INTRODUCTION

Guava (Psidium guajava Linn.), a part of Myrtaceae family, is a widespread tropical and subtropical plant with a long history of traditional usage. Several fragments of guava (P. guajava Linn.) have a lot of medicinal properties that were not only consumed as food but also traditional medicine to remedy various ailments [1].

There has been a long history in the utilization of traditional medicine in half of the world, and it circumscribes a simple, reachable, and affordable source of treatment [2]. Some researchers have revealed that the depletion of fruits, vegetables, and seed can be advantageous to prevent risk factors of several ailments related to the circumstance of chronic disorders, and for many other purposes due to their bioactive compounds [3,4]. Therapies have shown auspicious potential agents with several benefits in herbal products. However, many herbal medicine utilizations which remain untested are poorly monitored or worse are not monitored at all. The limited information of their action mechanism, potential harmful reactions, contraindications, and interactions with existing common pharmaceuticals encourage many researchers to promote the safety and rational utilization of these agents [5].

In pharmacology, it is very important to perform toxicity test for new drug candidates. The test was conducted to evaluate the safety or hazards of several substances including industrial chemicals, pharmaceutical, and consumer care products. The Organization for Economic and Development (OECD) introduced acute toxicity as the advance effect occurring in a short time of oral administration after a simple or multiple doses is given [6-8].

Up-and-Down Procedure (UDP) of OECD 425 is one of the methods for LD₅₀ acute toxicity test. UDP permits to reduce the number of animals substantially, which is required for determining LD₅₀ values as well as ED₅₀ values of a variety of other listings. Animals in the UDP methods are observed individually at least once during the first 30 min after dosing, and periodically during the first 24 h–14 days. All observations including toxic signs, body weight, and pathology are systematically recorded, with individual records being maintained for each animal [7,9]. The aim of our recent study is to know the phytochemical screening and histology appearance of acute oral toxicity, and sign and symptom of toxicity after P. guajava Linn. extract treatment to the mice.

MATERIALS AND METHODS

Preparation of plant extract

Fruit samples were collected from guava trees grown at Dukuh Waluh Village, Purwokerto, Central Java, Indonesia. Random ripe fruit samples were collected into plastic bags with appropriate labeling and were stored in an ice cooler box to be transported to the laboratory for extraction. The fruit samples were constituted by Central Laboratory of Universitas Padjadjaran.

Extraction methods for guava fruits

The fruit samples were washed in tap water and were placed into a blender to be grounded. 96% ethanol solvent was used for maceration extraction procedure. Then, the filtering was conducted using a funnel buncher. The filtral produced from the filtration was concentrated using a rotary evaporator at 40°C to obtain the result of concentrated extract and was suspended using distilled water as needed. The extract was afterward collected and stored at 4°C until use.

Phytochemical screening

Chemical test for the screening of bioactive chemical constituents in the guava was carried out with extracts using a guide of phytochemical methods as described by Harborne [10]. The extract was chemically

ABSTRACT

Objective: The main objectives of the research are to investigate the phytochemical screening, histology appearance, and safety of acute oral toxicity study on the extract of the fruit of Psidium guajava Linn. in mice.

Methods: Mice that were administered by oral feeding with different and controlled dose were divided into three groups, with dose limits of both 2000 and 5000 mg/kg b.w. We analyzed the P. guajava Linn. extract with specific methods before treating the subject. The methods were followed with acute oral toxicity study of Up-and-Down Procedure Organization for Economic and Development 425. The mice were then observed for signs and symptoms of toxicity. In addition, toxicity in the liver and kidney was analyzed through histology observation.

Results: Phytochemical screening revealed the presence of flavonoids, quinone, triterpenoid/steroid, tannins and saponins, and the absence of alkaloids. We found that the treatment with 2000 and 5000 mg/kg b.w. of the extract did not show any differences in body weight changes, number of hepatocyte in liver, and podocyte in kidney compared with control (*p>0.05). Moreover, we noticed all mice lived and were healthy during observation.

Conclusion: Our finding indicates that the extract of the fruit of P. guajava Linn. is safe and it was not toxic to the liver and kidney.

Keywords: Phytochemical screening, Histology, Acute toxicity, Psidium guajava Linn.
tested for the presence of flavonoids, quinone, triterpenoid/steroid, alkaloids, tannins, and saponins.

**Experimental animals**
A total of 12 healthy female albino mice of the Swiss Webster that weighed 20–30 g that were 8–12 weeks old and that were nulliparous and non-pregnant were selected as the subject. The mice were procured from the Laboratory of Pharmacology and Therapy, Universitas Padjadjaran. The mice were housed in cages in a temperature-controlled room (22±3°C) and were provided with conventional rodent laboratory fed an unlimited supply of drinking water ad libitum. The procedures taken passed the Ethical Clearance from Health Research Ethics Committee (No. 1104/UN6.C.10/PN/2017), Universitas Padjadjaran.

