ACUTE TOXICITY OF NANO BERBERINE GEL IN WHITE MICE

Nguyen Ngoc Tuan[1], Le Quoc Chieu[1], Le Thi Hong Hanh[2]

[1]Le Huu Trac National Burn Hospital
[2]Military Medical University

ABSTRACT

Berberine is isolated from Coscinium fenestratum and some plants in the family Ranunculaceae which has the effect of treating burn wounds. The study determined acute oral toxicity administration in white mice of nano Berberine gel (NBBR) produced by the Le Huu Trac National Burns Hospital.

***Research methods**: The research method was accorded to the guidelines of the Ministry of Health, World Health Organization. Evaluation of 120 mice, divided into 10 groups, NBBR dosages were gradually increased from 30.9g/kg to 123.96g/kg by oral administration.*

***Results:** No dead mice, no abnormal disorders in movement and digestion; no abnormal histopathological found in livers, spleens and kidneys.*

***Conclusion:** LD_{50} (Median Lethal Dosage) of NBBR gel had not been determined in white mice by oral administration with the highest dose that could be given 123.96g/kg body weight/day.*

***Keywords:** Acute toxicity, white mice, nano Berberine gel (NBBR), pathology.*

1. INTRODUCTION

Acute toxicity is a toxic effect that occurs immediately after the administration of one or more doses of chemicals in 24 hours. The acute toxicity study was conducted to determine the primary toxicity of a chemical substance (including a pharmaceutical product), the sensitivity of animal toxicity, target organs, and risk assessment information after acute exposure to chemical substances [1, 2].

Burn injuries are common, with serious consequences, prolonged treatment, and expensive costs. Infection of the burn wound is a typical consequence, hurting the local area as well as the entire body. Local treatment of burn has a critical position to restrict the contamination, creating good conditions for the burn wound healing.

Some normal topical antibacterial drugs that treat at the burn after using a

Corresponding author: Nguyen Ngoc Tuan, Le Huu Trac National Burn Hospital
Email: ngoctuan64@gmail.com
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long time such as Betadine (Povidone-iodine), Silver Sulfadiazine, Sulfamylon (Mafenide), Silver Nitrate, Chlorhexidine (Hibiscrub)... in the clinical field has shown drug resistance. Moreover, these medications have to be imported and are very expensive, when washing or applying to the burn wound, its side effect still causes pain or cannot be used for a long time (due to toxicity), having little effect on stimulating the epithelial process, or some topical medications (such as Hydrogen Peroxide) are exclusively used for regular wounds and not for burns.

Berberine is extracted from *Coscinium fenestratum* and various *Ranunculaceae* plants. Berberine solution or cream to treat burns has been proven to be beneficial for a long time. The medication possesses anti-infective, anti-inflammatory, and wound-healing properties (also known as plant antibiotics). However, there is a danger of resistance because of long-term usage and low concentration (limited solubility in water).

Due to their benefits, nano-based active substances are becoming increasingly popular. Nanostructured systems and nanoparticle-based systems have a large contact surface, active components are released quickly, boosting medication bioavailability.

We researched to make NBBR gel with basic standards. It is required to follow WHO and the Ministry of Health's general rules in order to use a pharmaceutical product in clinical practice. As a starting point for future research, we need to assess the acute toxicity of NBBR gel.

2. MATERIALS AND METHODS

2.1. Materials

NBBR gel product passes basic standards, provided by Le Huu Trac National Burn Hospital.

2.2. Experimental animals

Adult Swiss mice, 120 individuals supplied by the Center for Experimental Animal Research, regardless of breed, healthy, and satisfying experimental criteria, weighing 18 - 22g. Mice were housed in laboratory settings for 5 days before being tested and were provided conventional animal feed and boiling water (cooled). To eliminate respiratory and contact cross-contamination, study animals were maintained in separate cages. Room temperature: 23°C, humidity: 50 - 60%, and alternate time of day (light): 12 h light/dark cycle (OECD 423, 2001). Experimental outcomes were monitored and recorded daily [3].

