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Research Article

Evaluation of Biochemical and Histological Effects on Liver of Swiss Albino Mice Due to Acute Oral Toxicity of Aqueous Leaf Extract of *Phyllanthus niruri*

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ABSTRACT

In modern era, plant extracts are being used extensively as a source of medicinal agents. However, there is limited data available about the toxicity of herbal products used in remedies, are essential for the study of toxicity profile of medicinal plants. Therefore, aim of this study was to observe the biochemical and histopathological changes in liver due to acute oral toxicity of aqueous leaf extract of *P.niruri*(L.) Mice were divided into six experimental groups of 6 mice each comprises Group-I as (control) and 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), 2000mg/Kg bw (Group-IV), 2500 mg/Kg bw (Group-V) and 3000 mg/Kg bw (Group-VI) for 7 consecutive days. The experimental mice were sacrificed and serum was collected for the analysis of serum ALP (alkaline phosphatase), SGPT (serum glutamic pyruvic transferase), total protein, albumin and total bilirubin. The liver was dissected, weighed and processed for histological analysis. Serum level of SGPT, total protein and albumin showed significant ($P \leq 0.05$) increase in the treated Group-V and Group-VI as compared to control. Serum ALP levels showed significant decrease ($P \leq 0.05$) in Group-V and Group-VI. Alterations in hepatocytes were observed only at higher doses of Group-V and Group-VI. The median acute toxicity (LD_{50}) of the compound was determined to be 2590.984 mg/Kg bw.

Key words: Aqueous leaf extract, Biochemical, Histological, *Phyllanthus niruri*, Swiss albino mice.

INTRODUCTION

In recent years plant metabolites have been extensively investigated as a source of medicinal agents[1]. However, there are paucity of information about the pharmacology and toxicology for the most common herbal medicinal plant extracts used in remedies. Therefore, there is need for the study of toxic profile of medicinal plants[2].

In the modern era, the demand of medicinal plant is increasing globally. Herbal drugs have gained popularity in recent years because of their safety, efficacy and cost effectiveness[3,4]. There has been growing interest in the phytochemical analysis of plant products because of its potential health benefits. In recent years, secondary plant metabolites (phytochemicals) have been extensively investigated as a source of medicinal agents[5].

Phyllanthus niruri (*Euphorbiaceae*) commonly known as stone breaker, used for therapeutic purpose in traditional system of medicine. The whole plant is used as a remedy for many conditions such as jaundice, dysentery, influenza, tumours, diabetes, diuretics, kidney stone etc. The plant is also useful for treating hepatotoxicity, hepatitis B, hyperglycaemia, viral and bacterial diseases.[6,7]. These medicinal plants have curative properties due to the presence of bioactive phytochemicals. Polyphenols are the most numerous and widely distributed class of phytochemicals which include flavanoids, chromones, coumarins, lignans, stilbenes, and xanthones and flavonoids[8]. Flavonoids have relatively potent anti-oxidant, anti-atherosclerotic, anti-inflammatory, anti-mutagenic, anti-tumor and anti-viral activities[9].

P.niruri has been excessively used for treating various diseases. But, limited data is available about its toxicity at different doses. Preliminary studies are required to evaluate its possible risks such as adverse effects, overdose or poisoning[10]. Therefore, the study aimed to evaluate the biochemical and histological alterations in liver due to acute oral toxicity of aqueous leaf extract of *P.niruri* in Swiss albino mice.

MATERIALS AND METHODS

Plant material

P.niruri plant was collected from the campus of B. M. D College, Vaishali, Bihar, identified taxonomically by Dr. S. Bedi, (Professor, Department of Botany, PWC, Patna University, Patna, Bihar). It was kept in the herbarium having voucher specimen number: B.M.D/BOT/16/10.

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Table 1: Environmental Protection Agency PROBIT analysis program used for calculating LC/EC values (Version 1.5) for P.niruri

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: Changes in body weight and organ weight of control and P.niruri treated mice.

