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*Original Article*

# Acute toxicity study of *Phyllanthus niruri* and its effect on the cyto-architectural structure of nephrocytes in Swiss albino mice *Mus-musculus*

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## ABSTRACT

**Background:** In the era of herbal renaissance, world is moving towards the medicinal plant that repairs and strengthening the body system without any toxic side effects. Popular medicinal plant *Phyllanthus niruri* contains various bioactive molecules, the present study aimed to observe the biochemical and cyto-architectural alterations in kidney associated with acute oral toxicity ($LD_{50}$) of aqueous extract of *P.niruri* in Swiss albino mice. However, limited data is available about the toxicity of herbal remedies used for medication, which is a critical constrain. **Materials and Methods:** For the acute oral toxicity study, the animals were divided into six groups of 6 mice each. Group–I was named control group and the treatment groups were administered aqueous leaf extract of *P.niruri* orally at different doses of 500 mg/Kg bw (Group-II), 1000 mg/Kg bw (Group-III), 2000 mg/Kg bw (Group-IV), 2500 mg/Kg bw (Group-V) and 3000 mg/Kg bw (Group-VI) for 7 consecutive days. The mice were sacrificed on and serum was collected for the biochemical analysis. The kidney was dissected and processed for histological analysis. **Results:** The $LD_{50}$ dose of *P. niruri* was found to be 2590.984 mg/Kg bw in Swiss albino mice model in laboratory condition. The result showed the elevated serum level of urea in treated group of mice at higher doses which was found to be statistically significant as compared to the control (Group–I). There were no any significant increase in serum creatinine has been observed. Histological alteration were observed at higher dose more than 2500 mg/Kg bw (Group-VI). **Conclusion:** It is evident from our study that *P. niruri* may have toxic effect at high doses. Therefore, it should be ingested with precautions.

**Key words:** *P. niruri*, $LD_{50}$, Kidney, Biochemical, Histological analysis.

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## INTRODUCTION

In the era of herbal renaissance, the demand for medicinal plant is increasing globally in both developed and developing countries. The world is now moving towards the medicinal plant that repairs and strengthening the body system without any toxic side effects.[1] Recently, scientific interest in medicinal plants has burgeoned due to the increased efficiency of plant derived drugs and raising concern about the side effects of modern medicine and resistant to the drugs. Impressive number of modern drugs has been isolated from natural sources, is being used as traditional medicine. As a result, plant based traditional medicine continues to play an essential role in health care, with 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care.[2] According to World health organization, about three quarters of the world's population currently use traditional medicine. Traditional medicine is an important part of Indian culture and herbs are now very popular in developing countries on account of their improved knowledge about their safety, efficacy, and quality assurance of ethno medicine. In recent years, secondary plant metabolites (phytochemicals) have been extensively investigated as a source of medicinal agents.[3] Therefore, in order to have standard natural plant products, preliminary studies have to be conducted in order to evaluate possible risks such as, undesirable effects, overdose or poisoning.[4]

In the *Phyllanthaceae* family one of the common herb that is widely used is *P.niruri* and proven therapeutic effects.[5] This species is indigenous to S. America, India, and China, widely distributed in many tropical countries where they are considered as weeds. The whole plant is used as a remedy for many conditions such as tumours, diabetes, diuretics, jaundice, kidney stone and dyspepsia etc. The plant is also useful for treating hepatotoxicity, hepatitis B and hyperglycaemia, viral and bacterial diseases.[6,7] Curative properties of the medicinal plant are due to the presence of bioactive phytochemicals, among which polyphenols are the most numerous and widely distributed class of phytochemicals. Polyphenols include classes of chromones, coumarins, lignans, stilbenes, xanthones and flavonoids.[8] Flavonoids had been found to possess relatively potent antioxidant, anti-atherosclerotic, anti-inflammatory, anti-mutagenic, anti-tumor and anti-viral activities.[9]

Despite wide spread use of *P.niruri* for treating various diseases, mainly it is known as a potent stone breaker. Therefore, this study aimed to observe the biochemical, haematological and histological alterations in kidney associated with acute oral toxicity ($LD_{50}$) of aqueous leaf extract of *P. niruri* in Swiss albino mice. However, there is limited data available regarding its toxicity.

## MATERIALS AND METHODS

### *Experimental Animal*

Healthy Swiss albino mice, weighing between 30-35 g were obtained from a random-bred colony at the Mahavir Cancer Sansthan, Patna, Bihar, India. They were maintained under the optimal condition at the temperature-(24 ±1°C), humidity-(55 ± 5%), and lighting- (12-h light/dark cycle). Food and distilled water were given *ad libitum* throughout the study. Study were carried out as per CPCSEA guidelines (Approval No.-1129/bc/07/CPCSEA).