2.3. Methods

According to decision No. 141/BYT 2015 of the Ministry of Health and WHO [4-6] on determining the safety of natural-source products. 120 mice were randomly divided into 10 groups, in each group were 12 mice. The mice were fasted for 16 hours before receiving the medication.

A special curved feeding needle was used to orally dose. the research drugs were administered in escalating dosages ranging from 0.3ml/10g body weight to 1.2ml/10g body weight (up to 4 times a day). Mice were grown and monitored according to the recommended protocol after receiving the medication. The follow-up period was continuous for 72 hours and 14 days later.

Fig. 2.1. A special syringe and drinking manipulation NBBR gel

- Target assessment:

+ The number of mice that died throughout the research.

+ Clinical monitoring includes automatic movement (normal walking, cornering in a mouse cage, movement disorders); unusual manifestations such as convulsions, tremors, sweating, cyanosis; gastrointestinal (abnormal changes in digestion: diarrhea). Time of assessment: Before taking the medicine (T0), After 1 day (T1), After 2 days (T2), After 3 days (T3), After 5 days (T5), After 7 days (T7), After 14 days (T14).

+ Histopathology: If a mouse died at any time it could be examined to establish the cause of death. At the end of the study, mice were killed for histopathological analysis: livers, spleens, and kidneys. General observation, making specimen HE staining of material, and microscopic observation with a standard microscope.

- Calculation of median lethal dose (LD_{50}): Each mouse was observed for clinical symptoms of toxicity and death in each group throughout 24 hours. Among the dose levels tested, there was no mortality in any mice between the lowest and maximum dose levels in the mice groups studied. Calculation: The lethal dose of 50% of experimental animals - LD_{50} according to the improved method of Lichtfiel - Wilcoxon et al [3].

2.4. Statistical analysis

Statistical methods for biomedical research, the data were statistically analyzed by Microsoft Excel, Stat view 501 software, $P < 0.05$ was accepted as statistical significance.

2.5. Research ethics

The experimental research on white mice with the aims of determining the product's safety in order to provide a community-beneficial product. Experimental animals were always kept in hygienic conditions and given an excess of food and water. The procedures and manipulations were carried out following standard laboratory animal procedures.

3. RESULTS

3.1. Results to determine LD_{50} orally

Table 3.1. Weight of white mice during the study

Group	Oral volume (ml/kg/24h)	Weight of white mice (g)						
		T0	T1	T2	T3	T5	T7	T14
1	120	19.75±1.14	21.17±1.32	19.58±1.16	20.17±1.03	21.50±1.09	21.42±0.51	25.58±1.44
2	110	20.00±0.00	21.08±1.08	20.08±1.51	20.08±0.90	21.75±1.82	22.33±1.83	25.58±2.50
3	100	19.33±0.65	19.83±1.27	18.58±1.31	19.33±0.78	20.50±1.09	20.75±0.75	24.50±1.00
4	90	19.00±1.21	19.75±2.22	18.00±0.95	18.00±2.17	22.08±2.75	21.58±1.83	24.33±1.92
5	80	19.50±1.38	18.67±0.86	17.92±0.51	18.17±0.94	19.58±1.00	21.75±0.87	24.42±1.16
6	70	20.60±0.47	21.30±0.90	20.10±0.90	21.40±0.47	23.40±0.63	24.67±0.59	26.45±1.29
7	60	20.50±0.62	21.40±1.43	19.50±1.63	21.20±2.00	21.20±1.39	22.68±1.73	26.85±1.75
8	50	20.15±0.68	21.88±0.51	20.80±1.11	20.37±1.28	22.10±0.78	21.88±2.13	25.78±1.82
9	40	19.75±0.87	21.78±1.37	19.93±1.40	19.93±1.40	22.10±1.84	23.29±2.11	26.17±1.62
10	30	19.83±0.81	22.97±1.01	21.23±1.01	21.23±1.01	21.45±1.80	22.64±2.90	26.41±1.96
P		> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

The weight of the white mice steadily grew; however, on the second day following the research, the weight of the mice in all groups declined somewhat since the mice had fasted before taking the product. The weight had grown greatly after two weeks when compared to the starting point.

The weights in the groups were compared at the time of the research, there were no statistically significant differences with $p > 0.05$.

