Pharmacy Education (2024) 24(9) 71 - 76
https://doi.org/10.46542/pe.2024.249.7176

ICSM SPECIAL EDITION

RESEARCH ARTICLE

The effect of acute toxicity test of red dragon Fruit (*Hylocereus polyrhizus*) peel extract on body weight and organ index of Wistar rats

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Keywords
Acute toxicity
Dragon fruit
Peel extract
Organ index

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Abstract

Background: Indonesian plant-based products are potential natural resources that can be developed as raw medicine materials, including dragon fruit peel extract. The peel of dragon fruit contains such high levels of antioxidants. The safety of dragon fruit plant-based products must be ensured by an acute toxicity test. **Objective:** This study aimed to determine the relationship between dose and toxicity in acute oral administration of dragon fruit plant-based products on the heart, lungs, liver, spleen, and kidney organ index. **Method:** Each six male and six female with a total of 24 rats were divided into four test groups, i.e., control group, 1250 mg/kgBW; 2500 mg/kgBW; and 5000 mg/kgBW. A single-dose test was given, and effect observation continued for 14 days. Body weight and organ index between male and female rats were compared statistically by the Independent T-test at a 95% confidence level. **Result:** There were no animal deaths during the test, thus the LD50 value of dragon fruit peel extract was > 5000 mg/kgBW. In general, there was an increase in the body weight of animals. There was no significant difference in body weight among test groups in male (p 0.429 > 0.05) and female rats (p 0.721 < 0.05). There was also no statistical difference in organ index between the test and control groups (p > 0.05). The animals' behaviours were also still normal manner. **Conclusion:** The extract was practically non-toxic in acute treatment. The organs and the behaviour were not altered by the extract.

Introduction

Herbal preparations for treating various ailments continue to be widely used in rural areas, where they were widely used in folklore before the development of synthetic or semi-synthetic medicines (Leonti & Casu, 2013). In developing countries, botanical remedies have gained prominence in primary healthcare (Engel *et al*., 2014). In recent times, natural products have also been instrumental in pursuing novel therapeutic agents for treating diverse human diseases or discovering new drugs (Hossen *et al*., 2017). zat, carotenoids, and anthocyanin, all of which are natural antioxidant compounds. Antioxidants can potentially mitigate the detrimental effects of elevated free radical levels, safeguard cells against toxicity, and aid in preventing a wide range of diseases. According to a study by Wahdaningsih, *et al*. (2017), the antioxidant activity of methanol extract, ethyl soluble fraction acetate and ethyl acetate insoluble fractions have antioxidant activity are 241.19 ppm, 8.34 ppm, 46.84 ppm, respectively. In addition, dragon fruit peel extract has been widely reported to have other pharmacological activities, such as antibacterial and anti-inflammatory agents. (Setiani, 2020; Wahdaningsih & Untari, 2021; Yuna *et al*., 2023)

Despite their widespread use, a limited number of traditional herbal medicines have undergone efficacy and safety testing (Dicson *et al*., 2015). No research has been conducted on the safety of extra red dragon fruit peels. For this reason, this study aimed to determine the relationship between dose and toxicity in acute oral

administration of dragon fruit plant-based product peel extract on the heart, lungs, liver, spleen, and kidney organ index.

Methods

Sample collection and processing

Sarang Burung Kolam Village, Matang Tangkit Hamlet, Jawai District, Sambas Regency, and West Kalimantan Province were the origins of the *Hylocereus polyrhizus* peel utilised in this study. The researchers gathered the peel of *Hylocereus polyrhizus* while it was still fresh, clean, red, and undamaged. The *Hylocereus polyrhizus* peel that was collected under clean running water and subsequently slit underwent a wet sorting process amounting to ten kilograms. The authors then decreased the size of the sample by chopping it. Dry sorting was then performed after the sample had been dried in an oven preheated to a temperature of ±50 °C until it was breakable without becoming too rigid, which reduced its water content. The authors then ground 500 mg of dried simplicia. To mitigate the risk of impurities and preserve the simplicia powder, the authors kept it in a sterile and dry receptacle (Wahdaningsih *et al*., 2017).