### Collection and Identification of Plant Materials

Leaf of *P.niruri* was collected from the campus of B.M.D College, Vaishali, taxonomically identified by Dr. S. Bedi, (Associate Professor, Department of Botany, PWC, Patna University, Patna). It is kept in the herbarium of the laboratory under the voucher specimen number: B.M.D/BOT/08/10. The leaves were shade dried at room temperature (25°C) for 10 days, powdered and stored.

### Acute Toxicity Study

After acclimatization, the animals were divided into 6 groups of 6 mice each. The control (group-I) received food and distilled water *ad libitum*, while the experimental groups in addition received aqueous leaf extract of *P.niruri* orally at different doses of 500 mg/ Kg bw(Group-II), 1000 mg/Kg bw (Group-III), 2000 mg/Kg bw (Group-IV), 2500 mg/Kg bw (Group-V) and 3000 mg/Kg bw (Group-VI). The extract was prepared by dissolving 500 mg-3000 mg of dried powder of *P.niruri* leaves in 10 ml of distilled water. The volume of aqueous extract to be administered was determined based on body weight and given to the mice for 7 consecutive days. The toxicological effects were observed in terms of mortality expressed as $LD_{50}$. Based on the experimental observations, the acute oral $LD_{50}$ of the extract was calculated by the use of software for probit analysis (Environmental Protection Agency PROBIT ANALYSIS PROGRAM, used for calculating LC/EC value, Version 1.5).

### Biochemical Parameters Analysis

The serum was obtained by centrifugation (3000 rpm for 15 minutes). Urea and creatinine levels was determined by the use of standard kit method using fully Automated Biochemistry Analyzer (Model No-SELECTRA-"E",VITALAB BY MERCK) in the Biochemistry Department of Mahavir Cancer Sansthan and Research Centre, Patna.

### Histological Examination

Mice were sacrificed by cervical dislocation and kidneys were extracted out, washed thoroughly in normal saline, fixed in 10% formal saline, trimmed, processed, embedded in paraffin wax, sectioned at a thickness of 4-5 µm, stained by double staining method (H&E) and observed under light microscope for histological changes.

### Statistical Analysis

Data was analyzed and experimental values ware expressed as the mean ± SEM and *P* value was calculated using one way analysis of variance (ANOVA) by using SPSS software. $P \leq 0.05$ was considered statistically significant.

## RESULTS

Swiss albino mice treated orally with different doses (500 mg/Kg bw, 1000 mg/Kg bw, 2000 mg/Kg bw, 2500 mg/Kg bw, 3000 mg/Kg bw of aqueous leaf extract of *P. niruri*. The median acute toxicity ($LD_{50}$) of the compound was determined to be 2590.984 mg/Kg bw as per the observations using software of probit analysis (EPA PROBIT ANALYSIS PROGRAM, used for calculating LC/EC value, Version 1.5) as shown in Table 1. Results showed decrease in body weight in treated groups which was statistically significant ($P \leq 0.05$) in Group-V and statistically very significant ($P \leq 0.01$) in Group-VI as compared to Group-I (Table 2). Relative kidney weight (K:BW) was also decreased which was statistically very significant ($P \leq 0.01$) at higher doses (Group-V and Group-VI).

The biochemical parameters which reflect the functioning of kidney are level of urea and creatinine (Figure 1a, 1b). In the present investigation, it was recorded that the serum urea level in treated Group-II and Group-III increased but it was not statistically significant ($P \geq 0.05$). However, treatment at higher doses of aqueous leaf extract of *P.niruri* revealed statistically significant ($P \leq 0.05$) increase in Group-V and Group-VI as compared to control (Group-I). There were no any marked alteration has been recorded in any groups in serum creatinine level in comparison to control group.

Light microscopic study of the cross section of kidney tissues of control Group-I revealed normal architecture of renal cells. In Group-II, Group-III and Group-IV (Figure 2b, 2c, 2d), renal cells are relatively normal, mild edema and mild congestion were observed. In Group-V (Figure 2e) congestion in renal cells were observed whereas, in Group-VI (Figure 2f) tubular edema, congestion, desquamation and degeneration were also observed.

**Table 1: Estimation of $LD_{50}$**

| Concentration of aqueous extract | Number exposed | Number responding | Observed proportion responding | Proportion responding adjusted for controls | Predicted proportion responding |
|---|---|---|---|---|---|
| **500.000** | 6 | 0 | 0.0000 | 0.0000 | 0.0000 |
| **1000.000** | 6 | 0 | 0.0000 | 0.0000 | 0.0005 |
| **2000.000** | 6 | 1 | 0.1667 | 0.1667 | 0.1841 |
| **2500.000** | 6 | 3 | 0.5000 | 0.5000 | 0.4506 |
| **3000.000** | 6 | 4 | 0.6667 | 0.6667 | 0.6947 |

Estimated LC/EC 50.0 of aqueous leaf extract of *P. niruri* was 2590.984.