Table 3.2. Rate of dead mice after 14 days of drinking NBBR gel

Group	Dosage (g/kg/24h)	Oral volume (ml/kg/24h)	n	Number of dead (after 14 days)	Number of alive (after 14 days)
1	123.96	120	12	0	12
2	113.63	110	12	0	12
3	103.30	100	12	0	12
4	92.97	90	12	0	12
5	82.64	80	12	0	12
6	72.31	70	12	0	12
7	61.98	60	12	0	12
8	51.65	50	12	0	12
9	41.32	40	12	0	12
10	30.99	30	12	0	12

14 days follow-up found no dead mice. The white mouse ate and drank normally, had smooth hair, clean eyes, dry anus, and normal mouse droppings.

Note: The greatest dosage which can be administered to mice is also the most concentrated suspension that can be pushed via the needle for mice to drink; it is also the most medicine a mouse has drinkable within 24 hours.

2.2. Research results identify some symptoms of poisoning drug

Table 3.3. Rate of mice with an abnormality of automatic movement

Group	Criteria	Research time					
		T_1	T_2	T_3	T_5	T_7	T_{14}
1	Walking normally	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	Clustered at the cage corner	0	0	0	0	0	0
	Movement disorder	0	0	0	0	0	0
2	Walking normally	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	Clustered at the cage corner	0	0	0	0	0	0
	Movement disorder	0	0	0	0	0	0
3	Walking normally	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	Clustered at the cage corner	0	0	0	0	0	0
	Movement disorder	0	0	0	0	0	0
4	Walking normally	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	Clustered at the cage corner	0	0	0	0	0	0
	Movement disorder	0	0	0	0	0	0
5	Walking normally	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	Clustered at the cage corner	0	0	0	0	0	0
	Movement disorder	0	0	0	0	0	0
6	Walking normally	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	Clustered at the cage corner	0	0	0	0	0	0
	Movement disorder	0	0	0	0	0	0
7	Walking normally	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	Clustered at the cage corner	0	0	0	0	0	0
	Movement disorder	0	0	0	0	0	0
8	Walking normally	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	Clustered at the cage corner	0	0	0	0	0	0
	Movement disorder	0	0	0	0	0	0
9	Walking normally	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	Clustered at the cage corner	0	0	0	0	0	0
	Movement disorder	0	0	0	0	0	0
10	Walking normally	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	Clustered at the cage corner	0	0	0	0	0	0
	Movement disorder	0	0	0	0	0	0

After 24 hours, no mice showed any movement abnormality, 100% of mice were acting normally.

Table 3.4. Rate of rats showed signs of convulsions, tremors, increased sweating, cyanosis

Group	Criteria	Research time					
		T_1	T_2	T_3	T_5	T_7	T_{14}
1	Convulsions, tremors	0	0	0	0	0	0
	Increased sweating	0	0	0	0	0	0
	Cyanosis	0	0	0	0	0	0
2	Convulsions, tremors	0	0	0	0	0	0
	Increased sweating	0	0	0	0	0	0
	Cyanosis	0	0	0	0	0	0
3	Convulsions, tremors	0	0	0	0	0	0
	Increased sweating	0	0	0	0	0	0
	Cyanosis	0	0	0	0	0	0
4	Convulsions, tremors	0	0	0	0	0	0
	Increased sweating	0	0	0	0	0	0
	Cyanosis	0	0	0	0	0	0
5	Convulsions, tremors	0	0	0	0	0	0
	Increased sweating	0	0	0	0	0	0
	Cyanosis	0	0	0	0	0	0
6	Convulsions, tremors	0	0	0	0	0	0
	Increased sweating	0	0	0	0	0	0
	Cyanosis	0	0	0	0	0	0
7	Convulsions, tremors	0	0	0	0	0	0
	Increased sweating	0	0	0	0	0	0
	Cyanosis	0	0	0	0	0	0
8	Convulsions, tremors	0	0	0	0	0	0
	Increased sweating	0	0	0	0	0	0
	Cyanosis	0	0	0	0	0	0
9	Convulsions, tremors	0	0	0	0	0	0
	Increased sweating	0	0	0	0	0	0
	Cyanosis	0	0	0	0	0	0
10	Convulsions, tremors	0	0	0	0	0	0
	Increased sweating	0	0	0	0	0	0
	Cyanosis	0	0	0	0	0	0

