Open Journal of Forestry, 2015, 5, 749-753
Published Online October 2015 in SciRes. http://www.scirp.org/journal/ojf
http://dx.doi.org/10.4236/ojf.2015.57067

Toxicolological Test of Saponins from *Sapindus mukorossi* Gaerth

Menghao Du*, Sumei Huang, Jinping Zhang, Jingwen Wang, Lisong Hu, Jingmin Jiang

Research Institute of Subtropical Forestry, CAF, Fuyang, China
Email: *xiaoduchongcn@aliyun.com.cn

Received 30 September 2015; accepted 18 October 2015; published 21 October 2015

Abstract

The study has been carried out to investigate acute oral toxicity, acute dermal toxicity in SPF rats and dermal irritation in rabbits. The result shows: 1) acute oral toxicity test shows that LD_{50} of saponins from *Sapindus mukorossi* is 9260 mg/kg (95% confidence interval is 6360 - 13,500 mg/kg) and 7940 mg/kg (95% confidence interval is 4890 - 12,900 mg/kg); 2) acute dermal toxicity test shows that LD_{50} of saponins from *Sapindus mukorossi* is more than 5000 mg/kg in both female and male Wistar rats; 3) dermal irritation test in rabbits shows that the average score of dermal irritation per day of each rabbit is zero after 14 days of continuous dermal irritation. According to the classification standard of toxicity in "Hygienic Standard for Cosmetics" (2002 version), the sample is classified as "practical nontoxic" and "non dermal irritation". Thus, we can conclude that the saponin extraction from *S. mukorossi* Gaerth is safe for cosmetics.

Keywords

Saponins, Acute Oral Toxicity, Acute Dermal Toxicity, Dermal Irritation

1. Introduction

The species of the genus of Sapindus belonging to the Sapindaceae has about five to twelve species of shrubs and small trees (FRPS, 1998). Members of the genus are commonly known as soapberries or soapnuts because the fruit pulp is used to make soap (FRPS, 1998). There are about 10% saponins in fruit pulp (FRPS, 1998), which makes it an ideal resource for the extraction of saponins. Saponin, a natural non-ionic surfactant, not only has a good emulsifying, separating and dispersing capability but also is a good foaming and foam stabilizer with a great cleaning capacity (Zhang et al., 1993). Thus, it can be used as foam stabilizer for building concrete (Lin, 1977) pesticide synergist (Hong & Tokunaga, 2000) and antiviral, and reducing blood pressure (Huang et al.,

*Corresponding author.

How to cite this paper: Du, M. H., Huang, S. M., Zhang, J. P., Wang, J. W., Hu, L. S., & Jiang, J. M. (2015). Toxicolological Test of Saponins from *Sapindus mukorossi* Gaerth. *Open Journal of Forestry, 5,* 749-753.
http://dx.doi.org/10.4236/ojf.2015.57067

2007; Ibrahim et al., 2006, 2008; Kuo et al., 2005; Yukiyoshi, 2001; Yata et al., 1986). So it is widely used in daily chemical industries, building materials, food industries and agriculture. In this study we evaluated saponins from *Sapindus mukorossi* to study acute oral toxicity, acute dermal toxicity in SPF rats and dermal irritation in rabbits.

2. Materials and Methods

2.1. Plant and Chemical Material

Fruits pulps of *S. mukorossi* were collected from Tiantai of Zhejiang Province, China, in November 2008. Prior to all extractions, fruits pulp was dried at 60˚C for 48 h and was ground in a Wiley mill to pass a 0.5 mm pores-ize screen. Chromatograph solvents used during the study were of HPLC grade and the other solvents and reagents used during the study were of AR grade.

2.2. Extraction Process

1 kg ground fruits pulps of *S. mukorossi* was mixed with 6 times the amount by weight of 85% EtOH, homogenized and transferred to an extraction flask. Reflux extraction was carried out at 85˚C water bath temperature for 2 hours. After cooling the extract solution is filtered through a folded filter. The filtrate is concentrated to dryness in a rotary evaporator at 75˚C water bath. The sample of saponins were washed sequentially with chloroform, ethyl acetate and then dried. The solid matter of sample was re-dissolved in 85% EtOH and mixture was centrifuged at 6000 g for 10 min at room temperature. The supernatant was dried by lyophilization. The samples were prepared for the following test (Du et al., 2014).

2.3. Experimental Animals

SPF Wistar rats, weight 175 - 213 g, provided by Zhejiang Provincial Research Center for Experimental Animals. Quarantined and observed for one week before testing. Caged feeding, five rats in one cage (20˚C ± 2˚C, 40% ± 5%). Grain whole-nourishment feed for Experimental animals. Environment is held constant. We ensure the welfare of Experimental Animals.

