International Journal of Research
Available at https://journals.pen2print.org/index.php/ijr/
e-ISSN: 2348-6848
p-ISSN: 2348-795X
Volume 06 Issue 03
March 2019

Investigation of Acute Toxicity of Methanolic Extract of Root of *Grewia nervosa* (Lour.) Panigrahi

Arnt Win[1], Aye Mon Thida Nyo[2], Swe Swe Mon[3]
[1]Associate professor, Department of Chemistry, Kyaukse University, the Republic of the Union of Myanmar
[2]Associate professor, Department of Chemistry, University of Mandalay, the Republic of the Union of Myanmar
[3]Assistant Lecturer, Department of Chemistry, Sagaing University of Education, the Republic of the Union of Myanmar

Abstract: *In this research work, the root of Grewia nervosa (Lour.) Panigrahi, one of Myanmar indigenous medicinal plants was selected for chemical analysis. The roots of G. nervosa (Lour.) Panigrahi (Myanmar name- Mya yar) were collected from Pyin Oo Lwin Township, Mandalay Region, Myanmar. The sample was cut into small pieces and dried in the air for about four weeks. Furthermore, the acute toxicity study on methanol extract of the root of G. nervosa (Lour.) Panigrahi was done by OECD guideline 425 (Organization for Economic Co-operation and Development) at Pharmacology Research Division, Department of Medical Research (Pyin Oo Lwin Branch), Mandalay Region, Upper Myanmar.*

Keywords : *Grewia nervosa* (Lour.) Panigrahi, indigenous medicinal plants, acute toxicity, OECD guideline 425.

1. Introduction

Medicinal plants are being used since centuries to treat different diseases [1]. Phytotherapy is gaining popularity as WHO encourages the appropriate ethnomedicinal use and signifies safety evaluation of herbal medicines [2-5]. FDA and WHO emphasize the validation of efficacious and safe use of herbal therapies through conduction of scientific based studies [2, 6]. *G. nervosa* (Lour.) Panigrahi, belonging to the family Malvaceae, is widely distributed in Myanmar. The leaves boiled along with turmeric and snail shell is being used for the treatment of jaundice, cold, heat stroke and dyspepsia [7]. Additionally, aqueous extract of bark of *G. nervosa* is used to treat Hepatitis B infection [8]. Leaves, bark, root and fruit of *G. nervosa* have been reportedly used to treat fever, dirrhoea as well as sprayed as insecticidal agent [9-11]. Leaves paste of the *G. nervosa* is also reported to cure digestion problems [12]. Since, ancient times the leaves of *G. nervosa* have been added in the Chinese herbal tea. Additionally, warm paste of fresh leaves of *G. nervosa* is also known to be applied on fractured body parts [13].

During the past few decades, traditional system of medicine has received marvelous attention for *in vivo* studies [14]. Toxicology is the important part of pharmacology which deals with the undesirable effect of phytocompounds on living organisms previous to the use as drug or chemical in clinical use [15]. Several studies are concentrated on toxicity analysis so as to determine the safeness of medicinal plants and their products. Toxicity analysis is essential, as some herbs consumed might have some toxic effects and many reports have been published for toxicity caused due to long term consumption of herbs. The occurrence of toxicity mechanism could differ depending on the cell membrane and chemical properties of the toxicants in human beings. It might happen within the cell membrane or on the cell surface or tissue underneath as well as at the extracellular matrix. According to OECD guidelines, in order to ascertain the protection and effectiveness of a new drug, toxicological studies are extremely significant in animals like mice, rat, guinea pig, dog, rabbit, monkey etc. Toxicological studies aid to extend decision whether a new drug must be adopted for clinical use or not. OECD guidelines such as 401, 423 and 425 do not permit the use of drug clinically without its clinical trial as well as toxicity studies [16-17].

Preliminary toxicological evaluation is necessary for authentication of safety of herbal medications. Although the roots of *G. nervosa* (Lour.) Panigrahi have valuable pharmacological effects, the comprehensive awareness about its toxicity potential has been lacking in Myanmar. In order to assess the toxic nature of a bioactive compounds present in the plant extract, acute oral toxicity is the first step to be carried out [18]. Therefore, the current study was conducted to assess the acute toxicity of methanolic extract of root of *Grewia nervosa* (Lour.) Panigrahi in animal model by following OECD guidelines 425 as the acute oral toxicity study is necessary to determine the safer dose range to manage the clinical signs and symptoms of the drugs.