None of the mice showed signs of convulsions, tremors, increased sweating, cyanosis

Table 3.5. The rate of mice showed digestive disorders

Group	Criteria	Research time					
		T_1	T_2	T_3	T_5	T_7	T_{14}
1	Reduce eating and drinking	0	0	0	0	0	0
	Diarrhea	0	0	0	0	0	0
	Normal	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
2	Reduce eating and drinking	0	0	0	0	0	0
	Diarrhea	0	0	0	0	0	0
	Normal	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
3	Reduce eating and drinking	0	0	0	0	0	0
	Diarrhea	0	0	0	0	0	0
	Normal	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
4	Reduce eating and drinking	0	0	0	0	0	0
	Diarrhea	0	0	0	0	0	0
	Normal	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
5	Reduce eating and drinking	0	0	0	0	0	0
	Diarrhea	0	0	0	0	0	0
	Normal	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
6	Reduce eating and drinking	0	0	0	0	0	0
	Diarrhea	0	0	0	0	0	0
	Normal	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
7	Reduce eating and drinking	0	0	0	0	0	0
	Diarrhea	0	0	0	0	0	0
	Normal	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
8	Reduce eating and drinking	0	0	0	0	0	0
	Diarrhea	0	0	0	0	0	0
	Normal	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
9	Reduce eating and drinking	0	0	0	0	0	0
	Diarrhea	0	0	0	0	0	0
	Normal	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
10	Reduce eating and drinking	0	0	0	0	0	0
	Diarrhea	0	0	0	0	0	0
	Normal	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

All groups of mice, after 24 hours of normal operation, did not show any symptoms of digestive abnormalities.

Table 3.6. Results of histological on livers, spleens and kidneys after 14 days

Group	Macroscopic and microscopic organs		
	Liver	Spleen	Kidney
1	Normal	Normal	Normal
2	Normal	Normal	Normal
3	Normal	Normal	Normal
4	Normal	Normal	Normal
5	Normal	Normal	Normal
6	Normal	Normal	Normal
7	Normal	Normal	Normal
8	Normal	Normal	Normal
9	Normal	Normal	Normal
10	Normal	Normal	Normal

The images illustrated the histological on the liver, spleen and kidney of a mouse.

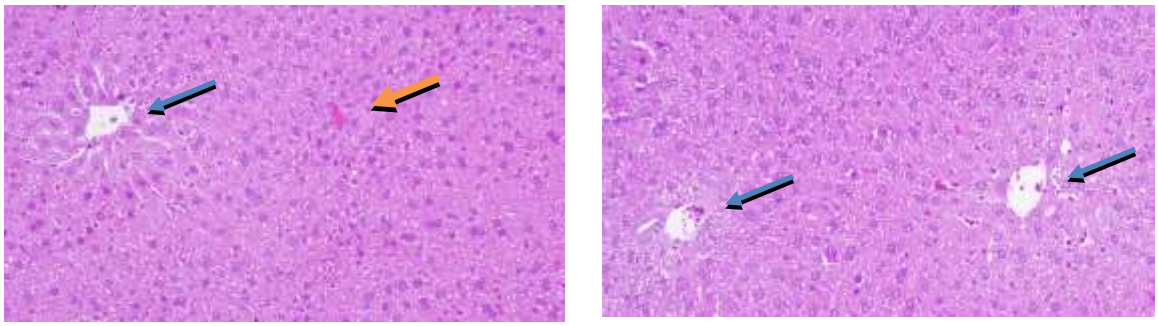

Fig. 2.2. Microstructure of mouse liver, HE 20X; Liver cells were not degraded; Sortinto strips, rafts; There was a sinus in the middle. Mildly congested vascular sinuses (yellow arrow). The lobular central vein was not congested (blue arrows).

