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Original Research Article

Oral and Acute Dermal Toxicity with *Passiflora edulis* Sims Aqueous Extract in Sprague-Dawley Rats

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ABSTRACT

Passiflora edulis **(**Passion fruit) seeds is rich in phenolic compounds (bioflavonoids) that can inhibit lipid peroxidation and have a strong antioxidant effect. The present study investigated the acute oral and acute dermal toxicities of the ethanol extract of *Passiflora edulis* Sims fruit seeds. Acute oral toxicity of *P. edulis* S. extract was investigated in rats according to the Organisation for Economic Co-operation and Development (OECD) Guideline. The median lethal dose (LD_{50}) was determined to be >15,000 mg/kg body weight. There was a significant increase in body weight ($p < 0.05$), and no death was recorded during the 14 days of the study. No overt changes were observed in the treated animals compared to controls. Overall, the results showed that oral administration of ethanol extract of *P. edulis* Sims. did not produce significant toxic effects in rats, indicating that the extract is relatively safe.

Keywords: Acute toxicity, Antioxidant, Fruit seeds, *Passiflora edulis*, Skin toxicity.

Introduction

Plants that contain phenolic compounds inhibit lipid peroxidation and produce a strong antioxidant effect by breaking the radical chain reaction of peroxy radical through a scavenging effect.[1] Consuming a bioflavonoid been published of mixture in food or as a supplement provides an antioxidant and protective effect against cardiovascular diseases.[2-4] Passion fruits (*Passiflora edulis* Sims/*P.edulis* Sims) is tropical fruit that can be found in tropical countries and has antioxidant actions. Studies have shown that passion fruit contains bioflavonoids, including reports of other functions of passion fruit seeds, especially their effects on oxidative stress and lipid profiles.[4-6] In addition to acting as an antioxidant supplement, passion fruit seed extract is used as a skin softener[7]. *Passiflora edulis* Sims extract readily penetrates the skin to deeply moisturize and reduce dry skin[7]. *P. edulis* Sims (Passifloraceae) is a creeping plant that is commonly found in tropical countries.[8] This plant is found in several colours including yellow, red, and purple, and the flesh of the fruit is the part most often consumed.[8, 9] Passionfruit seeds are reported to contain many polyphenol compounds that can provide an antioxidant effect superior to that of the fruit flesh or skin[8, 9]. Research on passionfruit flesh and skin has been widely published,[7] but research on passionfruit seeds is lacking. In middle- and low-income countries, information about passion fruit seeds poisoning is scarce.[10] Plants or medicines must be confirmed to be safe before they can be used as medicine.[11] As such, the goal of this study was to evaluate the acute oral toxicity of the ethanol extract of *P. edulis* fruit seed and the acute dermal toxicity of the ethanol extract.

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Materials and Methods

Experimental animals

Healthy adult female Sprague-Dawley rats that were nulliparous, weighing 150–160 g, and aged 3–4 months were used. Female rats were chosen because of their sensitivity to both oral and dermal treatment administration. They were purchased from the Pharmacology Laboratory (Faculty of Medicine, Padjadjaran University, Bandung, Indonesia).

The animals were housed in stainless steel cages under standard conditions at 25–27°C, with a 12-hour light/dark cycle, and at 40–60% relative humidity. The rats were acclimatized for 7 days in the laboratory. The animals were fed with standard animal pellets with water *ad libitum*. The rats were allotted into two groups of five rats each. They were fasted for 17–20 h with free access to water.

All procedures were performed in accordance with the European Community Guidelines (EEC Directive 1986; 86/609 / EEC) and were approved by the Health Ethics Committee of the Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia (No. KET-931/UN2.F1/ETIK/PPM.00.02/2019).

Plant Material and Extraction

Fresh *P. edulis* S. seeds (1 kg) were collected from the *P. edulis*. plantation in North Sumatra, Indonesia, in April 2019. The seeds were collected and cleaned of dirt, then washed under running water and dried. The seeds were initially air-dried in open air, protected from direct sunlight, followed by a drying process in an oven at 50°C. The dried seeds were powdered using a blender and sieved with 20-mesh filters. The powder was stored in a clean and sealed container. The extract was prepared by diluting about 8.0 g powder in 100 mL water, which was indirectly orally administered to rats.