1.1 Botanical Description

Family : Malvaceae
Genus : *Grewia*
Botanical name : *Grewia nervosa* (Lour.) Panigrahi
Myanmar name : Mya yar
Part used : Root

International Journal of Research
Available at https://journals.pen2print.org/index.php/ijr/
e-ISSN: 2348-6848
p-ISSN: 2348-795X
Volume 06 Issue 03
March 2019

Figure 1. Leaves and Root of *Grewia nervosa* (Lour.) Panigrahi.

2. Materials and Methods

2.1 Collection and Preparation of Sample

The roots of *G. nervosa* (Lour.) Panigrahi (Myanmar name Mya yar) were collected from Pyin Oo Lwin Township, Mandalay Region, Myanmar. It was cut into small pieces and dried in the air for about four weeks. It was stored in a well-stoppered bottle and used throughout the experiment.

2.2 Study on Acute Toxicity of Methanol Extract of Root of *Grewia nervosa* (Lour.) Panigrahi (OECD- 425, 2008)

2.2.1 Site of Study

Study on acute toxicity of methanol extract of root of *G. nervosa* (Lour.) Panigrahi was done at Pharmacology Research Division, Department of Medical Research (Pyin Oo Lwin Branch).

2.2.2 Preparation of Plant Extract for Acute Toxicity Test

Materials

- Air dried sample of root of *G. nervosa* (Lour.) Panigrahi
- Methanol
- Beakers, measuring cylinder
- Funnel, filter paper
- Rotary evaporator

Method

Air dried sample (500 g) of root of *G. nervosa (Lour.)* Panigrahi were percolated with methanol (1.5 L) and stored in a stoppered bottle. After four weeks, it was filtered with filter paper and the resulting filtrate was evaporated by using Rotary evaporator. Totally 2.2 g of methanol extract was obtained. The resulting dry methanol extract was used for acute toxicity study.

2.3.3 Acute Toxicity Test

Materials

- Albino ICR (Institute of Cancer Research) strain mice (female and body weight between 30±5 g)
- Mice cage
- Animal balance
- Drinking water bottles
- Surgical gloves and masks
- Disposable syringe
- Cannula
- Beakers
- Distilled water
- Dry methanol extract

Method

As the experimental model for an acute toxicity study, albino mice were used and this experiment was carried out on methanol extract of root of *G. nervosa (Lour.)* Panigrahi. The study was performed to assess the acute toxicity on oral administration. Study protocol is give below.

Name of the study	-	Acute oral toxicity study
Test material	-	Methanol extract of root of *G. nervosa* (Lour.) Panigrahi
Animal model	-	Albino mice
Animal strain	-	ICR (Institute of Cancer Research) strain
Animals produced from	-	Laboratory Animal Services Division, Department of Medical Research (Pyin Oo Lwin)
Sexes	-	Female
Weight of animals	-	Between 30 ± 5g
No. of test drug dose	-	4 doses
Vehicle for administration	-	Distilled water
Type of administrations	-	Single oral administration
Concentration of dose	-	175, 550, 1750 and 5000 mg/kg body weight
Acclimatization period	-	5 day
Observation period	-	14 days

According to OECD guideline 425 (Organization for Economic Co-operation and Development) (2008), healthy female albino (ICR) strain mice (30 ± 5 g) were randomly selected for an acute oral toxicity study and kept in their cages for 5 days before the experiment to allow for acclimatization of laboratory conditions. Firstly, the selected animal was weighed and then fasting about 4 hours before the test, but they were allowed with free access to water. Then, crude extract was dissolved in distilled water for required concentration and calculated dose was administered orally in a single dose using cannula. One mouse was used for each dose level. Since no estimate of the substance's lethality is available. Dosing was initiated at 175 mg/kg and up and down procedure was carried out. Test doses were selected from the sequence of 175, 550, 1750 and 5000 mg/kg. Firstly, starting dose of 175 mg/kg of sample solution was given to a test animal. Mice were observed after dosing at least

International Journal of Research
Available at https://journals.pen2print.org/index.php/ijr/
e-ISSN: 2348-6848
p-ISSN: 2348-795X
Volume 06 Issue 03
March 2019

once during the first 30 minutes, periodically during the first 24 hours with special attention given during the first 4 hours and daily up to 14 days.

