

NEUROPHARMACOLOGICAL STUDY OF *PHYLLANTHUS NIRURI* IN SWISS ALBINO MICE

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ABSTRACT

Objective: The objective of this study is to evaluate the neurological, behavioral, and autonomic changes of *Phyllanthus niruri* in Swiss albino mice using Irwin's method.

Methods: A total of 24 mice was divided into four groups of six each (3-male, 3-female in each group). Aqueous extract of *P. niruri* was prepared. Based on body weight aqueous extract was given to the mice by orally through gavage tube (Group I – 300 mg/kg, Group II – 600 mg/kg, Group III – 900 mg/kg, and Group IV – 1200 mg/kg). Neuropharmacological profile is studied for each mice using Irwin's observational test, the mice were observed for 4 h after oral administration for various behavioral, neurological, and autonomic changes at 0, 1, 2, 3, 4 h.

Results: *P. niruri* showed negligible actions at 300 mg/kg and 600 mg/kg body weight. At 900 mg/kg and 1200 mg/kg *P. niruri* showed certain behavioral and neuronal changes. *P. niruri* increased alertness, stereotypy, restlessness, irritability/aggressiveness in behavioral profile indicating that the drug is a CNS stimulant. Furthermore, it showed mild tremors in neurological profile indicating CNS excitation.

Conclusion: Aqueous extract of *P. niruri* at 900 mg/kg and 1200 mg/kg showed changes in behavioral profile, neurological profile, showing it as CNS stimulant properties. Since it is an observational study further research should be done to explore CNS stimulant properties in various *in vivo* studies.

Keywords: *Phyllanthus niruri*, Ayurvedic, Neuropharmacological profile, CNS stimulant.

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INTRODUCTION

The utilization of herbal plants to treat various human diseases is common in developing countries due to their easy access and low cost, compared with advanced Western medicine [1]. These herbal formulations were routinely used by humans throughout history, without any scientific evidence, but now are being used in practical clinical trials to find their optimum and beneficial and toxic therapeutic dose ranges [1]. This led to the development of numerous alkaloid drugs such as digitoxin in heart failure patients and anticancer drugs such as vincristine and vinblastine for various cancer [2].

Phyllanthus belongs to largest genus in the family Phyllanthaceae, with 11 sub-genus that comprise over 700 well-known species and are cosmopolitan in distribution, mainly in the tropics and subtropics. Plants of the genus *Phyllanthus* were utilized as herbal formulations for hundreds of years in many Southeast Asian countries, Brazil, India, and China. It is used in the Indian ayurvedic systems from the ancient times (more than 2000 years) [3].

Among the *Phyllanthus* species, *Phyllanthus niruri* is a small annual herb growing of height 30–60 cm. It is identified in Amazon rainforest and other tropical areas, including South East Asia, Southern India, and China. *P. niruri* L. has been extensively reported in traditional and folk medication systems such as Ayurveda and Siddha to treat various diseases including diabetes mellitus, jaundice, asthma, joint pain, immunomodulator, loss of appetite, constipation, injuries, as antimicrobial, conjunctivitis, gonorrhoeal diseases of males and females, inflammatory diseases, skin itching, kidney stones or failures, and urogenital disease [4-16]. Curative properties of *P. niruri* are due to the presence of polyphenols, include classes of chromones, coumarins, lignans, stilbenes, xanthenes, and flavonoid [17-19].

Methanolic extract of *P. niruri* has been shown to mitigate the sensorimotor and cognitive deficit engendered by subacute chlorpyrifos

exposure in rats. Studies on *Phyllanthus* species have been done in recent decades, but while most of the individual studies have focused in the composition of the constituents in various plant parts and the biological activities of plant extracts, hardly any study exists showing neuropharmacological profile using Irwin's method [20]. Hence, this study aims to evaluate the behavioral, neurological, and autonomic changes of *P. niruri* in Swiss albino mice using Irwin's test [21].

Objective

Evaluate neuropharmacological profile of *P. niruri* in Swiss albino mice using Irwin's method.