Acute oral toxicity test

The animals were randomly divided into two groups of five rats each. They were marked to facilitate observation. The extract was administered in a single dose by oral gavage. The dose used was 15,000 mg/kg body weight (BW) to determine the degree of toxicity. We decided to use the maximum dose to determine the LD_{50}. We calculated the dosage as 15,000 mg extract per 1 kg BW of rats weighing 200 g so that the resulting extract weight was ~3000 mg for

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each rat. This test was conducted for 14 days. Every day, the rats received ~214.29 mg extract, administered in two doses per day. So, ~107.14 mg of extract was dissolved in 10 mL of water and administered to each rat for each dose. After administration of the extract, food was withheld for 2 hours. In this study, fresh extract was made each time it was administered to the rats.

The rats from both trials were clinically examined. The rats were monitored for 24 hours, with special attention paid to the first 6 hours and once a day for a further 14 days. Rats were weighed and observed until death. Changes in the weight of each animal were calculated and compared with control animals, as stated in the OECD 402 guidelines.[612] The time of death was recorded. The patterns of behavior, such as salivation, tremors, convulsions, diarrhoea, lethargy, sleep, and coma, were recorded. Changes in physical appearance, injuries, pain, and signs of the disease were recorded once per day during the study period, along with any changes to the skin, eyes, and mucous membranes, as well as the rate of breathing, blood circulation, and autonomic and central nervous systems.

Animals were observed individually at least once at 1, 2, and 4 hours after administration. Observation continued every day thereafter for 14 days. Individual animal body weights were recorded before administration of the drug on the first day of the study, and afterward on the 14th day of the experiment. Changes in the weight of each animal were calculated and compared with control animals. On the last day of observation, all rats were euthanized with ether inhalation followed by cervical dislocation and examined macroscopically. Anomalies in internal organs such as the heart, liver, kidney, lungs, and stomach were documented and examined microscopically. After the examination, fat tissues were removed.

Acute dermal toxicity test

The acute oral toxicity test was followed by an acute dermal toxicity test following the same animal allocation procedure as in the acute toxicity test. The acute dermal toxicity test is important for determining the skin's reaction to the extract. The back of the rat was chosen as a patch area because the area is broad, has minimal movement, and contact of the extract with the skin can be prolonged.

A new group of healthy young adult female Sprague-Dawley rats that were nulliparous, 170–184 g, and aged 3–4 months were used. All animals were placed into stainless steel cages, in rooms maintained at standard conditions of temperature (25–27°C), 12-hour light/dark cycle, and at 40–60% relative humidity. The rats were acclimatized for 5 days in the laboratory. The animals were fed with standard laboratory animal feed with water *ad libitum*. After the acclimation period, the rats were categorized into two groups (each containing 5 rats). They were starved for 17–20 hours with free access to water. All procedures were performed in accordance with the European Community Guidelines (EEC directives 1986; 86/609 / EEC) and were approved by the Animal Ethics Committee of the Faculty of Medicine, Padjadjaran University, Bandung, Indonesia, No. 62/KEP/UNPAD/2019.

The back of each animal was shaved (6 × 8 cm) using a razor 24 hours before extract application. The rats were randomly selected and assigned to the treatment or control groups. Twenty-four hours before the test, fur was removed from the dorsal area of the test animal's trunk by clipping. About 10% of the body surface area was cleaned for the application of *P. edulis* S. extract. The extract was applied to the dorsum area, then covered with a non-irritating porous gauze bandage and plaster for 24 hours. The positive control group received a dose of 15,000 mg/kg of 10% white soft paraffin as a vehicle. This dose was applied locally only once on the first day of the study. At the end of the exposure period, the residual test material was removed. The positive control group received the same dose. This dose was applied locally only once on the first day of the study.

Rats in both groups were examined once on the first day of the study. Rats were further monitored for 24 hours, with focus on the first 6 hours, and were then monitored once a day for 14 days. On the last day of observation which showed intact or eroded skin, all rats were sacrificed and examined macroscopically, with a focus on the skin reaction. Anomalies in the skin were documented and examined microscopically.