Preparation of extract

In this study, maceration was utilised to prepare extracts. The authors extracted using a jar containing powdered *Hylocereus polyrhizus* peel simplicia. Then, methanol solvent was added and stirred multiple times until the simplicia powder was completely submerged. During the 3x24-hour maceration period, the authors conducted a filtration process every 1x24 hours using flannel cloth. The authors also redissolved the residue using a fresh methanol solvent. Another time, the macerate underwent filtration through a Buchner. Afterwards, the substance was evaporated using a rotary evaporator (Buchi) until a viscous extract was obtained, as determined through qualitative and quantitative analysis (Wahdaningsih *et al*., 2017).

Approval from ethics committee

The research was carried out after obtaining approval from the Ethics Committee of the Faculty of Medicine, Tanjungpura University, with approval number 4452/UN22.9/PG/2023.

Experimental animal

The authors randomly allocated n (number) female and male Wistar rats among the treatment groups. The inclusion criteria were non-pregnant status, 2-3 months of age, and 100–200 grams of body weight. The exclusion criteria, on the other hand, consisted of rats exhibiting physical abnormalities or defects. The authors kept the experimental room in pristine condition every day. Following the OECD rule on number 425 (Organisation for Economic Co-operation and Development), the room temperature was maintained within the range of 22.3oC, the relative humidity was maintained between 50 and 60%, and the lighting cycle consists of 12 hours of light and 12 hours of darkness. The OECD officially released the Guideline for Testing of Chemicals, Acute Oral Toxicity Up-and-Down Procedure (UDP) on October 3, 2008. Six male and six female groups were formed by dividing the test animals using a random sample of 24 rats. Details of the grouping of rats are shown in Table 1. Picric acid facilitated the differentiation of individual test animals by assigning numbers to each one. Marking is applied to the head for rat number one, the back for rat number two, the tail for rat number three, the head and back for rat number four, the head and tail for rat number five and marking on the back and tail for rat number six.

Table I: Animals experimental group

Group	Treatments
Control	Given food and drink (ad libitum)
Lower dose	Given a dose of 1250 mg/kgBW *Hylocereus polyrhizus* peel extract
Middle dose	Given a dose of 2500 mg/kgBW *Hylocereus polyrhizus* peel extract
Upper dose	Given a dose of 5000

Acute toxicity study

This investigation assessed acute toxicity using the following parameters: body weight, behaviour, organ index, and LD50 value. The LD50 value was ascertained by quantifying the number of viable and non-viable rats for each experiment within 48 hours after treatment. Observations of body weight were conducted on days 1, 7, and 14. The animal's behaviour and motor activity (platform activity, pineal reflex, corneal reflex, hanging posture, flexion response, Hafner response, grooming, defecation and urination) were assessed at the following time points: 0 hours (before test preparation administration), 30 minutes, 1 hour, 2 hours, 24 hours, and before its termination. Observations of the organ index were conducted after termination.

The OECD 425 Limit Test method was employed to conduct the acute toxicity test in this investigation. The limit test was conducted using three dosing stages: 1250 mg/kgBW, 2500 mg/KgBW, and 5000 mg/KgBW. The initial dose of 1250 mg/KgBW was administered to

the rat. As the rat remained alive after 24 hours, observations were maintained for 14 days. The 14-day observation period was designed to detect any delayed toxic effects that were not apparent within the initial twenty-four hours. Throughout the fourteen days of observation, the test animals exhibited no delayed toxic effects on any recorded parameters. Continued testing was conducted on three rats in the same group receiving the identical treatment at 1250 mg/KgBW. Following administration of the test preparation at 1250 mg/KgBW, all four rats remained alive for 24 hours, as indicated by the test results. Consequently, experimentation was maintained at 5000 mg/kgBW and 2500 mg/kgBW, utilising the identical testing methodology. Following OECD 425 guidelines and The Indonesian Food and Drug Authority regulations, testing was terminated when the test animal exhibited signs of survival after administering the maximum dose of 5000 mg/KgBW (Organisation for Economic Co-operation and Development., 2022).

