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Research Article

PHYTOCHEMICAL SCREENING AND HISTOLOGY APPEARANCE OF ACUTE ORAL TOXICITY STUDY ON ETHANOL EXTRACT OF *PSIDIUM GUAJAVA* LINN. FRUIT IN MICE

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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.

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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 LD_{50} acute toxicity test. UDP permits to reduce the number of animals substantially, which is required for determining LD_{50} values as well as ED_{50} 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 substantiated 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

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_{50} was given more than the limit dose [7].

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. fruit extract understudy on a qualitative basis. Phytochemical analysis revealed the presence of flavonoids, quinone, triterpenoid/steroid, tannins, and saponins in the *P. guajava* Linn. and the absence of alkaloids.

Table 1: Phytochemistry of *P. guajava* Linn. Extract

Constituents	Qualitative tests	Result
Flavonoids	HCl 2M and Amyl alcohol	+
Quinone	NaOH 30%	+
Triterpenoid/steroid	Acetic acid anhydride and H_2S0_4	+
Alkaloid	Dragendorff	-
Tannins	$FeCl_3$	+
Saponins	H_20	+

(+) present and (-) absent

Clinical observations
Assessment of animal behavior was performed by general observation to the animals on a daily basis from the first dosing treatment until the end of the study. The recorded changes or abnormalities could be indications of toxicity. We found that there were no significant changes in behavior on all mice from different treatments. In addition, all mice survived during observation, starting from treatment until the end of observation (Table 2).

Body weight changes
Body weight is an important parameter to monitor the animal's health status. Weight loss is frequently used as the first indicator of the adverse effect of the drugs. The dosage is considered toxic if the drug causes 10% or more body weight reduction. This condition could be taken as a sign of toxicity even though there are no other changes occurred [9]. We found that all of the mice from the treatment groups did not show any significant decrease in body weight from 0 to 14 days (Fig. 1). Furthermore, there was no significant difference between control and treatment group ($p=0.074$) (Table 3).

Body weights of mice from each group observed before treatment until 14 days.

Histology analysis
We proceeded our observation in the liver and kidney to know the toxicity effect after the treatment. All the tissue sections collected from the liver and kidney were observed under the microscope to know the number of hepatocytes and podocytes. We found that the number of normal hepatocytes was not significantly different ($p=0.630$) between control and treatment group (Tables 4 and 5). Similar results were also found when we analyzed the number of podocytes from glomerulus area in the kidney ($p=0.553$) (Tables 6 and 7). In addition, there was no difference between hepatocytes and podocytes morphology in the control and treatment group (Fig. 2).

Morphological analysis of liver (A, B, and C) and kidney (D, E, and F) by HE-stained paraffin sections from control and treatment mice (Bar 50 μm). The hepatocytes and podocytes were counted and compared from each group (G and H, respectively). CV: Central vein. H: Hepatocyte. G: Glomerulus; P: Podocyte; NS: Not Significant. Bar 50 μm.

DISCUSSION

This study showed phytochemical screening of the presence of flavonoids, quinone, triterpenoid/steroid, tannins, and saponin in *P. guajava* Linn. extract. All these bioactive compounds have some beneficial effects in remedying including antidiarrhea, antimicrobial, acne lesion, and thrombocytopenia, to name a few [1,14-16].

Toxicity test procedures measured several parameters such as body weight, clinical signs, and symptoms. The observation was necessary for determining the LD_{50}. Our study showed that there were no toxic

Table 2: Clinical observation

Group	Toxic Signs		Group	Toxic Signs		Group	Toxic Signs	
Control	Cyanosis	-	Dose 1	Cyanosis	-	Dose 2	Cyanosis	-
	Tremor	-		Tremor	-		Tremor	-
	Salivation	-		Salivation	-		Salivation	-
	Piloerection	-		Piloerection	-		Piloerection	-
	Feces	-		Feces	-		Feces	-
	Vomiting	-		Vomiting	-		Vomiting	-
	Death	-		Death	-		Death	-

(-) normal. Control=distillated water; dose 1=2000 mg/kg b.w.; dose 2=5000 mg/kg b.w.