Phytochemical analysis

Phytochemical screening of *P. edulis* S. extract was conducted using standard method using sphectrophotometry and colour visualization.[6]

Antioxidant activity screening

Passionfruit extract was made into several concentrations (6, 8, 10, 12 and 14 ppm) which were tested using α, α-diphenyl-*β*-picrylhydrazyl (DPPH) radicals. The purpose of using these concentrations was to find the IC_{50} values using mathematical equations obtained from the correlation between radical inhibition and the extract concentration. Inhibition is determined as the presence of purple discoloration and can be calculated from measurement of its absorbance at 516 nm. At each extract concentration (10 and 15 mg/mL), 2 mL free radicals (DPPH, 0.1 mM) were added and allowed to react with the extract for 30 minutes.

Statistical analysis

Statistical analysis of lethal dose values (LD_{50}) was performed using Thompson–Weil with a 95% confidence interval. Comparisons of before and after treatment were performed using the paired t-test in one group. To test between-group differences, an independent t-test was used. Values less than 0.05 were considered significant. All data are expressed as means ± standard deviations.

Results and Discussion

The phytochemical analysis results showed the presence of flavonoids in the passionfruit seed extract. In addition, tannin, saponin, and triterpenoid substances were also present, as shown in Table 1.

The effectiveness of a sample to counteract free radicals from the DPPH method is determined using the IC_{50} value. The IC_{50} is defined as the concentration that can reduce 50% of the DPPH free radicals. The smaller the IC_{50} value, the stronger the antioxidant activity. Results show that the IC_{50} values for the DPPH radica scavenging activity of the ethanol extract of passion fruit seed is <31.25 ppm.

No deaths or signs of poisoning were observed in the rats for either the acute oral or acute dermal toxicity tests.

One hour after administration of 15,000 mg/kg BW of *P. edulis* S. extract in the acute oral toxicity test, the rats were less active for 30 minutes. Weight loss was observed on day 2, but body weight increased again in the following days. At the end of the first week, an increase in the body weight was recorded, but with no significant differences between the groups (Table 2).

After that, weight gain was observed until the end of the observation period. No mortality was observed for 14 days after treatment with *P. edulis* S. water extract. The differences in weight gain between the two groups before and after the administration of *P. edulis* S. water extract are shown in Table 3.

Table 1: Phytochemical constituents of *Passiflora edulis* ethanol seed extract

Phytochemical	Inference
Flavonoids	+
Alkaloids	-
Tannins	+
Saponins	+
Quinones	-
Steroids	-
Triterpenoids	+

+ = present; - = absent

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There were no abnormal findings in the gross pathological examination of all internal organs in the groups. Based on these results, the recommended oral LD_{50} of *P. edulis* S. extract is 15,000 mg/kg BW for female rats. Therefore, the extract can be labeled as non-toxic in the hazard category according to the globally harmonized system.[12] The autopsy showed no macroscopic anomalies in the internal organs.

In the acute dermal toxicity test, no sign of irritation on the skin, erythema, eschar, edema, or other reactions on the skin area in either intact or eroded rats were observed.

Products from traditional medicinal plants have become popular in primary healthcare, especially in developing countries, and some products may be mistakenly considered safe merely because they originate from a natural source.[11] Historically, herbal medicines were exclusively used for the treatment of various diseases, and are still used today in rural communities.[10] However, in some circumstances, the use of herbal medicines can produce some adverse reactions.[3, 11] With the increase in the use of medicinal plants, scientific studies have become imperative for validating their folkloric use.[11] *P. edulis* S. has not been used in many countries as herbal medicine, especially in developing countries, except for the juice of passion fruit.

Passiflora edulis Sims seeds have antioxidants that can remove oxidants in the blood.[13, 14] Free radicals, or reactive oxygen species (ROS), have been linked to the pathogenesis of several degenerative diseases and cancers.[15] Antioxidants can slow or stop the uncontrolled generation of ROS, thereby helping to reduce stress-induced oxidative diseases.[15] Our findings showed that apart from weight gain, there were no significant changes in the parameters used for the evaluation of toxicity. All animals survived until the end of the observation period. Given the lack of significant weight difference between the test group and the control group before and after the experiment, we concluded that administration of the extract also did not affect animal growth.