Organ index

The following organs were examined: spleen, heart, lungs, liver, and kidneys. Evaluations were conducted to determine whether or not each organ was damaged. Before obtaining their weight, organs must be dried using absorbent paper. Subsequently, the weight of the organ must be determined. The internal organ index percentage was calculated by dividing the organ's weight by the test animal's body weight. The formula for calculating the organ index is as follows (The Indonesian Food and Drug Authority, 2014):

$$\text{Organ Index} = \frac{\text{Organ weight (gram)} \times 100\%}{\text{Rat Body weight (gram)}}$$

Analysis data

The testing yielded both qualitative and quantitative information. The qualitative data were acquired by examining the motor activity and behaviour of the test animals and assessing the occurrence of fatalities or toxic effects. Using the AOT425StatPgm software (Westat Inc., ver.1.0), quantitative data in the form of Lethal Dose (LD50), organ weight, and organ index values were analysed. The statistical analysis was conducted through Statistical Program for Social Science (SPSS) software (version 25) by comparing the body weight and organ index of male and female rats using the Independent T-test with a 95% confidence level. A one-way ANOVA test assessed differences in body weight and organ index between acute toxicity test dose groups.

Results

The results of the acute toxicity assessment conducted using the OECD 425 method on dragon fruit peel extract (*Hylocereus polyrhizus*) revealed that the initial dosing levels of 1250 mg/kgBW, 2500 mg/kgBW, and 5000 mg/kgBW did not induce fatalities (Table II). Data about the incidence of fatalities was entered into the AOT425statpgm software application. It was determined that the LD50 of red dragon fruit peel extract is greater than 5000 mg/kgBW, as the extract satisfies the Hayes & Loomis (1996) criteria for practically non-toxic toxicity.

Table II: The comparisons of body weight between male and female rats (gram)

Groups	Male			Female			P^c
	Mean	SD	P^a	Mean	SD	P^b	
Control	146.78125	20.698114	0.429	129.51417	3.241044	0.721	0.150
1250 kg/BW	138.35250	23.588819		127.34500	12.719053		0.443
2500 kg/BW	160.30500	9.101591		125.22167	3.031398		<0.001
5000 kg/BW	140.70583	22.226750		131.68000	9.621684		0.484

Notes: [a] = *p*-value between males group; [b] = *p*-value between females group; [c] = *p*-value between males vs females in each test dose

The impact of the extract on body weight in male and female rats during 14 days is illustrated in Figure 1, respectively. The control groups exhibited the most substantial increase in body weight, while the group administered the 2500 mg/kg BW dosage demonstrated the least weight gain. Table II illustrates the magnitude of the effect that administering the extract to rats would have. The treatment groups did not differ significantly in terms of average body weight between male (p 0.429 > 0.05) and female rats (p 0.721 > 0.05) rats. A similar pattern was observed concerning the average body weight of male and

female rats across all experimental dosages except for 2500 mg/kg BW. A notable disparity in body weight was noted between male and female rats at this dosage, with female rats demonstrating a comparatively lower body weight than male rats.

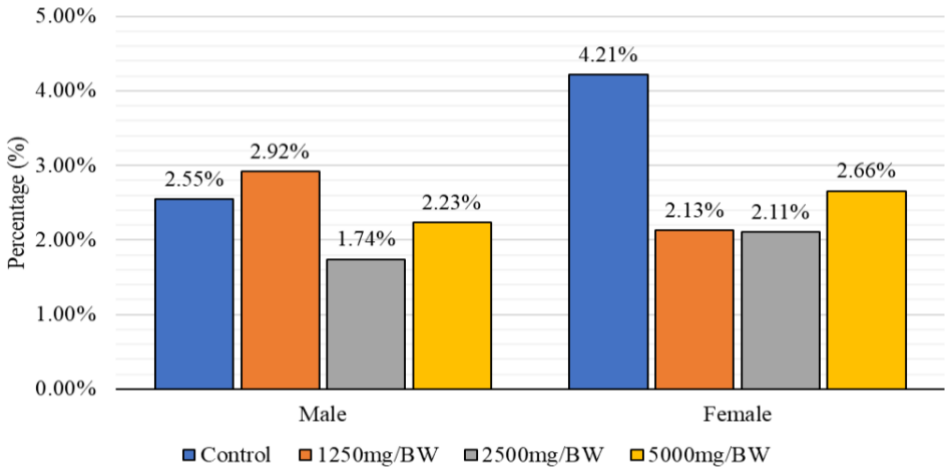

Figure 1: Percentage of body weight gain in (a) male rats and (b) female rats

Table III presents the investigation outcomes about the effects of dragon fruit peel extract on the conduct of the experimental animals. The male rats in the control group exerted the most sustained activity on the platform, averaging 7 minutes. Conversely, the activity duration on the platform was the shortest for male rats administered doses of 1250 and 2500 mg/kgBW. Positive behaviour was observed in every animal's pineal reflex, corneal reflex, flexion, Hafner, and reestablishment ability. The extract did not induce defecation or urination in most test animals. The majority of both male and female mice lacked grooming behaviour. It was observed that as the test dose increased, the motor activity of the animals decreased. In contrast, male mice administered 2500 and 1250 mg/kgBW exhibited normal appearance.