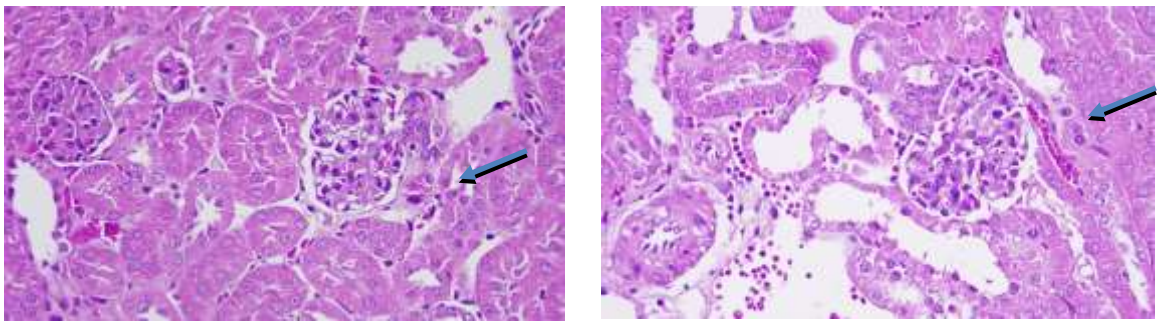

Fig. 2.3. Microstructure of mouse kidney, HE 20X. The renal cortex had glomeruli (blue arrows), the tubules and the blood vessels between the tubules. Renal tubular epithelial cells were not degraded. The blood vessels were mildly congested.

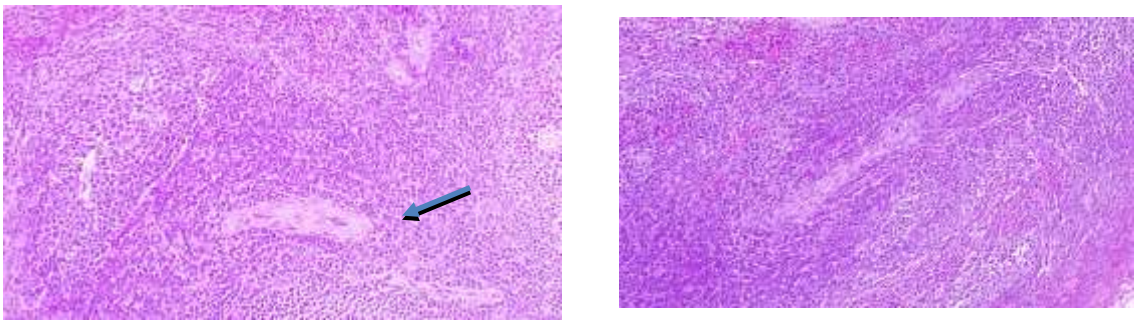

Fig. 2.4. Microstructure of mouse spleen; HE 20X. Splenic parenchyma with white and red pulp. The white pulp region had fairly uniform lymphoid follicles with a central quill artery (blue arrow). The red pulp region contains the Billroth cord and the vascular sinuses.

3. DISCUSSION

The acute toxicity test is the first 14-day toxicity evaluation based on a single dose. The test estimates a lethal dose or concentration (e.g., LD_{50} or LC_{50}) as intrinsic toxicity of the substance [7].

The acute toxicity research of the medication in experimental animals primarily determines the Median Lethal Dosage, which is the amount that kills 50% of the experimental animals under particular conditions (denoted LD_{50}). The LD_{50} value is an essential parameter for determining drug toxicity. LD_{50} dose that has a pharmacological effect on experimental animals is one of the bases for inferring the dose in humans. LD_{50} measures the possibility of short-term toxicity (acute toxicity) of drug toxicity, deciding the applicability in humans. The lower LD_{50} is a more dangerous chemical; the higher the LD_{50} is a less hazardous chemical [8].

- *Experimental animals*: A variety of animals can be employed to examine acute toxicity. Animals such as dogs, hamsters, cats, guinea pigs, rabbits, monkeys, and so forth... but most commonly rats and mice were used in the experiments [7, 8]. In the study, BBRN gel is a novel product, and we assessed acute toxicity in white mice using the technique recommended by the Ministry of Health, WHO, and the OECD [4-6].

