaction to hazardous chemicals can be observed in glomerular shrinkage, cell necrosis, and loss of brush border [14]. The results of this investigation

demonstrated no significant difference between the relative organ weight of the kidneys in the control group and the group that was given a dose of mangosteen rind extract. It revealed that the mangosteen rind ethanol extract was not harmful because one of the parameters for a toxic effect was a weight change of the Relative organs. Previous research conducted by Chayaburakul et al [11] indicated that the subchronic toxicity test of mangosteen extract at dosages of 0, 10, 50, 100 mg/kg BW did not create a significant variation in the relative organ weight of the kidney.

3.2. Ureum levels

Table 2: Average levels of urea after administration of ethanol extract of mangosteen rind.

Groups	Ureum average ± SD (mg/dL)				p
	Control	250mg/kgBW	500mg/kgBW	1000mg/kgBW	
Male	39.84±4.93	38.48±6.51	40.16±4.43	44.98±4.81	0.260*
Female	31.38±1.05	37.12±2.98	33.52±2.84	36.54±3.56	0.019**

*Analyzed with one-way Anova
**Analyzed with Kruskall Wallis

The findings of the One Way Anova test analysis indicate that there is no statistically significant difference ($p > 0.05$) in the impact of administering ethanol extract of mangosteen rind at doses of 250 mg/kgBW, 500 mg/kgBW, and 1000 mg/kgBW on urea and creatinine levels in male rats. The Kruskal-Wallis test revealed a significant difference in the effect of ethanol extract of mangosteen rind at doses of 250 mg/kg BW, 500 mg/kg BW, and 1000 mg/kg BW on the urea levels of the female group ($p \leq 0.05$). Consequently, the analysis proceeded with the Mann-Whitney test.

3.3. Creatinine levels

The One Way ANOVA test conducted on the creatinine levels of male rats and the Kruskal Wallis test performed on females revealed no statistically significant difference ($p > 0.05$). The information is presented in Table 1.

The urea levels of female rats exhibited statistically significant results, warranting the use of a Post Hoc Mann-Whitney test to compare the control group with the three different doses. The study results presented in Table 1 indicate a notable disparity between the control group and the groups administered doses of 250 mg/kgBW and 1000mg/kgBW, respectively. The substantial elevation in urea levels seen at a dosage of

Table 3: The average creatinine levels of male and female rats after administration of ethanol extract of mangosteen rind.

Groups	Creatinine average ± SD (mg/dL)				p
	Control	250mg/kgBW	500mg/kgBW	1000mg/kgBW	
Male	0.426±0.076	0.426±0.038	0.360±0.047	0.440±0.032	0.066*
Female	0.378±0.046	0.364±0.040	0.364±0.038	0.422±0.023	0.086**

*Analyzed with one way Anova
**Analyzed with Kruskall Wallis

1000 mg/kg BW may not be attributable to impaired renal function. As per the Laboratory of Physiology at the Faculty of Biology, Gadjah Mada University in Indonesia, the typical urea levels in female rats were found to be within the normal range of 23.19-44.61 [20].

The kidney is a vital organ responsible for the elimination of potentially harmful metabolites and foreign chemicals from the body, making it crucial for medication processing [14,15]. Factors that can elevate urea levels include eating of high protein diets, gastrointestinal bleeding, corticosteroid use, dehydration, hypovolemic conditions, or kidney injury [21]. Urea and creatinine levels serve as indicators of renal function. However, their sensitivity decreases if the kidney problem is still anticipated. Measuring renal clearance can be significantly influenced by a rise in urea and creatinine levels, particularly when the glomerular filtration rate reduces by around 50-70% [22]. The elevation in urea levels may be attributed to the rat meal, which had a protein composition ranging from 18.5% to 20.5%.

Protein undergoes a process of conversion into peptides and amino acids. The acids present in the colon are absorbed and transported to the liver, with over 90% of them being successfully absorbed. The hepatocytes undergo deamination and transaminases of these amino acids. The surplus nitrogen will be incorporated into the urea cycle. Protein that is not absorbed by the small intestine, along with urea, will undergo conversion into ammonia by intestinal microbes, particularly in the large intestine. Ammonia will permeate through the portal circulation and reach the liver in order to participate in the urea cycle [18,21]. Mangosteen is also rich in xanthone chemicals, which serve as antioxidants. Antioxidants can mitigate oxidative stress caused by elevated reactive oxygen species (ROS) generation, so safeguarding the kidneys from harm marked by heightened levels of urea and creatinine [23,24].

In a study conducted by Chayaburakul et al [11], it was found that administering doses of 0, 10, 50, and 100 mg/kg BW of mangosteen extract did not result in elevated levels of urea and creatinine, indicating no subchronic toxicity. This study employed doses of

250 mg/kg BW, 500 mg/kg BW, and 1000 mg/kg BW to observe the effects of escalating doses on female rats. The results showed significant increases in urea levels [11].

4. Conclusion

The administration of ethanol extract derived from the peel of the mangosteen fruit, at dosages of 250 mg/kg BW, 500 mg/kg BW, and 1000 mg/kg BW, did not result in mortality or alterations in the relative weight of the kidneys. However, it did have a toxic impact on the kidneys, as seen by an elevation in urea levels in female rats. Additional investigation is required through the implementation of renal histological examination and the continuation of chronic toxicity studies.

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