Weight Parameters	Group-I (Normal mice)	Group-II (500mg/kg bw)	Group-III (1000mg/Kg bw)	Group-IV (2000 mg/Kg bw)	Group-V (2500 g/Kg b. wt)	Group-VI (3000 mg/Kg bw)
Weight change	3.33±1.21	3.54±1.14	3.47±0.45	3.45±1.81*	2.92±1.12*	2.25±1.423**
Liver to body weight ratio	0.052±0.32	0.054±0.123	0.051±0.13	0.051±0.187	0.049±0.15	0.042±0.098

Route of administration: Oral, Values are mean ± SEM, *P≤0.05(Significant), **P≤0.01(very significant) compared to normal, n = 6.

The leaves of plant dried in shade at room temperature (25 ±1ºC) for 10 days, grinded, powdered and stored.

Experimental Animal

Swiss albino mice reared in the animal house of Mahavir Cancer Sansthan, Patna and weighing 30-35g (age 6-8 weeks) were randomly divided into six groups. Mice were kept in polypropylene cages lined with husk in a well ventilated room at temperature- 25 ±1°C, humidity- 55 ± 6%, and lighting- 12-h light/dark cycle. Food (Amrut Laboratory Animal Feed, Mysore Feed Limited, Bangalore, India) and tap water were given *ad libitum* throughout the study. All animal experiments were carried out as per CPCSEA guidelines (Approval No.-1129/bc/07/CPCSEA)**.**

Acute Toxicity Study and Dose Selection

The control (Group-I) received food and distilled water *ad libitum*, while the experimental groups received the aqueous leaf extract of *P.niruri* orally at different doses in addition to food and tap water *ad libitum*: Group-II (500 mg/Kg bw), Group-III (1000 mg/Kg bw), Group-IV (2000 mg/Kg bw), Group-V (2500 mg/Kg bw), and Group-VI (3000 mg/Kg bw). 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.

Biochemical Study

The serum was obtained from the blood (ocular vein puncture) by centrifugation (3000 rpm for 15 minutes). SGPT, ALP, total protein, albumin, and total bilirubin were determined by the use of standard kit method using fully Automated Biochemistry Analyzer (Model No-SELECTRA-"E",VITALAB BY MERCK) in the department of Biochemistry, Mahavir Cancer Sansthan and Research Centre, Patna, Bihar.

Histopathological Examination

Mice were first anaesthesized and sacrificed. The liver was dissected out, washed thoroughly in normal saline, preserved and 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 at 400X 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*≤ 0.05 was considered statistically significant.

RESULTS

Swiss albino mice administered aqueous leaf extract of *P.niruri* orally at different doses (500 mg/Kg bw, 1000 mg/Kg bw, 2000 mg/Kg bw, 2500 mg/Kg bw, 3000 mg/Kg bw). 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.

There was no mortality recorded at the doses of 500mg/Kg bw 1000mg/Kg bw, 2000mg/Kg bw. But, the mice treated orally with aqueous leaf extract of *P.niruri* at the dose of 2500mg/Kg bw (Group-V) and 3000mg/Kg bw (Group-VI) showed marked alterations in body weight as well as ratio of organ(liver) and body weight (L:BW)as compared to control (Group-I) [Table-2].

There were not any marked changes observed in biochemical and histological examination at the doses of 500mg/Kg bw 1000mg/Kg bw, 2000mg/Kg bw. But, There were marked increase in the serum levels of SGPT,

Table 3: Biochemical changes in liver of Swiss albino after administration of different concentration of aqueous leaf extract of P.niruri for seven days exposure period.

	P. niruri aqueous leaf extract					
Biochemical parameters	Group-I (Normal mice)	Group-II (500mg/Kg bw)	Group-III (1000 mg/Kg bw)	Group-IV(2000 mg/Kg bw)	Group-V (2500 mg/Kg bw)	Group-VI(3000 mg/Kg bw)
SGPT (IU/l)	30.63±0.977	30.98±0.45	31.2±1.21	39.0±0.56*	54.35±4.04**	55.20±0.32**
ALP (IU/l)	236.6±1.28	235±1.4	231.2±1.23	234.23±0.65*	224.67±0.23**	210.25±30.56**
Total Protein (gm/dl)	6.14±0.039	6.19±0.25	6.2±0.23	6.01±0.32	6.925±0.23**	6.895±0.65**
Albumin (gm/dl)	3.07±0.44	3.05±0.33	3.14±0.21	3.3±0.123	3.4±0.16**	3.42±0.12**
Total Bilirubin (mg/dl)	0.41±0.012	0.40±0.07	0.41±0.030	0.42±0.0794	0.42±0.079	0.41±0.079