**Table 2: Effect of aqueous leaf extract of *P.niruri* for seven days exposure period**

| Weight (gram) | Group-I (Control) | Group-II (500 mg/ Kg) | Group-III (1000 mg/ Kg) | Group-IV (2000 mg/ Kg) | Group-V (2500 mg/ Kg) | Group-VI (3000 mg/ Kg) |
|---|---|---|---|---|---|---|
| **Body wt. before dose** | 30.12±0.22 | 30.05±0.28 | 30.10±0.22 | 30.13±0.17 | 30.12±0.19 | 30.09±0.22 |
| **Body wt. after dose** | 30.65±0.12 | 30.14±0.23 | 30.17±0.18 | 27.43±0.33* | 26.33±0.39* | 24.75±0.42** |
| **K: BW (%)** | 1.2±0.22 | 0.92±0.33 | 0.96±0.28 | 0.85±0.30 | 0.69±0.33** | 0.66±0.314** |

Route of administration: oral, values are mean± SEM, *Significant ($P< 0.05$), **Highly Significant ($P< 0.01$) compared to control, n=6.

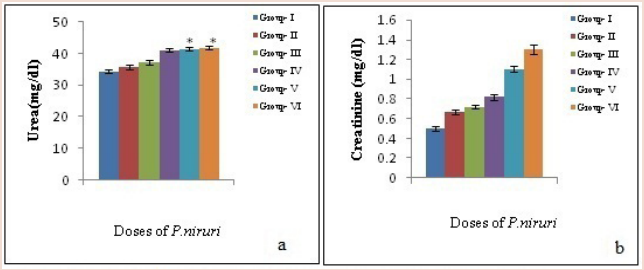


**Figure 1: (a)** Levels of urea (mg/dl) in different experiment groups Group-I-Normal, Group-II - 500 mg/Kg w Group-III - 100 mg/Kg bw, Group-IV-2000mg/kg bw, Group-V-2500 mg/Kg bw and Group-VI-3000 mg/Kg bw of aqueous leaf extract of *P. niruri* treated mice. *$P = 0.05$, compared to control value (Group-I). **(b)** Levels of creatinine (mg/dl) in different experimental groups Group-I-Normal, Group-II-500 mg/Kg bw Group-III-1000 mg/Kg bw, Group-IV-2000mg/Kg bw, Group-V-2500 mg/Kg bw and Group-VI-3000 mg/Kg bw of aqueous leaf extract of *P. niruri* treated mice. *$P = 0.05$, compared to control value (Group-I).

## DISCUSSION

There is growing concern about the toxicity profile of herbal plant during last decade due to the bioactive ingredients. Acute tubular ne-

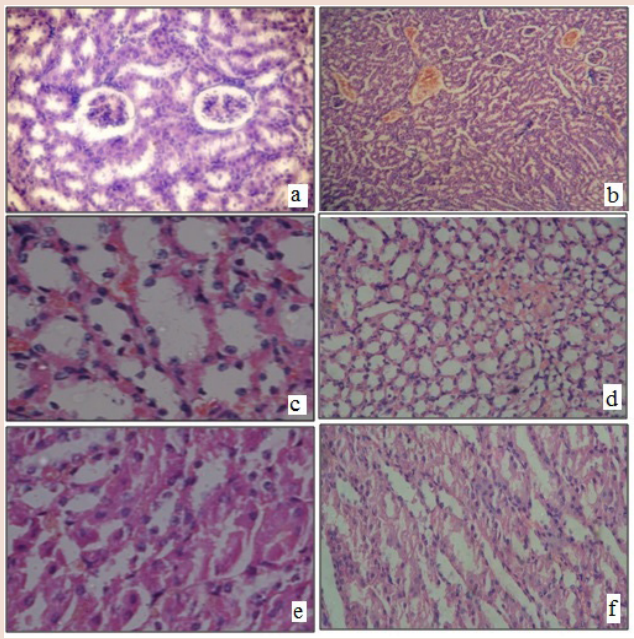


**Figure 2: a:** Section of control mice kidney showing normal glomeruls, proximal tubules (PT) and distal tubules (DT). **b:** Section of kidney of 500mg/Kg bw of *P. niruri* treated mice (Group-II) showing mild edema in distal tubues (DT). **c:** Section of kidney of 1000 mg/Kg bw of *P. niruri* treated mice (Group-III) showing mild edema in tubules. **d:** Section of kidney of 2000mg/Kg bw of *P. niruri* treated mice (Group-IV) showing mild edema and congestion in tubules. **e:** Section of kidney of 2500 mg/Kg bw of *P. niruri* treated mice (Group-IV) showing edema in tubules. **f:** Section of kidney of 3000 mg/Kg bw of *P. niruri* treated mice (Group-V) showing tubular degeneration, edema and congestion in tubules.

crosis, Acute renal failure, nephropathy, tubular necrosis, acute interstitial nephritis are serious complications resulting from the use of herbs like *Pithecellobium labatum, Aristolochia fangchi, Uncaria tomentosa*.[10-14] Therefore, the present investigation has been conducted to understand the biochemical and histopathological alterations associated with acute oral toxicity of aqueous leaf extract of *P. niruri*.