- *Routes of drug entry*: Some method research routes of drug entry can be used to determine LD_{50}, such as skin or ocular mucosal contact, oral (ingestion), respiration (inhalation), muscular injections, injecting veins, or injecting into the peritoneal cavity. However, transdermal (topical) and oral techniques are the most often used. Chemical feeding is far simpler and less costly than other methods. Oral toxicity studies are often applied to studies of drugs, food poisoning and accidental poisoning in water [8].

- LD_{50} *of NBBR gel*: Even at the maximal dose (Group 1, oral volume 120ml/kg mouse weight/24h, corresponding to a dosage of preparations 123.96g/kg/24h), the experimental findings did not indicate LD_{50} of the preparation in white mice orally. This was also the thickest suspension that could be administered to mice by needle, as well as the most medication that mice could consume in 24 hours.

In all research groups, no mice died. No abnormal movement disorders, 100% of mice walked normally. No disorders such as convulsions, tremors, increased sweating, cyanosis; did not experience digestive disorders such as decreased appetite, diarrhea... All groups of mice, after 24 hours of normal operation. Macroscopic and microscopic observations of liver, kidney and spleen after 14 days did not reveal any pathological changes.

*Based on the results of this study, we can conclude that the oral LD_{50} for NBBR gel is more than **123.96g/kg**.*

Extrapolating the human dose with a conversion factor of 6 [9], the human dose is 743.76mg/kg. A person weighing 50kg can drink 37.188g NBBR gel without toxicity.

Extrapolating human dose by a factor of 12 [4, 10]: Using NBBR gel preparations from a dosage level of 30.99g/kg/24h (extraction to human dose 129.13g/50kg/24h) to the maximum oral dosage for mice were 123.96g/kg/24h, equivalent to 516.50g/50kg/24h in humans.

Our results are also similar to other publications. Berberine was less harmful in clinical dosages. Berberine sulfate's LD_{50} value in cats was around 25mg/kg, whereas doses of 45mg/kg are not poisonous in dogs. The administration of 10ml of Berberin-saturated solution intravenously to rabbits had toxic effects. Instilling 0.5% Berberin solution once every half hour into the rabbit's eyes minimized congestive corneal inflammation caused by 0.05% silver nitrate solution [11, 12].

Berberine's LD_{50} varies depending on the active component, dosage, and method of administration, such various values are reported previously. The toxicity of *Berberis vulgaris* (European origin, contains berberine) was mild [13]. The oral LD_{50} value for powdered root of *Berberis vulgaris* in mice was 2600mg/kg [14].

Gardner Z. reported that Berberine sulfate (isolated from *Berberis aristata*) had LD_{50} of 205mg/kg when administered injecting into peritoneal in rats, LD_{50} of berberine sulfate oral administration in rats and mice was greater than 1000mg/kg and 329mg/kg, respectively. Berberine sulfate was well tolerated at an oral dosage of 100mg/kg in rats [15].

Berberine's LD_{50} was determined in rats using three distinct injection routes: intravenous (IV), intraperitoneal (IP), and intragastric (IG) with doses of 10.4g; 20.8g; 41.6g; and 83.2g/kg. LD_{50} of IV injection and IP were 9.0386mg and 57.6103mg/kg, respectively; however, LD_{50} was not found in the IG oral group. Berberine has a safe oral dosage of 20.8g/kg in rats and 2.97g/kg in humans, which was 100 times larger than the amount normally administered in clinical trial research [16]).

Berberine was extracted from *Rhizoma cottidis* hah an oral LD_{50} 713.58mg/kg in white mice, that was categorized as slightly hazardous [17]. Regarding the mouse White mice drank the fibrous root extract of *Rhizoma cottidis* at > 7g/kg without experiencing any acute toxicity, making it had the least toxic [18].

Muhammad A. (2014) studied acute oral toxicity of the methanol extract from *Berberis vulgaris* in white mice and discovered that dosages of 100mg/kg did not cause any death and changes in health such as behavior, increased secretion,

sleep, coma... LD_{50} was 666.66mg/kg. According to Hodge and Sterner's toxicity scale (2005), the extract was slightly poisonous. Histopathology revealed no liver alterations [3].