Route of administration: Oral, Values are mean ± SEM, *P≤ 0.05(Significant), **P≤ 0.01(very significant) compared to normal, n = 6; SGPT- Serum Glutamate Pyruvate Transaminase, SGOT- Serum glutamic oxaloacetic transaminase, ALP = Serum Alkaline Phosphatase.

total protein, and albumin recorded in the mice of Group-V and Group-VI treated orally with aqueous leaf extract of *P.niruri* at the dose of 2500mg/Kg bw and 3000mg/Kg bw and that was statistically very significant ($P \leq 0.01$) as compared to control (Group-I)[Table-3].The serum level of alkaline phosphatase (ALP) in Group-V and Group-VI decreased and found to be statistically significant ($P \leq 0.05$) as compared to control (Group-I). There were not any marked changes in the serum level of total bilirubin in all the treated groups as compared to Group-I [Table- 3].

Light microscopic study of the cross section of liver tissues of control (Group-I) revealed normal architecture of hepatocytes with cytoplasm. The shape and size of nucleus with nuclear membrane was appropriate [Fig- 1a]. Group-II showed no sign and symptoms of inflammation, and adequate number of bile ducts and intact blood vessels without any haemorrhage [Fig- 1b]. No significant alterations in liver microsections of treated Group-III [Fig-1c], Group-IV[Fig- 1d] as compared to control Group-I. There were marked architectural alterations have been observed such as haemorrhage foci with marked dilation in the central vein and fatty degenerations in Group-V[Fig-1e] and Group-VI[Fig- 1f]. Dilation in blood vessels, pleiomorphic change in hepatocytes and lymphocytes surrounding ducts and ductules were also observed in Group-V[Fig- 1e] and Group-VI[Fig- 1f].

DISCUSSION

There is growing concern about the toxicity of herbal plant due to substantial amounts of pharmaceutically bioactive ingredients whose mechanisms of actions and adverse effects are mostly unknown[12]. Severe liver injury, acute and chronic abnormalities, cirrhotic transformation and liver failure, have been observed after the ingestion of a wide range of herbal products such as mushrooms, germander (*Teucriumchamaedrys*), chaparral (*Larrea tridentate*) etc.[13]. Hence, investigations on biochemical and histological alterations associated with acute oral toxicity of aqueous extract of *P.niruri* were conducted.

Investigation of the acute oral toxicity is the first step in the toxicological investigation of the unknown substance.[14]In the present investigation the median lethal 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). Our results were in agreement with the report of Dreisbach and Robertson indicating that the LD_{50} is the amount of chemical that will kill approximetly 50% of the group of animals[15,16].

Body weight and organ weight are important factors to monitor the health of an individualand to analyze the toxic impact of herbal plant extracts[17]. Organ-to-body weight (L:BW) ratios are indices which are often used in toxicological evaluations[18]. 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].

It can be concluded that decrease in body weight and organ:body weight (L:BW) ratio after administration of aqueous leaf extract of *P.niruri* was at the doses of 2500 and 3000 mg/Kg bw[19,20] indicating its toxic potential only at high doses. Alterations in liver weight suggests that due

