

## HEPATOPROTECTIVE EFFECT OF *PHYLLANTHUS NIRURI* ALKALOID FRACTION IN D-GALACTOSAMINE INDUCED HEPATITIS IN RATS

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### ABSTRACT

**Objective:** The current study was designed to explore the hepatoprotective activity of *Phyllanthus niruri* (PN) alkaloid fraction in induced hepatitis in rats.

**Methods:** The rats are divided into 5 groups as per the experimental design. Pre alkaloid treatment of PN was given to the hepatitis rats for 21 d. After completion of the treatment, the serum was collected, and biochemical analysis was carried out. The hepatic markers like alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities, bilirubin (BL) urea (UR), creatinine (CR) levels are estimated in serum.

**Results:** ALT, AST activities, BL, CR levels are increased, and UR levels are decreased in hepatitis rats. However with PN alkaloid fraction pre-treatment all these hepatic markers significantly ( $P < 0.01$ ) came back to near normal levels. Histopathological studies also prove that PN protected the hepatic tissue from hepatitis.

**Conclusion:** The outcome of the present study showed that the alkaloid fraction of PN possesses hepatoprotective effect in hepatitis rats.

**Keywords:** *Phyllanthus niruri*, Hepatitis, Liver markers

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### INTRODUCTION

Hepatitis remains as a clinical challenge and a problem of great importance. Hepatitis can have serious health effects including mortality. There is no specific treatment for hepatitis. According to World Health Organisation (WHO) report, more than 600,000 peoples die each year due to hepatitis [1]. There are many models and many chemicals for inducing hepatitis in rats. Chemicals like CCL<sub>4</sub>, D-galactosamine (D-GalN) and others. D-GalN induced liver damage is very similar to human viral hepatitis in its morphological and functional features [2]. Hence in this study, we used D-galactosamine for the induction of hepatitis in rats.

*Phyllanthus niruri* is one of the most important medicinal plants belongs to Phyllanthaceae family. PN has many bioactive compounds, which possesses pharmacological properties. PN shows antioxidant, antimicrobial and other pharmacological properties [3-5].

Until now, there was no literature on the effect of an alkaloid fraction of PN on hepatic stress markers in hepatitis rats. Hence, the present study was designed to explore the hepatoprotective effect of an alkaloid fraction of PN in D-galactosamine induced hepatitis in rats.

### MATERIALS AND METHODS

#### Plant material collection

5 Kilograms of *Phyllanthus niruri* was locally collected in the month of October and November 2014 at seven hills, Tirupati, Andhra Pradesh, India, Identified and authenticated by botany taxonomist, Dr. Madhava Chetty, Department of Botany, S. V. University. A voucher specimen No.2151 was retained in the Department of Botany, S. V. University, Tirupati. The plant material was washed with water and rinsed with sterile distilled water; shade dried and powdered using a mechanical grinder. The Alkaloid fraction of PN was extracted by using method Houghton and Raman [6]. Of 5 Kilo grams of *Phyllanthus niruri*, we got only 10grms of the alkaloid fraction. The alkaloid fraction was stored in air tight container and maintained at 4 °C in a refrigerator.

#### Chemicals

All the chemicals are obtained from Sigma, Fisher Scientific (Pittsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India) and Qualigens (Mumbai, India).

#### Animals and experimental design

Male Wistar rats (180±200 g) are obtained from Indian Institute of Science (IISc), Bangalore. The animals were kept under standard laboratory conditions (temperature (27±2 °C), natural light-dark cycle (photoperiod of 12 h light and 12 h), and humidity 55–60%. The rats were maintained on a standard pellet diet (M/s Hindustan Lever Ltd., Mumbai) and provided access to water *ad libitum*. The study design was approved by the animal ethical committee (Resolution No.10/08/a/CPCSCA/IAEC/SVU/09-10/ZOOL/KRS/Dt.25.09.2009). After 7 d of acclimatization, animals were divided into five groups of six rats in each group.