A drug is desirable if it has a therapeutic effect at low doses and the least amount of undesirable secondary and toxic effects in individuals.[4, 14] The results of the study, following the OECD guidelines, showed that the *P. edulis* S. extracts are non-toxic at a dose of 15,000 mg/kg BW. *Passiflora edulis* Sims constituents are known to have beneficial effects on the skin, suggesting a possible use in the cosmetics industry.[7, 14, 16] Topical drugs, the mainstay of treatment in dermatology, are applied with the hope that percutaneous absorption will be minimal and systemic side effects will not occur. The superficial layer of the epidermis, the stratum corneum, provides almost all of the skin's barrier properties.[7, 14, 16] Most of the absorption of the drugs is transcellular; it is unlikely that absorption occurs between cells or through sweat pores and hair follicles. This is a passive diffusion process, the amount of which depends on the integrity of the epidermal barrier, but is influenced by the drug.[13]

Skin toxicity showed that low-molecular-weight drugs (under 800 Dalton) with a high water content and high fat solubility show the most penetration.[7] Vehicles containing the drugs used are important. The level of hydration of the stratum corneum, so that the epidermis increases its water content, increases drug absorption. The rate of transport of the drug depends on its aqueous solubility, which is directly proportional to the coefficient of the oil/water partition, its concentration in the formulation vehicle, and the surface area of the skin to which it is exposed; it is inversely related to the thickness of the stratum corneum.

Flavonoids are polyphenolic compounds that are categorized according to their chemical structure into flavonols, flavones, flavones, isoflavones, catechins, anthocyanidins, and chalcones.[5] Flavonoids are powerful antioxidants, and they act through radical scavenging or metal chelating activities[3,4]. This metabolite is commonly used in a variety of pharmaceutical and cosmetic products, which is an indication that this metabolite may be non-toxic.

Toxicity testing is needed for each natural ingredient if it is to be used as a herbal ingredient in alternative treatments. The results of this study demonstrated the oral and dermal safety of the use of passionfruit seeds in rats. Research on humans can be performed to determine their effects on human organs.

Table 2: The effect of *P. edulis* S. water extract on Sprague-Dawley rats at baseline, day 1, and day 14 on intergroup body weight

Treatment time	C (g) Mean ± SD	P (gram) Mean ± SD
Baseline	150.8 ± 1.09	154.2 ± 1.64
Day 1	179.0 ± 7.91	185.6 ± 1.14
Day 14	202.8 ± 2.28	206.2 ± 0.84*

* $p < 0.05$, C, control group; P, *P. edulis* Sims group; SD, standard deviation

Table 3: Effect of *P. edulis* S. aqueous extracts on Sprague-Dawley rats at 15,000 mg/kg body weight in each group

Group	Treatment	Body Weight	
		Before treatment Mean ± SD	After treatment Mean ± SD
C	Water for injection	150.8 ± 1.09	202.8 ± 2.28*
P	15,000 mg/kg of extract	154.2 ± 1.64	206.20 ± 0.84*

* $p < 0.05$.

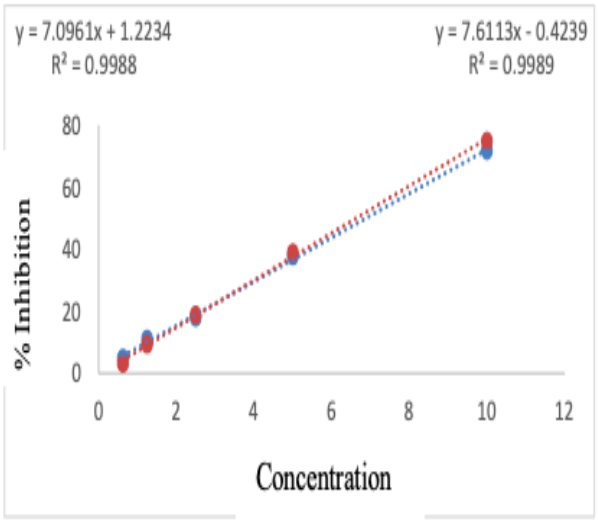

Figure 1: Comparison between concentration and inhibition of free radical (blue lines: *P. edulis* S. extract; red lines: vitamin C)

Conclusion

The study results showed that the aqueous extract of *P. edulis* S. has an acute non-toxic effect with oral and dermal administration in Sprague-Dawley rats. However, a comprehensive study is required to ascertain the toxicity and safety of the aqueous extract of *P. edulis* S. in humans.

Conflict of interest

The authors declare no conflict of interest.

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Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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