Azmat A. (2014) found that an ethanolic extract of oral *Berberis vulgaris* did not produce toxicity up to 100mg/kg, in mice a dosage of 1000mg/kg/day for 14 days did not cause toxicity or mortality [19].

4. CONCLUSION

The LD_{50} of NBBR preparation in white mice had not been measured orally, with the greatest potential dosage for white mice being 123.96g/kg/24h.

REFERENCES

1. OECD guideline for testing chemicals, OECD/OCDE 404; Acute Dermal Irritation/ Corrosion, Adopted 24th April 2002.
2. OECD guideline for testing chemicals, OECD/OCDE 423; *Repeated Dose 28-day Oral Toxicity Study in Rodents*, Adopted 3rd October 2008
3. Muhammad Ahmed, Aisha Azmat, *Acute toxicity (lethal dose 50 calculation) and histopathological effects of methanolic extract of berberis Vulgaris in mice*; World J. of Pharmaceutical Research, Nov. 2014, 3 (8), 1439-1448
4. Ministry of Health (2015). *Guidelines for preclinical and clinical trials of traditional medicines and drugs from medicinal herbs*, Decision 141/BYT-QD.
5. Do Trung Dam (2006), *Method of determining acute toxicity of drugs*, Medical Publishing House.
6. WHO (2000). *Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines*, Geneva: World Health Organization.
7. Eaton DL, Steven G. Gilbert, *Principles of Toxicology. In: D. Kilassen C, editor. Casarett &Doull's Toxicology the basic science of poisons. 8th ed. New York: MC Grow Hi education;* 2013: p. 34-37
8. Hodge A. and Sterner B. (2005).*Toxicity classes*. In: Canadian center for occupational health and safety. Copy right @1997-2010. Retrieved from (http://www.ccohs.ca/oshanswers/chemicals/id50.htm) 0n 3/5/2010.
9. Shannon Reagan-Shaw, *Dose translation from animal to human studies revisited*, The FASEB Journal, 2008; vol. 22 no. 3 659-661.
10. National Institute of Medicinal Materials (2006), *Methods of studying pharmacological effects of herbal drugs*, Science and Technology Publishing House, Hanoi
11. Timothy C. Birdsall ND, Gregory ND (1997), *"berberine: Therapeutic potential of an Alkaloid found in several medicinal plant*", Alt Med Rev;2 (2): 94-103.
12. Amin AH, Subbaiah TV, Abbasi KM (1969), *"Berberine sulfate: antimicrobial activity, bioassay, and mode of action"*, Can J Microbiol; 15:1067-1076
13. Seyede Zohre Kamrani Rad, Maryam Rameshrad, and Hossein Hosseinzadeh, *Toxicology effects of Berberis vulgaris (barberry) and its active constituent, berberine: a review,* Iran J Basic Med Sci. , 2017. **20**(5): p. 516-529.
14. L. Peychev, *Pharmacological investigation on the cardiovascular effects of Berberis vulgaris on tested animals.* Pharmacia, 2005. **52**: p. 118-121
15. Gardner Z, Michael McGuffin, *American Herbal Products Association's Botanical Safety Handbook. 2nd ed. New York: CRC Press;* 2013. 130-132.
16. Kheir MM, Yugang Wang, Hua L, Hu J, Li L, Lei F, et al., *Acute toxicity of berberine and its correlation with the blood concentration in mice.* Food Chem Toxicol., 2010 April. 48(4): p. 1105-10.
17. Yi J, Xiaoli Ye, Wang D, He K, Yang Y, Liu X, et al., *Safety evaluation of main alkaloids from rhizoma coptidis.* J Ethnopharmacol., 2013 jan 9, 145 (1): 303-10.
18. Ning N, Yan Zhi Wang, Zou ZY, Zhang DZ, Wang DZ, Li XG, *Pharmacological and safety evaluation of fibrous root of rhizoma coptidis.* Environ Toxicol Pharmacol., 2015 Jan. 39 (1): p. 53-69.
19. Azmat A and Ahmed M. *Hypotensive activity, toxicology and histopathology of different extracts of Berberis vulgaris*. Journal of Medicinal Plant Research. 2014; 8(8):378-385.