MATERIALS AND METHODS

The study was initiated after getting approval from the Institutional Animal Ethical Committee (IAEC) of Coimbatore Medical College. Proposal number is CMCH/PH/007/2019 on 29.4.2019. 24 Swiss Albino mice of both sexes, weighing 25–30 g were bought from authorized breeder. The animals were kept in central animal house, Coimbatore medical college for 1 week to acclimatization as per CPCSEA guidelines. Animals were maintained in clean polypropylene cages and fed with standard pellet diet and water ad libitum during the study. The animals were maintained under controlled temperature with 12 h dark cycle and 12 h light cycle. Twenty-four mice were divided into four groups [1-4] of six animals in each group for the study. Each group contains three male and three female mice.

Preparation of plant extract

Leaves of *P. niruri* were collected from local gardens of Coimbatore. Leaves were identified and authenticated by Botanist from Tamilnadu agriculture university, Coimbatore. Leaves are washed and shade dried at room temperature (30°C) for 10 days. Leaves are powdered and stored. The aqueous extract was prepared by the Pharmacy department in Coimbatore Medical College. Based on body weight aqueous extract given to the mice oral by gavage tube (Table 1).

Neuropharmacological profile (Irwin test)

The Irwin test is an observation test, which is commonly used to evaluate the effects of a new substance on behavior and physiological function [22]. The main aim of Irwin test is to determine potential toxicity and to select doses for specific therapeutic activity. The Irwin test can also be used in safety pharmacology, as an approach for detecting untoward effects of a new compound on general behavior and for evaluating its acute neurotoxicity [21].

Changes in neuropharmacological profile that is divided into behavioral, neurological, and autonomic profile were observed after administration of test drug in increasing doses.

Neuropharmacological profile score

Effects of the test substance were scored on a scale of 0–8. Base score was 4 for the normal signs or effects. Score below 4 indicates subnormal response and score above 4 are supernormal. The basal score for abnormal sign was 0 and maximal score was 8.

METHODOLOGY

A total of 24 mice were divided into 4 groups of 6 each (n=6, 3-male, 3-female in each group). The mice were observed for 4 h after oral administration for various behavioral, neurological, and autonomic changes at 0, 1, 2, 3, 4 h according to Table 2. Each day two mice from different group was studied by Irwin’s method.

OBSERVATION AND RESULTS

In Group I mice - there were no significant changes in behavioral profile, neurological profile, autonomic profile at 0, 1, 2, 3, 4 h for all six mice (Fig. 1).

Table 1: Drug, dose, and route of administration for different groups

Groups	Drug and dose	Mice	Route
Group 1	<i>P. niruri</i> 300 mg/kg	6	Oral
Group 2	<i>P. niruri</i> 600 mg/kg	6	Oral
Group 3	<i>P. niruri</i> 900 mg/kg	6	Oral
Group 4	<i>P. niruri</i> 1200 mg/kg	6	Oral

P. niruri: *Phyllanthus niruri*

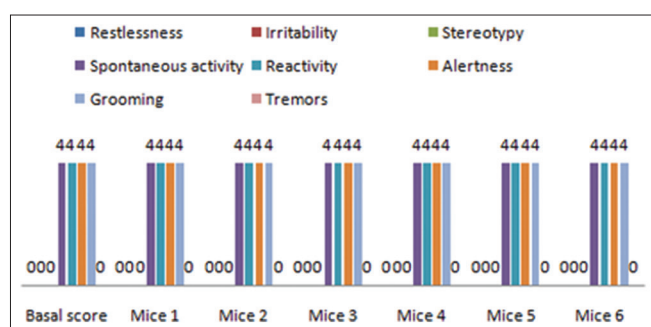


Fig. 1: Group I *Phyllanthus niruri* 300 mg/kg

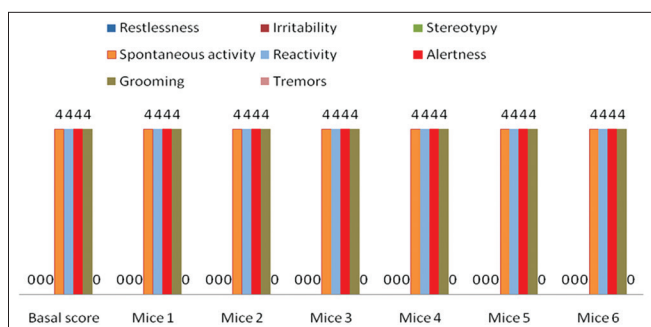


Fig. 2: Group II *Phyllanthus niruri* 600 mg/kg

In Group II mice - there were no significant changes in behavioral profile, neurological profile, autonomic profile at 0, 1, 2, 3, 4 h for all six mice (Fig. 2).