Group I Normal Control (NC): rats received saline for 21 d and served as normal control.

Group II Alkaloid Fraction treatment (Aft): rats were given an alkaloid fraction of *Phyllanthus niruri* (100 mg/kg b/w) for 21 d.

Group III Hepatitis Control (HC): A single injection of D-galactosamine (800 mg/kg b/w) was given intraperitoneally for the induction of hepatitis 48 h before sacrifice.

Group IV Hepatitis+Alkaloid Fraction treatment (H+Aft): Pre-treatment of an alkaloid fraction of *Phyllanthus niruri* was administered orally for 21 d and a single injection D-galactosamine (800 mg/kg b/w) was given intraperitoneally for the induction of hepatitis 48 h before sacrifice.

Group V Hepatitis+Silymarin treatment (H+St): Pre-treatment of standard drug silymarin (100 mg/kg b/w) was administered orally for 21 d and a single injection of D-galactosamine (800 mg/kg b/w) was given intraperitoneally for the induction of hepatitis 48 h before sacrifice.

After 21 d of treatment, all the rats were sacrificed, and blood samples were collected. Serum was separated and was frozen at 80°C until further analysis.

**Estimation of hepatic markers**

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin (BL), urea (UR), creatinine (CR) are measured in serum by using commercially available kits following the manufacturer’s protocol.

**Histopathological studies**

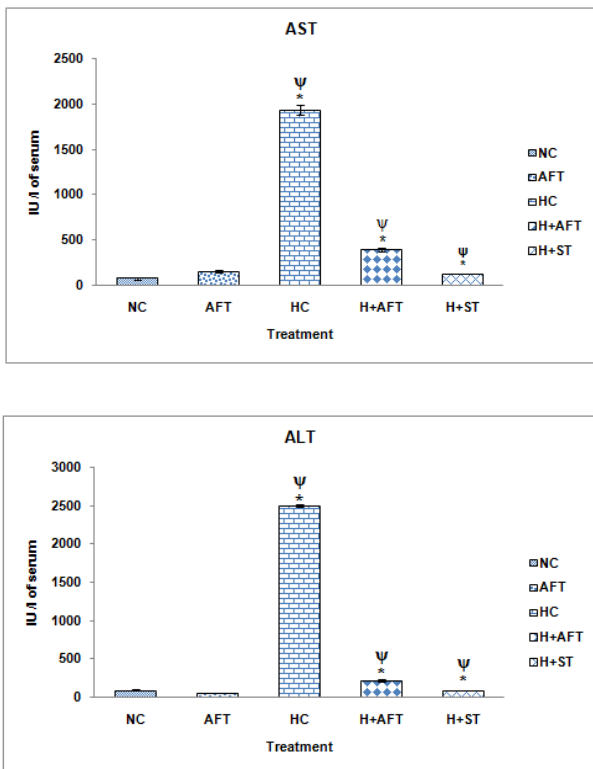
The liver tissues were fixed for 48 h in 4% paraformaldehyde, were dehydrated by passing them successively in different mixtures of ethyl alcohol-water, cleaned in xylene, and embedded in paraffin. Sections of the liver (3μ thick) were prepared, stained with haematoxylin and eosin (H&E 10×), and mounted using neutral (DPX) mountant for microscopic observation.

**Statistical analysis**

Results are expressed as means±standard deviation (SD). Variance analysis was done with Duncan’s multiple comparison tests among data were carried out using the SPSS (Version 15; SPSS Inc., Chicago, IL, USA) and M. S. Office, excel software for the significance of the main effects (factors), and treatments along with their interactions. Statistical significance was set at P<0.01. The p-values are presented with obtained data.