**Acute oral toxicity test**
The mice were divided into three sets that were administered by oral feeding to different sets, at dose limits of both 2000 and 5000 mg/kg b.w. and control. All mice were observed for toxic signs, body weight, and mortality for 14 days for qualitative data.

Group 1 served as a control and received distilled water. Group 2 received a dose limit of 2000 mg/kg b.w. of fruit extract (0.2 ml/kg b.w., p.o). Each mouse was given one dose test. If it survives, another four mice were given a dose sequentially. If three or more animal survives, the test was proceeded to a dose limit of 5000 mg/kg b.w. Group 3 received a dose limit of 5000 mg/kg b.w. of fruit (0.2 ml/kg b.w., p.o). If the mouse survives, another two mice were given a dose sequentially. If both mice survive, the LD 

**Histology analysis**
The mice were sacrificed, and afterward, liver and kidney collection was conducted. The organs were fixed with 3% (w/v) paraformaldehyde and were processed as previously described. We performed hematoxylin-eosin (HE) staining according to the previous methods [11-13].

**Statistical analysis**
Quantitative data were expressed as mean±SD. The data were determined and analyzed using one-way ANOVA. The statistical significance was accepted if p<0.05.

**RESULTS**

**Phytochemical analysis**
Phytochemical screening is an early stage to give a scheme of compound classification in plant samples. Table 1 shows the summarized phytochemical screening of chemical constituents of *P. guajava* Linn.

| Constituents          | Qualitative tests | Result |
|-----------------------|-------------------|--------|
| Flavonoids            | HCl 2M and Amyl alcohol | +      |
| Quinone               | NaOH 30%          | +      |
| Triterpenoid/steroid  | Acetic acid anhydride and H₂SO₄ | +      |
| Alkaloid              | Dragendorff       | -      |
| Tannins               | ReCl₃             | +      |
| Saponins              | H₂O               | +      |

(+*) present and (-) absent

Table 2: Clinical observation

| Group | Toxic Signs | Group | Toxic Signs | Group | Toxic Signs |
|-------|-------------|-------|-------------|-------|-------------|
| Control | Cyanosis | - | Dose 1 | Cyanosis | - |
|        | Tremor     | - |          | Tremor | - |
|        | Salivation | - |          | Salivation | - |
|        | Piloerection | - |          | Piloerection | - |
|        | Feces | - |          | Feces | - |
|        | Vomiting | - |          | Vomiting | - |
|        | Death | - |          | Death | - |

(-) normal. Control=distilled water; dose 1=2000 mg/kg b.w.; dose 2=5000 mg/kg b.w.
Fig. 1: Body weight observation

Fig. 2: Histology of the liver and kidney

Table 3: Statistical analysis of body weight

| Treatment group | Mean±SD      | p value |
|-----------------|--------------|---------|
| Control         | 27.60±2.69   |         |
| Dose 1          | 29.60±1.18   | 0.074   |
| Dose 2          | 28.20±2.90   |         |

Control=distilled water; dose 1=2000 mg/kg b.w.; dose 2=5000 mg/kg b.w.

Table 4: Anova test of hepatocytes number

| Treatment group | Mean±SD      | p value |
|-----------------|--------------|---------|
| Control         | 111.00±9.41  |         |
| Dose 1          | 123.40±24.92 | 0.630   |
| Dose 2          | 20.67±16.92  |         |

Control=distilled water; dose 1=2000 mg/kg b.w.; dose 2=5000 mg/kg b.w.
Table 5: Post hoc test of hepatocytes number

| Treatment group | p value |
|-----------------|---------|
| Control versus dose 1 | 0.361 |
| Control versus dose 2 | 0.527 |
| Dose 1 versus dose 2 | 0.85 |

Control=distilled water; dose 1=2000 mg/kg b.w.; dose 2=5000 mg/kg b.w.

Table 6: ANOVA test of podocytes number

| Treatment group | (Mean±SD) | p value |
|-----------------|-----------|---------|
| Control         | 39.75±19.20 | 0.793 |
| Dose 1          | 46.20±5.16   | 0.527 |
| Dose 2          | 37.33±3.05   | 0.527 |

Control=distilled water; dose 1=2000 mg/kg b.w.; dose 2=5000 mg/kg b.w.

Table 7: Post hoc test of podocytes number

| Treatment group | p value |
|-----------------|---------|
| Control dose 1  | 0.432 |
| Control dose 2  | 0.793 |
| Dose 1 dose 2   | 0.327 |

Control=distilled water; dose 1=2000 mg/kg b.w.; dose 2=5000 mg/kg b.w.

CONCLUSION
Our finding indicates that the *Psidium guajava* Linn. fruit extract contains some bioactive compounds that have a medicinal effect. Toxicity test with a dose limit of 2000 and 5000 mg/kg b.w. of the extract administration showed that it is nontoxic.

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AUTHORS' CONTRIBUTIONS
NA, IM, and DHD designed the experimental study and carried out the analysis. NA, IM, ARR, and AC contributed in preparing the manuscript and revision. All authors have read and approved the final manuscript.

CONFLICTS OF INTEREST
The authors have none declare.

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