2.4. Acute Oral Toxicity Test

Rats were randomly assigned to 5 dosage groups. See **Table 1**. Density of the sample is 1.05 g/ml, Oral dosing capacity is 10 ml/kg BW in the 2150, 4640, 10,000 mg/kg BW dosage groups, in which the sample is diluted to 215, 464, 1000 mg/ml by distilled water. In 21,500 mg/kg BW group the oral dasing capacity is 20.48 ml/kg BW without dilution of the sample. Feed is withheld 14 hour before dosing with supply of water. The rats are weighed before administration. The sample is administration through oral intubations. Feed is given two hours after dosing. In the first hour after oral administration continuous observation is conducted, followed by two times of observation per day till 14th day. The character of poisoning, recovery and death were recorded in detail. All dead rats were anatomized and examined.

Table 1. Dosage and weight of rats in five different dosage groups.

via	xeS	egasoD (mg/BWkg)	rat no.	weight (g)
mouth	♀	2150	5	175.3 - 195.0
		4640	5	176.0 - 197.7
		10,000	5	180.3 - 196.8
		21,500	5	180.9 - 199.6
	♂	2150	5	177.3 - 204.3
		4640	5	179.1 - 207.8
		10,000	5	182.7 - 208.2
		21,500	5	185.3 - 213.4

2.5. Acute Dermal Toxicity Test

Rats were weighed into one dosage group. See Table 2. Fur on the back of rats is removed by electric clippers 24 hours before dosing. The sample is smeared on a 5 × 6 cm^2 area of the naked skin at a dose of 0.95 ml/200 gBW (Using the sample directly) for 24 hours, with plastic covered and rubberized fabric fixed. Day after exposure terminated and the skin is cleaned with water. In the first hour after oral administration continuous observation is conducted, followed by two times of observation per day till 14th day. The character of poisoning, recovery and death were recorded in detail. All dead rats were anatomized and examined.

2.6. Dermal Irritation Test in Rabbits

Fur on the back of rabbits is removed by electric clippers 24 hours before dosing. The sample is smeared on a 5 × 6 cm^2 area of the naked skin at a dose of 0.95 ml/200 g BW (Using the sample directly) for 24 hours, with plastic covered and rubberized fabric fixed. Day after exposure terminated and the skin is cleaned with water. In the first hour after administration continuous observation is conducted, followed by two times of observation per day till 14th day. The character of poisoning, recovery and death were recorded in detail. All dead rats were anatomized and examined.

2.7. Statistical Analysis

The results are using Horn's table to find LD_{50}.

3. Results and Discussion

3.1. Oral Acute Toxicity in Wistar Rats by Saponins

30 minutes after administration, poisoning sign of sagged spirit were observed. Death of rats happen from 4 hours to 4 days after administration, swollen stomach and intestine were found by anatomy. Most of the survived rats were recovered from poisoning symptom 7 days after administration. No other significant pathogenic alteration was observed by naked eye. The result of acute oral toxicity test showed that LD_{50} of saponins from *Sapindus mukorossi* is 9260 mg/kg (95% confidence interval is 6360 - 13,500 mg/kg) and 7940 mg/kg (95% confidence interval is 4890 - 12,900 mg/kg) in female and male Wistar rats respectively (Table 3).

Table 2. Dosage and weight of rats.

sex	egasoD (mg/BWkg)	no. rat	weight (g)
♀	500	5	190.7 - 211.4
♂	500	5	194.8 - 214.5

Table 3. Oral acute toxicity in Wistar rats by saponins.

sex	dosage (mg/kg)	rats	death rats	LD_{50} and 95% CI (mg/kg)
♀	2150	5	0	9260 (6360 - 13,500)
	4640	5	0	
	10,000	5	3	
	21,500	5	5	
♂	2150	5	0	7940 (4890 - 12,900)
	4640	5	1	
	10,000	5	3	
	21,500	5	5	

3.2. Acute Dermal Toxicity in Wistar Rats by Saponins

No obvious intoxication sign was observed and death happened. The result of acute dermal toxicity test showed that LD_{50} of saponins from *Sapindus mukorossi* is more than 5000 mg/kg in both female and male Wistar rats (**Table 4**).

3.3. Grading Results of Dermal Irritation Test

The result of dermal irritation test in rabbits showed that the average score of dermal irritation per day of each rabbit is zero after 14 days of continuous dermal irritation by the saponins from *Sapindus mukorossi* (**Table 5**).