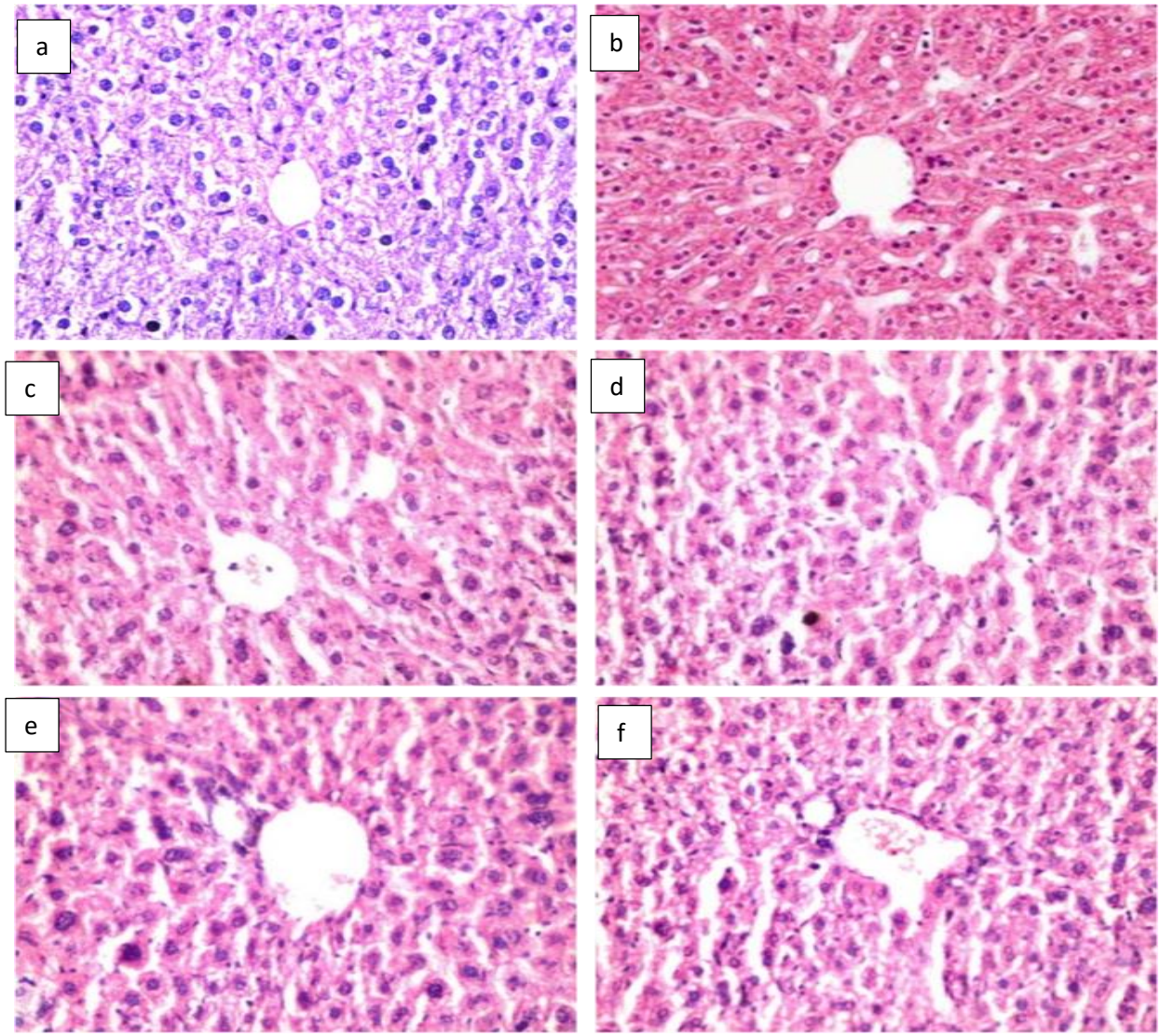

Figure 1a: Microphotographs of liver of Group-I showing round polygonal cells with spherical nucleus. H & E (400 X). Fig- 1b: Microphotographs of liver of Group-II showing hepatocytes are distinct and relatively normal, no fatty change, cytoplasm not vacuolated. H & E (400 X). Fig- 1c: Microphotographs of liver of Group-III showing no area of necrosis, no fatty degeneration and change only slight dilation of blood vessels. H & E (400 X). Fig- 1d: Microphotographs of liver of Group-IV showing fatty degenaration, and pleiomorphic nuclei. H & E (400 X). Fig- 1e: Microphotographs of liver of Group-V showing pleiomorphicnulei, dilated blood vessels and Lymphocytes surrounding ducts and ductules. H & E (400X). Fig- 1f: Microphotographs of liver of Group-VI showing pleiomorphicnulei, dilated blood vessels and Lymphocytes surrounding ducts and ductules. H & E (400X).

to treatment with high dose of over ≥2500mg/Kg bw of *P.niruri* caused hepatocellular hypertrophy[19].