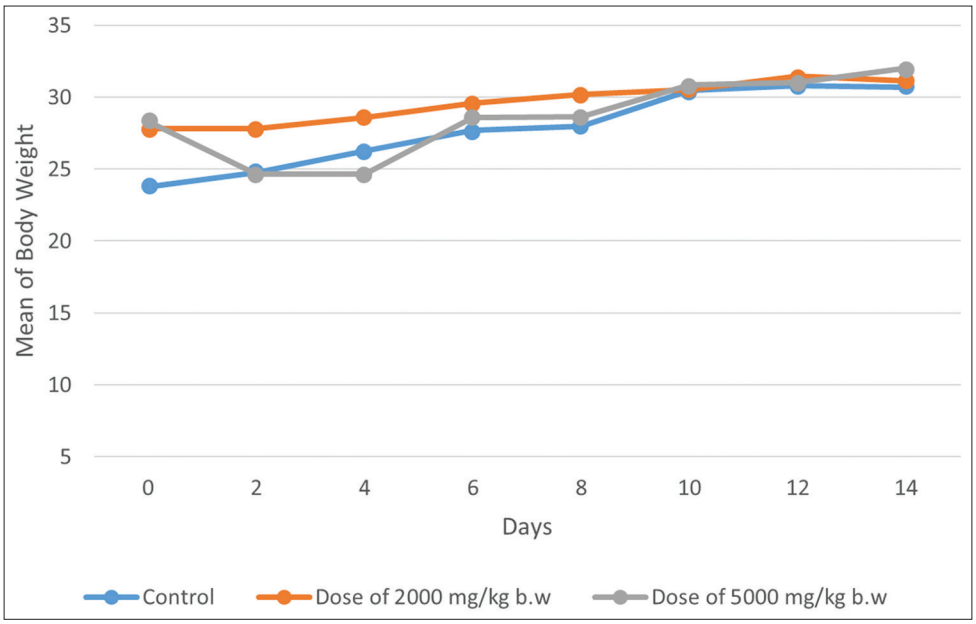

Fig. 1: Body weight observation

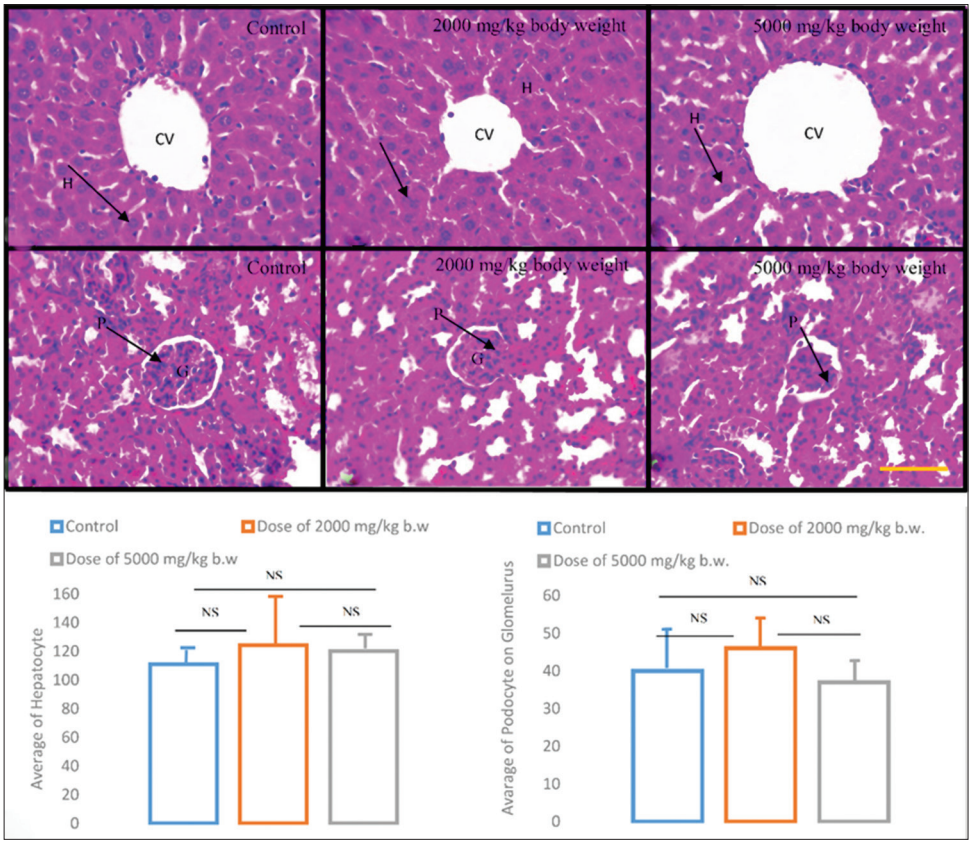

Fig. 2: Histology of the liver and kidney

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=distillated 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=distillated 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=distillated 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	
Dose 1	46.20±5.16	0.553
Dose 2	37.33±3.05	

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

signs and changes found on body weight in mice from treatment group within 4 h, 48 h, 7 days, and 14 days after treatment of guava fruit (*P. guajava* Linn.) extract, and there was no significant difference compared to control group. Previous studies also showed no differences in body weight, behaviors, and symptoms between control group that received distilled water compared to treatment groups that received guava leaves extract at the dose of 200, 1000, 2000, 2500, 5000, and 20,000 mg/kg/day for 14 days to 6 months [17,18].

Histological examination of the liver in the control group and the treatment group showed that there was no difference in the number of the hepatocyte. It may be because *P. guajava* plant has a hepatoprotective activity due to the bioactive compound in *P. guajava* Linn. [19]. *P. guajava* Linn. contains total polyphenols and dietary fiber such as flavonoids that may exert an antioxidant effect as radical-scavenging activity [20,21]. *P. guajava* Linn. also contains saponin that serves to reduce blood cholesterol levels. These substances could protect the stability of liver metabolic process [22]. Moreover, our previous data showed that there was no disturbance in the liver function on a biochemical test of liver function including alanine aminotransferase, aspartate aminotransferase, and total bilirubin at doses of 2000 and 5000 mg/kg body weight of the *P. guajava* Linn. extract [23,24].

Histological examination of the kidney in the control and treatment group showed no differences in the number of podocytes. The previous study mentions that the active content of guava fruit extract had kidney protective properties on ethanol extract of *P. guajava* Linn. in mice-induced doxorubicin [25,26]. During a development of a new drug, there could be a change in renal function which raises the question of nephrotoxicity. Sometimes it showed a positive effect on the kidneys; however, the incidence with negative effects is unavoidable [27,28].

CONCLUSION

Our finding indicates that the *P. 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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