4. Conclusion

The result shows: 1) acute oral toxicity test shows that LD_{50} of saponins from *Sapindus mukorossi* is 9260 mg/kg (95% confidence interval is 6360 - 13,500 mg/kg) and 7940 mg/kg (95% confidence interval is 4890 - 12,900 mg/kg); 2) acute dermal toxicity test shows that LD_{50} of saponins from *Sapindus mukorossi* is more than 5000 mg/kg in both female and male Wistar rats; 3) dermal irritation test in rabbits shows that the average score of dermal irritation per day of each rabbit is zero after 14 days of continuous dermal irritation. According to the classification standard of toxicity in "Hygienic Standard for Cosmetics" (Ministry of Public Health of China, 2002), the sample is classified as "practical nontoxic" and "non dermal irritation". Thus, we can conclude that the saponin extraction from *S. mukorossi* Gaerth is safe for cosmetics.

Table 4. Acute dermal toxicity in Wistar rats by saponins.

sex	dosage (mg/kg)	rats	body weight (g)			death rats
			beginning	7th day	14th day	
♀	5000	5	203.3 ± 8.3	210.1 ± 8.1	218.2 ± 6.4	0
♂	5000	5	204.2 ± 7.4	225.2 ± 7.1	259.7 ± 5.3	0

Table 5. Grading results of dermal irritation test in rabbits by saponins.

time (day)	rabbits	scoring of dermal irritation					
		samples			physiological saline solution		
		erythema	edema	total	erythema	edema	total
1	4	0	0	0	0	0	0
2	4	0	0	0	0	0	0
3	4	0	0	0	0	0	0
4	4	0	0	0	0	0	0
5	4	0	0	0	0	0	0
6	4	0	0	0	0	0	0
7	4	0	0	0	0	0	0
8	4	0	0	0	0	0	0
9	4	0	0	0	0	0	0
10	4	0	0	0	0	0	0
11	4	0	0	0	0	0	0
12	4	0	0	0	0	0	0
13	4	0	0	0	0	0	0
14	4	0	0	0	0	0	0
average score in 14 days of each rabbit		0	0	0	0	0	0
average score per day of each rabbit		0	0	0	0	0	0

Acknowledgements

The authors are grateful for the financial support by State Forestry Administration P. R. China and Forest Department of Zhejiang Province, China (Project number: 200804032, 2010SY03), for carrying out the above research work.

References

Du, M. H., Huang, S. M., & Zhang J. P. (2014). Isolation of Total Saponins from *Sapindus mukorossi*. *Gaerth, 4,* 24-27.

Editorial Committee of the Flora of China of Chinese Academy of Science (FRPS) (1998). *Flora of China*. Beijing: Beijing Science Press.

Hong, K. J., & Tokunaga, S. (2000). Extraction of Heavy Metals from MSW Incinerator Fly Ash Using Saponins. *Chemosphere, 41,* 345-352. http://dx.doi.org/10.1016/S0045-6535(99)00489-0

Huang, H. C., Tsai, W. J., Law, C. C. et al. (2007). Anti-Platelet Aggregation *Triterpene saponins* from the Galls of *Sapindus mukorossi*. *Chemical and Pharmaceutical Bulletin, 55,* 1412-1415. http://dx.doi.org/10.1248/cpb.55.1412

Ibrahim, M., Khaja, M. N., Aara, A. et al. (2008). Hepatoprotective Activity of *Sapindus mukorossi* and *Rheum emodi* Extracts: *In Vitro* and *in Vivo* Studies. *World Journal of Gastroenterology, 14,* 2566-2571. http://dx.doi.org/10.3748/wjg.14.2566

Ibrahim, M., Khan, A. A., Tiwari, S. K. et al. (2006). Antimicrobial Activity of *Sapindus mukorossi* and *Rheum emodi* Extracts against *H. pylori*: *In Vitro* and *in Vivo* Studies. *World Journal of Gastroenterology, 14,* 7136-7142.

Kuo, Y. H., Huang, H. C., Yang Kuo, L. M. et al. (2005). New Dammarane-Type Saponins from the Galls of *Sapindus mukorossi*. *Journal of Agricultural and Food Chemistry, 53,* 4722-4727. http://dx.doi.org/10.1021/jf047963s

Lin, Q. S. (1977). *The Chemical Composition of Chinese Herbal Medicine*. Beijing: People's Medical Publishing House.

Ministry of Public Health of China (2002). Hygienic Standard for Cosmetics.

Yata, N., Sugihara, N., Yamajo, R. et al. (1986). Enchanced Small Intestinal Absorption of Lactam Antibiotic in Rats in the Presence of Monodesmosides Isolated from Pericarps of *Sapindus mukorossi* (enmei-hi). *Journal of Pharmacobio-Dynamics, 9,* 211-217.

Yukiyoshi, T. (2001). The Spermicidal Affection of Sapindus. *Natural Medicines, 55,* 563-568.

Zhang, M. J., Liu, P. R., Zhao, J. Z. et al. (1993). Study on the Comprehensive Utilization of *Sapindus mukorssi*. *Natural Product Research and Development, 5,* 76-78.