In Group III mice – there was an observational change in behavioral profile at end of 4th h after *P. niruri* administration at dose of 900 mg/kg (Fig. 3).

Score of restlessness was 1 for 5 mice and 2 for 1 mouse, Irritability score was 1 for four mice and 2 for two mice, Stereotypy score was 1 for 4 mice.

Score for spontaneous activity, reactivity, alertness, grooming were 5 for six mice.

In Group IV mice – there was an observational change in behavioral profile at end of 4th h after *P. niruri* administration at dose of 1200 mg/kg (Fig. 4).

Score of restlessness was 2 for six mice, Irritability score was 2 for six mice, Stereotypy score was 1 for six mice. Score for spontaneous activity, reactivity, alertness, grooming were 6 for six mice.

There was also a change in neurological profile; score of tremors was 1 in all six mice at end of 4th h.

DISCUSSION

P. niruri showed negligible actions at 300 mg/kg body weight and 600 mg/kg body weight. At 900 mg/kg and 1200 mg/kg *P. niruri* showed the following behavioral, neuronal changes.

P. niruri at dose of 900 mg/kg and 1200 mg/kg showed increased alertness, stereotypy, restlessness, irritability/aggressiveness in the behavioral profile. Its indicating that the drug has some CNS stimulant properties. At dose of 1200 mg/kg *P. niruri* showed mild tremors in neurological profile indicating it has some CNS excitation properties. No gross changes were observed in autonomic profile at given dose of *P. niruri*.

P. niruri has been widely studied for its hepatoprotective activity in vitro and in vivo models. A previous study demonstrated that phyllanthus prevented memory impairment and possessed anticholinergic and

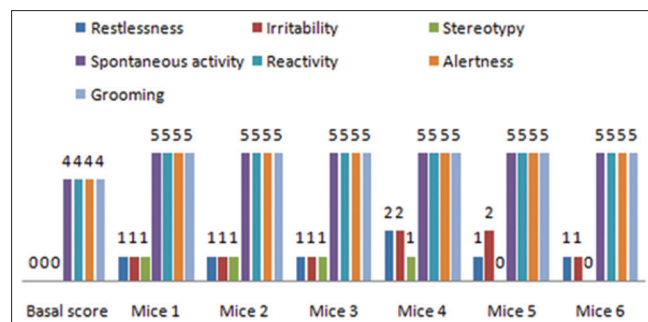


Fig. 3: Group III *Phyllanthus niruri* 900 mg/kg

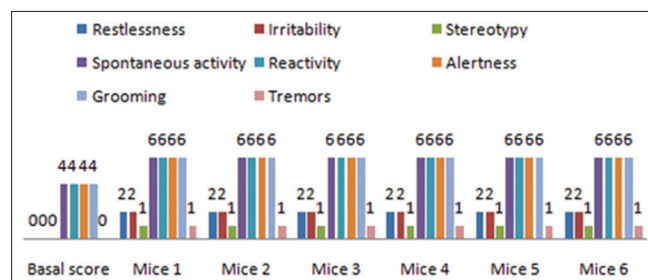


Fig. 4: Group IV *Phyllanthus niruri* 1200 mg/kg

Table 2: Neuropharmacological profile of *Phyllanthus niruri* (Irwin's method)