Acute toxicity ($LD_{50}$) test gives a clue on the range of doses that could be used in subsequent toxicity for testing and estimating the therapeutic index of drugs and xenobiotics.[10] In the study, the meadian lethal dose ($LD_{50}$) of the aqueous extract of *P. niruri* was found to be 2590.984 mg/Kg bw. In female Sprague-Dawley (S-D) rats, it was more than 5000 mg/Kg bw.[15] These variations in the acute toxicity study was due to difference in route of entry, duration and frequency of exposure, age, sex, variations between different species (interspecies) and variations among members of the same species (intraspecies).[16] Body weight and organ weight are important factors to monitor the health of an individual and to analyze the toxic impact of herbal plant extracts.[17] Loss in body weight is frequently the first indicator of the onset of an adverse effect. A dose, which causes 10% or more reduction in the body weight, is considered to be a toxic dose.[7] Organ weights are widely accepted for the evaluation of test article-associated toxicities.[18,19] Hence the decrease in body and organ weight in the present investigation at high dose of aqueous extract of *P.niruri* indicates its toxic potential. In the present study the decrease in body weight at higher doses in treated Group-V and Group-VI. The organ weight in relation to body weight revealed marked alteration and that was statistically significant. Various studies have been conducted to understand the toxic effect on the kidney.

Serum urea and creatinine acts as an indicators for kidney function tests as studied by Williams (1999).[20] Urea is the main end product of protein catabolism. Serum urea level varies directly with protein intake and inversely with the rate of excretion. Creatinine is the waste product formed in muscle by creatinine metabolism and it is synthesized in liver, passes into circulation and taken up almost entirely by skeletal muscles.[21] Increase in creatinine level may indicate the renal damage. In the present study, increased serum urea has been recorded in Group-V and Group--VI. The increased serum level may indicate renal function impairment due to adverse effect of higher doses of aquous leaf extract of *P.niruri* on the kidney.[22] Rise in serum creatinine depends upon the extent of tubular necrosis.[23] However, in the present study no any marked alteration in serum level of creatinine has been recorded. The histological alterations in tubular structure and glomeruli function lead the various architectural alteration in micro-section of the nephrocytes of aqueous leaf extract of *P.niruri* were observed, indicating toxicity and adverse.[24-27] The toxic effect of aqueous leaf extract of *P. niruri* on kidney may be due to anyone or more of the phytochemicals present in the extract.[17]

## CONCLUSION

These results provide evidence of toxicity profile of the aqueous leaf extracts of *P.niruri* at high doses. Hence, it should be ingested with caution. The acute toxicity study of $LD_{50}$ determination was due to active ingredients responsible for toxic effects. Therefore, observed biochemical and histological finding supports that it is be used or administered as nephroprotective agent at lower doses or definitely used with cautions.

## ACKNOWLEDGEMENTS

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## ABBREVIATION USED

**$LD_{50}$:** Lethal dose, **H & E:** Hematoxylin & Eosin, **PT:** Proximal tubules; **DT:** Distal tubules.

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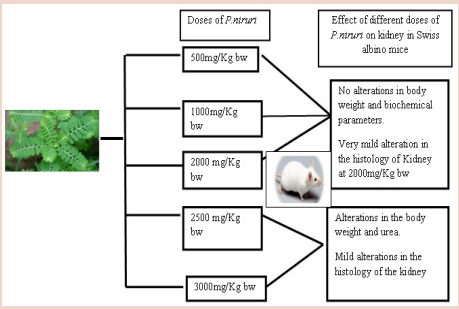
## PICTORIAL ABSTRACT




## SUMMARY


- $LD_{50}$ of the aqueous leaf extract of *P. niruri* was found to be 2590.984 mg/Kg bw in Swiss albino mice.
- No significant changes in biochemical and hostological indices was observed in lower dose that is 500, 1000, 2000 mg/Kg bw.
- Histological alterations were observed at higher doses 2500mg/Kg bw and 3000 mg/Kg bw.
- Biochemical and histological finding supports that it is be used or administered as nephroprotective agent at lower doses or definitely used with cautions.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