Signs of toxicity and mortality of the mice were recorded. Observation included changes in fur, eyes, mucous membranes, respiratory rate, autonomic central nervous systems and behavioral pattern. The time of death if any was also recorded. In this study, there are no signs of toxicity and lethality at the dose level of 175 mg/kg body weight. So, the sample solution 550 mg/kg was administered orally to another mouse. The observation for toxic signs were done as described above. Since the signs of toxicity and lethality were not observed at the dose level of 550 mg/kg if the sample solution, the upper dose level of 5000 mg/kg of the sample solution was given orally to another mouse. Then, the symptoms of toxicity were observed again as indicated above. Body weights of mice were measured and recorded shortly before fasting and once weekly after dosing the sample solution. At the end of the test period, the mice were weighed again [19].

Figure 2. Weighing fasted body weight of each female albino mice.

Figure 3. Female albino ICR strain mice (test mice)

Figure 4. Administration of methanol extract solution to the test mouse

3. Results and Discussion

3.1 Determination of Acute Toxicity on Methanol Extract of Root of *Grewia nervosa* (Lour.) Panigrahi

The mice administered with 175, 550, 1750 and 5000 mg/kg doses of methanol extract of root of *G. nervosa* (Lour.) Panigrahi were kept under observation for two weeks. The observation parameters used in this experiment were cage side observations and mortality record. The results obtained from this experiment were shown in the following tables.

Table 1. Acute toxicity study on the methanol extract of root of *G. nervosa* (Lour.) Panigrahi based on cage side observations.

No.	Parameters	Observations
1	Condition of the fur	Normal
2	Skin	Normal
3	Subcutaneous swellings	Nil
4	Abdominal distension	Nil
5	Eyes-dullness	Nil
6	Eyes-opacities	Nil
7	Pupil diameter	Normal
8	Ptosis	Nil
9	Color and consistency of the faeces	Normal
10	Wetness or soiling of the perineum	Nil
11	Condition of teeth	Nil
12	Breathing abnormalities	Normal
13	Gait	Nil

Table 2. Acute toxicity study on the methanol extract of root of *G. nervosa* (Lour.) Panigrahi based on daily body weight record (in grams) and mortality record.

Test dose	Dosage of extract (mg/kg)	Marking	Sex	Body weight of mice (g)			Mortality up to 14 days
				Day 1	Day 7	Day 14	
1	175	Head	Female	25	26.4	27.1	Nil

2	550	Back	Female	26	26.6	28.1	Nil
3	1750	Tail	Female	34	27	31.1	Nil
4	5000	Right foot	Female	30	31	31.3	Nil
5	5000	Left foot	Female	24	21.6	25.0	Nil
6	5000	Head and tail	Female	25	23.1	27.4	Nil

At the end of observation period, it was found that all mice were alive and did not show any toxic symptoms such as diarrhea, inactivity, restlessness, aggressiveness, eye-dullness, breathing abnormal-ities, etc... According to these resulted data, the medium lethal dose, LD_{50} is more than 5000 mg/kg body weight and thus the methanol extract of this plant is practically non toxic and may be relatively harmless.

4. Conclusion

In this study, one Myanmar indigenous medicinal plant, *G. nervosa* (Lour.) Panigrahi, was selected for the investigation of acute toxicity. The acute oral toxicity on methanol extract of root of *G. nervosa* (Lour.) Panigrahi was determined by OECD guideline 425 (Organization for Economic Co-operation and Development). At the end of the experiment, there are no toxic signs and no death record at the dose of 5000 mg/kg methanol extract of the sample. Therefore, the LD_{50} value of the test sample was found to be more than 5000 mg/kg. Thus, the test sample showed free from acute toxic effect up to the dose of 5000 mg/kg and can be considered relatively safe.

5. Acknowledgements

We are deeply thankful to Dr Kyae Mon Lwin, Professor, Head of Department of Chemistry, Kyaukse University, Mandalay Region, Myanmar for her kind permission and for providing research facilities. We also want to acknowledge Dr Kyaw Zin Thant, Director General, Department of Medical Research, and Dr Win Aung, Deputy Director General, Department of Medical Research (Pyin Oo Lwin Branch), for their permission and facilities to do acute toxicity test. Thanks are also due to Dr Ei Ei Htwe, Research officer and all staff in Pharmacology Research Division, Department of Medical Research (Pyin Oo Lwin Branch), for their helpful evaluation and analyzing data on acute toxicity test containing in my research.