Time (h) →	BS ↓	Group I phyllanthus 300 mg/kg					Group II phyllanthus 600 mg/kg					Group III phyllanthus 900 mg/kg					Group IV phyllanthus 1200 mg/kg					
		0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	
Behavioral	Alertness	4																				
	Visual placing	4																				
Awareness	Passivity	0																				
	Stereotypy	0																				
Mood	Grooming	4																				
	Vocalization	0																				
	Restlessness	0																				
	Aggression	0																				
Motor activity	Fearfulness	0																				
	Spontaneous activity	4																				
	Reactivity	4																				
	Touch response	4																				
Neurological	Pain response	4																				
	Startle response	0																				
CNS	Straub tail	0																				
	Tremors	0																				
Excitation	Twitches	0																				
	Convulsions	0																				
Posture	Body posture	4																				
	Limb position	4																				
Motor in coordination	Staggering gait	0																				
	Abnormal gait	0																				
	Righting reflex	0																				
Muscle tone	Limb tone	4																				
	Grip strength	4																				
	Body sag	0																				
	Body tone	4																				
	Abnormal tone	4																				
Reflexes	Pinna	4																				
	Corneal	4																				
	Ipsilateral flexor reflex	4																				
Autonomic	Writhing	0																				
	Pupil size	4																				
	Palpebral opening	4																				
	Exophthalmoses	0																				
	Urination	0																				
	Salivation	0																				
	Piloerection	0																				
	Hypothermia	0																				
	Skin color	4																				
	Heart rate	4																				
Misc.	Respiratory rate	4																				
	Lacrimation	0																				
Dead	Number	0																				

BS: Basal score

anti-inflammatory properties [23]. These findings suggested that *Phyllanthus* is neuroactive and can alter the brain functions. It had been also found that *Phyllanthus* at doses of 100–500 mg/kg for 14 days revealed non-toxic effect with no abnormalities in normal behavior and physiology of rats [24]. However, to the best of our knowledge, no studies regarding neuropharmacological profile for acute toxicity study for *P. niruri* was done. Therefore, the present study sought to investigate neuropharmacological profile of *P. niruri* using Irwin's method.

During this study, no death occurred to mice after drug administration. Limitation of this study is it's an observational test, which may prone to bias.

CONCLUSION

From the results of experiment conducted, aqueous extract of *P. niruri* at dose of 300 mg/kg and 600 mg/kg has no changes in behavioral profile, neurological profile, and autonomic profile in Swiss albino mice. However, at dose of 900 mg/kg and 1200 mg/kg *P. niruri* showed changes in behavioral profile, neurological profile, showing it as CNS

stimulant properties. Since it is an observational study further research should be done to explore CNS stimulant properties in various *in vivo* studies.

CONFLICT OF INTEREST

No conflict of interest to disclose.

FUNDING

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REFERENCES

1. Singh T, Singh A, Kumar R, Singh JK. Acute toxicity study of *Phyllanthus niruri* and its effect on the cyto-architectural structure of nephrocytes in Swiss albino mice *Mus-musculus*. *Pharmacogn J* 2016;8:77-88.
2. Haque T, Muhsin MD, Akhter T, Haq ME, Begum R, Chowdhury SF. Antimicrobial and analgesic activity of leaf extracts of *Phyllanthus reticulatus* Poir. (Family- *Euphorbiaceae*). *Jahangirnagar Univ J Biol Sci* 2016;5:81-5.