Table III: The behaviours of Rattus norvegicus after 60th minutes of first ingestion of extracts

Groups		Platform activity (minutes)	Pineal Reflex	Corneal reflex	Flexion	Haffner	Reestablishment ability	Defecate	Urinary	Grooming	Motoric activity
Control	Male	7	P	P	P	P	P	A	A	P	↑
	Female	4.75	P	P	P	P	P	A	A	P	↑
1250 mg/kgBW	Male	1	P	P	P	P	P	A	A	A	N
	Female	2	P	P	P	P	P	A	P	A	↑
2500 mg/kgBW	Male	1	P	P	P	P	P	P	A	A	N
	Female	6	P	P	P	P	P	A	P	A	↓
5000 mg/kgBW	Male	3.25	P	P	P	P	P	A	A	A	↓
	Female	5	P	P	P	P	P	P	A	A	↓

Table IV shows the effect of the extract on the test animal organs by calculating the organ index. The One-way ANOVA test results showed no significant differences in each organ index between the test groups ($pb > 0.05$). There were no significant differences in the percentage index of the kidney, spleen, liver, heart and lungs ($pa > 0.05$) in the test group of males compared to the control group. Likewise, in female rats, there was no difference in the percentages of organ index between treatment groups ($pb > 0.05$). Moreover, test the dose groups toward control ($pa > 0.05$). In comparison to other organs, the liver frequently has the highest index percentage, whereas the spleen exhibits the lowest organ index.

Table IV: The effects of *Hylocereus polyrhizus* peel extract on Rattus norvegicus organs

Group	Kidney			Spleens			Liver			Heart			Lungs		
	OI	*Pa*	*Pb*	OI	*Pa*	*Pb*	OI	*Pa*	*Pb*	OI	*Pa*	*Pb*	OI	*Pa*	*Pb*
Male															
Control	0.411± 0.032	Ref		0.253± 0.039	Ref		3.10 ± 0.359	Ref		0.298± 0.041	Ref		0.760 ± 0.232	Ref	
1250	0.355± 0.029	0.531	0.763	0.204± 0.030	0.997	0.972	3.461± 0.113	0.536	0.322	0.297± 0.026	0.902	0.703	0.521± 0.105	0.523	0.187
2500	0.356 ± 0.026	0.313		0.225± 0.053	0.993		3.276± 0.406	0.968		0.259± 0.021	0.603		0.537± 0.075	0.497	
5000	0.380± 0.027	0.481		0.265± 0.086	1.000		3.681± 0.390	0.349		0.287± 0.037	0.292		0.467± 0.120	0.142	
Female															
Control	0.336± 0.032	Ref		0.243± 0.024	Ref		3.098± 0.271	Ref		0.283± 0.017	Ref		0.576± 0.057	Ref	
1250	0.413± 0.055	0.217	0.320	0.362± 0.112	0.221	0.109	4.221± 0.535	0.086	0.208	0.362± 0.044	0.440	0.108	0.612± 0.135	0.792	0.170
2500	0.321± 0.026	0.603		0.188± 0.021	0.955		3.391± 0.273	0.842		0.261± 0.014	0.180		0.448± 0.056	0.215	
5000	0.343± 0.048	0.835		0.261± 0.038	0.995		3.588± 0.296	0.462		0.334± 0.018	0.303		0.612± 0.169	1.000	

Notes: OI= Organ Index (%); ^{y}= dose in mg/kgBW; p^{a}= *p*-value of difference towards control group; p^{b}= *p*-value of difference between male and female rat