References

[1].Ridtitid W., Sae-Wong C., Reanmongkol W., Wongnawa M. Antinociceptive activity of the methanolic extract of kaempferia galanga linn. In experimental animals. J Ethnopharmacol. 2008; 118(2): 225–230.
[2].WHO . World Health Organization; 1993. Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines; p. 94.
[3].Daswani G.P., Brijesh S., Birdi J.T. reclinical testing of medicinal plants: advantages and approaches. Workshop Proceedings on Approaches Towards Evaluation of Medicinal Plants Prior to Clinical Trial Citeseer. 2006
[4].Ogbonnia S.O., Mbaka G.O., Anyika E.N., Osegbo O.M., Igbokwe N.H. Evaluation of acute toxicity in mice and subchronic toxicity of hydroethanolic extract of chromolaena odorata (l.) king and robinson (fam. Asteraceae) in rats. ABJNA. 2010;1(5):859–865.
[5].Vaghasiya Y.K., Shukla V.J., Chanda S.V. Acute oral toxicity study of pluchea arguta boiss extract in mice. J. Pharmacol. Toxicol. 2011;6(2):113–123.
[6].Setzer R.W., Kimmel C.A. Use of noael, benchmark dose, and other models for human risk assessment of hormonally active substances. Pure Appl. Chem. 2003;75(11-12):2151–2158.
[7]. Kalita D, Deb B. 2004. Some folk medicines used by the Sonowal Kacharis tribe of the Brahmaputra valley, Assam. Nat Prod Radiance. 3(4): 240-246.
[8].Biswas A, Bari MA, Roy M, Bhadra SK. 2010. Inherited folk pharmaceutical knowledge of tribal people in the Chittagong hill tracts. Bangladesh. Indian J Tradit Know. 9(1): 77-89.
[9]. Luo JP, Zhang LP, Yang SL, Roberts MF, Phillipson JD. 2009. Seperation and structure elucidation of alkaloids from Chinese drug buzhaye, Folium Microcos. Acta Pharm Sin B. 44(2): 150-153.

[10].Bandara KP, Kumar V, Jacobsson U, Molleyres LP. 2000. Insecticidal piperidine alkaloid from *Microcos paniculata* stem bark. Phytochemistry. 54(1): 29-32.
[11].Feng SX, Lin LD, Xu HH, Wei XY. 2008. Two new piperidine alkaloids from the leaves of *Microcos paniculata*. J Asian Nat Prod Res. 10(12): 1155-1158.
[12].Rahman MM, Islam AMT, Chowdhury MAU, Uddin MA, Jamil A. 2012. Antidiarrhoeal activity of leaves extract of *Microcos paniculata* in mice. Int J Pharm. 2: 21-25.
[13].Debnath B, Debnath A, Shilsharma A, Paul C. 2014. Ethnomedicinal knowledge of Mog and Reang communities of south district of Tripura, India. Indian J Adv Plant Res. 1(5): 49-54.
[14]. Mazid M, Khan TA, Mohammad F. Medicinal plants of rural India: a review of use by Indian folks. Indo Global Journal of Pharmaceutical Sciences. 2012; 2(3):286-304.
[15].Aneela S, De S, Kanthal LK, Choudhury NS, Das BL, Sagar KV. Acute oral toxicity studies of *Pongamia pinnata* and *Annonas quamosa* on albino wistar rats. InternationalJournalof Research in Pharmacy and Chemistry. 2011; 1(4):820-4.
[16].Ecobichon Ansari SH. *Essential of pharmacognosy*. 1st edition, New Delhi: Birla Publications Pvt. Ltd.,2007.
[17].Dharmalingam S, Natesan G, Evaluation of acute toxicity of the methanolic extract of Tanacetum parthenium L. in albino wistar rats. "Journal of Scientific and Innovative Research" 2017; 6(3): 113-115
[18].Akhila JS, Deepa S, Alwar MC. Acute toxicity studies and determination of median lethal dose, Current Science. 2007; 93:917-920.
[19].OECD . Vol. 425. OECD; 2008. Acute oral toxicity: Up and down procedure; pp. 1–2. (Guideline for the Testing of Chemicals).