3. Mao X, Wu LF, Guo HL, Chen WJ, Cui YP, Qi Q, et al. The genus *Phyllanthus*: An ethnopharmacological, phytochemical, and pharmacological review. *Evid Based Complement Altern Med* 2016;2016:7584952.
4. Oudhia P. Bhuiaonla (*Phyllanthus niruri*): A Useful Medicinal Weed. Available from: <https://www.hort.purdue.edu/newcrop/cropfactsheets/phyllanthus.html>.
5. Kumar A, Rana AK, Singh A, Singh A. Effect of methanolic extract of *Phyllanthus niruri* on leptin level in animal model of diabetes mellitus. *Biomed Pharmacol J* 2019;12:57-63.
6. Janghel V, Patel P, Chandel SS. Plants used for the treatment of icterus (jaundice) in Central India: A review. *Ann Hepatol* 2019;18:658-72.
7. Mukherjee M, Gupta S. Evaluation of acute toxicity and anti-asthmatic activity of *Phyllanthus niruri* L. leaves extracts. *Asian J Pharm Pharmacol* 2018;4:615-9.
8. Porto CR, Soares LA, Souza TP, Petrovick PR, Lyra IL, Araújo Júnior RF, et al. Anti-inflammatory and antinociceptive activities of *Phyllanthus niruri* spray-dried standardized extract. *Rev Bras Farmacogn* 2013;23:138-44.
9. Tjandrawinata R, Susanto L, Nofiarny D. The use of *Phyllanthus niruri* L. as an immunomodulator for the treatment of infectious diseases in clinical settings. *Asian Pac J Trop Dis* 2017;7:132-40.
10. Njoroge AD, Anyango B, Dossaji SF. Screening of *Phyllanthus* species for antimicrobial properties. *Chem Sci J* 2012;2012:CSJ-56.
11. Sheila Maregesi M, Kauke B, Kagashe G, Kaali R. Traditional eye medicines in Tanzania: Products, health risk awareness and safety evaluation. *Herb Med* 2016;2:1.
12. Mollik AH, Islam T, Khatun A, Hossan S, Nasrin D, Jahan R, et al. Medicinal plants used against syphilis and gonorrhoea by traditional medicinal practitioners of Bangladesh. *Planta Med* 2009;75:PH40.
13. Pasha F, Irfan S. A study on antimicrobial properties of *Phyllanthus niruri* and *Ocimum sanctum*. *Biomed Pharmacol J* 2015;4:297-9.
14. Boim MA, Heilberg IP, Schor N. *Phyllanthus niruri* as a promising alternative treatment for nephrolithiasis. *Int Braz J Urol* 2010;36:657-64.
15. Kaur B, Kaur N, Gautam V. Evaluation of anti-helicobacter pylori (DSMZ 10242) activity and qualitative analysis of quercetin by HPLC in *Phyllanthus niruri* linn. *World J Pharm Pharm Sci* 2016;6:1691-706.
16. Patel JR, Tripathi P, Sharma V, Chauhan NS, Dixit VK. *Phyllanthus amarus*: Ethnomedicinal uses, phytochemistry and pharmacology: A review. *J Ethnopharmacol* 2011;138:286-313.
17. Kamruzzaman HM, Hoq MO. A review on ethnomedicinal, phytochemical and pharmacological properties of *Phyllanthus niruri*. *J Med Plants Stud* 2016;4:173-80.
18. Bagalkotkar G, Sagineedu S, Saad M, Stanlas J. Phytochemicals from *Phyllanthus niruri* Linn. and their pharmacological properties: A review. *J Pharm Pharmacol* 2007;58:1559-70.
19. Nijveldt RJ, van Nood E, van Hoorn DE, Boelens PG, van Norren K, van Leeuwen PA. Flavonoids: A review of probable mechanisms of action and potential applications. *Am J Clin Nutr* 2001;74:418-25.
20. Mathiasen JR, Moser VC. The Irwin test and Functional Observational Battery (FOB) for assessing the effects of compounds on behavior, physiology, and safety pharmacology in rodents. *Curr Protoc Pharmacol* 2018;83:e43.
21. Roux S, Sablé E, Porsolt RD. Primary observation (Irwin) test in rodents for assessing acute toxicity of a test agent and its effects on behavior and physiological function. *Curr Protoc Pharmacol* 2005;Chapter 10:Unit 10.10.
22. Irwin S. Comprehensive observational assessment: Ia. A systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. *Psychopharmacologia* 1968;13:222-57.
23. Joshi H, Parle M. Evaluation of the anti-amnesic effects of *Phyllanthus amarus* in mice. *Rev Colomb Méd* 2007;38:132-9.
24. Ilangkovan M, Jantan I, Mesaik MA, Bukhari SNA. Immunosuppressive effects of the standardized extract of *Phyllanthus amarus* on cellular immune responses in Wistar-Kyoto rats. *Drug Des Devel Ther* 2015;9:4917-30.