Discussion

An acute toxicity assessment was conducted on an extract derived from red dragon fruit peel. The peel, originally discarded as food, has since been recognised for its potential applications as a traditional medicine agent due to its antimicrobial, cosmeceutical, antioxidant, and food additive properties (Amalia *et al*., 2016; Manihuruk *et al*., 2017; MD *et al*., 2018; Hendra *et al*., 2020; Tanjung & Rokaeti, 2020). The OECD 425 method was utilised in this study to determine the LD50 value of the peel extract of H. *polyrhizus*. Following an initial 1250 mg/kg BW dose, the subsequent test doses were 5000 mg/kg BW and 2000 mg/kg BW. The effect of the extract on the gender of the animals was compared using male and female Rattus norvegicus specimens. No animals perished throughout the LD50 value test. The AOT425statpgm analysis revealed that the LD50 value of the extract derived from red dragon fruit peel exceeded 5000 mg/kg BW. The extract is designated as virtually non-toxic based on the toxicity classification proposed by Hayes and Loomis (1996). The red dragon fruit peel extract did not exhibit acute toxicity at doses of up to 5000 mg/kg BW. Previous investigations on the acute and chronic toxicity of the methanol extract of red dragon fruit (H. *polyrhizus*) deemed this extract "*virtually non-toxic*" (Hor *et al*., 2012) when its LD50 value exceeded 5000 mg/kg BW. After the experiment, the authors observed alterations in the body weight of the test animals due to the administration of red dragon fruit peel extract. Researchers have used changes in animal body weight as an indicator to determine the harmful effects of chemicals and treatments (Hubrecht, 2013). If organ damage occurs due to fluid accumulation or swelling, it will impact the weight and organ index. During the fourteen days of testing, the authors observed an overall increase in the body weight of the experimental animals. Compared to the control group and male and female rats, the increase in the weight of the animals did not reach statistical significance. The feed and water consumption of the experimental animals resulted in a concurrent rise in body weight. During the fourteen-day assessment of acute toxicity, the authors observed that the animals' intake of food and beverages remained within expected limits. According to some studies, lipid, carbohydrate, and protein metabolism are crucial processes in the animal body, as these nutrients contribute significantly to numerous physiological functions (Saleem *et al*., 2017; Stevens & Mylecraine, 1994). In the 2500 mg/kg BW dose group, male and female rats had significantly different body weights (160.30 g *vs*. 125.22 g), p 0.001. However, compared to the control group, there was no significant difference in organ index at the 2500 mg/kg BW dose. These results suggest increased body weight, but no significant effect on organ index was observed (refer to Table IV). After administering dragon fruit peel extract, no observable effects on animal behaviour were observed. The animals exhibited expected behaviours after receiving the medication. All the experimental animals retained the

capacity to engage in locomotion on the platform, demonstrated pineal and Haffner reflexes, and completed reestablishment. A restricted subset of animals defecated after receiving the test dose, but no diarrhoea or excessive defecation was reported. No detrimental effects were observed. The effects of the extract on various organs were assessed using organ indicators, including those of the kidney, spleen, liver, heart, and lungs. The analysis did not reveal any statistically significant differences in organ index between the control and test dose groups. The toxicity test results, consistent with the previous investigation, indicated that the organs of the test animals remained unaffected by dragon fruit extract for 28 days (Hor *et al*., 2012). The liver, kidney, heart, lungs, and spleen are the essential body organs most susceptible to the metabolic effects of potentially dangerous substances (Auletta & RAC, 1995). Toxicity refers to the condition of being hazardous, which implies the occurrence of harmful effects resulting from the interaction between toxic substances and cells. The contact between harmful substances and the cell membrane might vary depending on their chemical properties. This interaction can occur on the cell surface, within the cell body, or in the tissues beneath, including the extracellular matrix. Toxic effects can occur before harmful substances bind to vital organs, including the liver and kidney; furthermore, it will reveal the cumulative toxic effects on target organs (Jothy *et al*., 2011; Hor *et al*., 2012). Further toxicity tests should be conducted to determine the effects of prolonged extract administration.

Conclusion

The results of acute toxicity testing indicate that the ethanolic extract derived from the peels of H. *polyrhizus* does not exhibit toxic effects. Researchers reached this conclusion by considering the observed animals' body weight, the organ index, and the LD50 value. However, the initial findings indicated that additional research is required to confirm the safety of this extract concerning recurrent dose effects and long-term use.

Acknowledgement

The authors thank the Ministry of Education, Culture, Research, and Technology for providing the necessary financial resources to conduct this study. In addition, the authors would like to thank M.Rafly Agustriangga, Alfian Nur Huda, Intan Yap, and Amrina Rasyada Asmara, who helped carry out the study.

Source of funding

The Ministry of Education, Culture, Research, and Technology invested in the funding for this study.

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