Liver function tests conducted through serum assays give information about the state of the liver, describing its functionality (albumin), cellular integrity (transaminases) and its link with the biliary tract (alkaline phosphatase)[21]. The concentration of serum glutamic pyruvic transaminase (SGPT) is commonly used for the biochemical marker of hepatocellular damage[22]. The serum SGPT, a cytosolic enzyme, activities presumably increase as a result of cellular membrane damage and increased membrane permeability[23,24]. The results of present study showed that serum SGPT level was elevated in the treated mice. It indicates the hepatocellular damage or injury caused by higher doses of aqueous leaf extract of *P.niruri* in mice model.

ALP comprises a group of enzymes that catalyze the hydrolysis of phosphate esters in an alkaline environment, generating an organic radical and inorganic phosphate. In the present study the decreased serum level of ALP in the treated groups is supported by earlier reports and documentations in mice model that decrease in the level of serum ALP due to damage of hepatocytes, is associated with pernicious anemia, zinc deficiency and hypophosphatasia[25,26]. Several reports suggest that *Phyllanthaceae* contains less amount of phosphorous and zinc which causes hypophosphatasia and zinc deficiency and these factors in associations with others may lead to

the decreased serum level of ALP in our experimental groups of mice[26].

Liver is the main source of serum protein and also the only site of synthesis of albumin. Protein intake is directly related to the levels of total protein and albumin[27,28]. Earlier research reports suggest that increased protein and albumin level indicate liver dysfunctions which impair protein synthesis[29]. Similar results were observed in our study and hepatocyte damage was recorded at higher doses of aqueous leaf extract of *P.niruri* and it may be the cause of increased serum level of total protein and albumin. It has also been reported that in aerial part of the *P.niruri*,[30] amino acid, protein, total sugar, starch and phenol are present so it may affect the level of protein and albumin. Phytochemicals could be responsible for the increase in the serum protein and albumin. Similar results were reported by Prakash et al. (2011) that herbal extract contains phytochemicals mainly coumenstans polypeptides steroids and flavonoids, that may be responsible for altered protein and albumin[31].

Bilirubin is the metabolic product of the breakdown of heme derived from senescent red blood corpuscles. The degree of increase in serum bilirubin values has prognostic significance in chronic liver injuries, but not in mild liver injuries[32,33]. In the present investigation, levels of total bilirubin is within the normal range, which indicated that there were no any chronic liver injury due to administration of aqueous leaf extract of *P.niruri.*

The functional studies in toxicology should be coupled with the appropriate histological studies, because appropriate morphological studies are useful for the anatomical localization of action of toxin[33]. In normal liver the hepatic cells are round, polygonal and contain clear spherical nucleus[34]. In our study, liver architectural has been recorded at the doses of 2500 and 3000mg/Kg bw of aqueous leaf extract of *P.niruri*. The toxic effect of aqueous extract of *P.niruri* on liver may be due to phytochemicals present which have deleterious impact on the experimental model[35]. Similar findings in changes in liver architecture were reported at higher doses of aqueous extract of 2500mg/Kg bw and 3000mg/Kg bw of *E.alba* indicating toxicity and adverse effects on liver. The hepatotoxic effect may be due to anyone or more photochemicals present in the aqueous leaf extract[36].

All the above mentioned evidences indicate that oral administration of aqueous leaf extract of *P.niruri* had adverse effect on biochemical and histological indices of liver at higher doses of 2500 mg and 3000 mg/Kg bw in Swiss albino mice. In conclusion, the median lethal dose (LD_{50}) of aqueous extact of *P.niruri* was found to be 2590.984 mg/Kg bw in Swiss albino mice. Our results provide evidence for the toxicity profile of the aqueous leaf extract of *P.niruri* at higher doses and therefore, it should be ingested with precaution. This result can also form the basis for clinical trials in human however, further detail investigation are required to come to a definitive mechanism of above explained liver functioning.

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