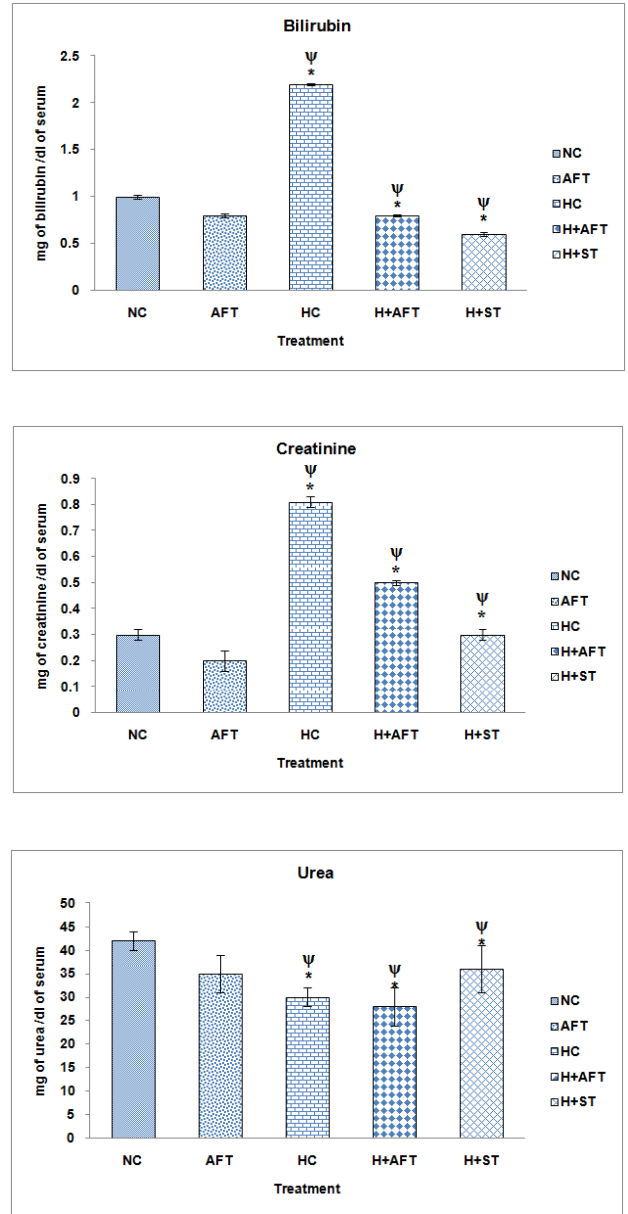
**RESULTS**

In the current study, we reported that in hepatitis rats, Aspartate aminotransferase, alanine aminotransferase activities are increased in hepatitis rats. However, with PN alkaloid fraction supplementation in hepatitis rats, we observed decreased activities of Aspartate aminotransferase, alanine aminotransferase significantly (P<0.01).



**Fig. 1: Effects of an alkaloid fraction of *Phyllanthus niruri* and silmyrin on AST & ALT, activities in the serum of normal and hepatitis rats. Data are expressed as means±SD (n = 6). \* The values are significant compared to the following: control (\* p<0.001), hepatic (\*p<0.01) (Dunnett’s multiple comparison test)**

Bilirubin, creatinine levels increased, and urea levels decreased in hepatitis rats. However treatment with an alkaloid fraction of *Phyllanthus niruri* in hepatitis rats, bilirubin, and creatinine levels decreased significantly (P<0.01) during hepatitis rats. Whereas Urea levels increased with an alkaloid fraction of *Phyllanthus niruri* in hepatitis rats.



**Fig. 2: Effects of an alkaloid fraction of *Phyllanthus niruri* and silmyrin on BL, UR and CR levels in the serum of normal and hepatitis rats. Data are expressed as means±SD (n = 6). \* The values are significant compared to the following: control (\* p<0.001), hepatic (\*p<0.01) (Dunnett’s multiple comparison tests)**

In the Current study, in normal control rats, we observed normal central vein, normal hepatocytes, and normal sinusoids. But in Hepatitis rats, degeneration of central vein (DCV), degeneration hepatocytes (DHC) and degeneration sinusoids (DSS) are seen. However, with PN supplementation in hepatitis rats these hepatic cells like central vein (RCV), hepatocytes (RHC) and sinusoids (RSS) are regenerated.

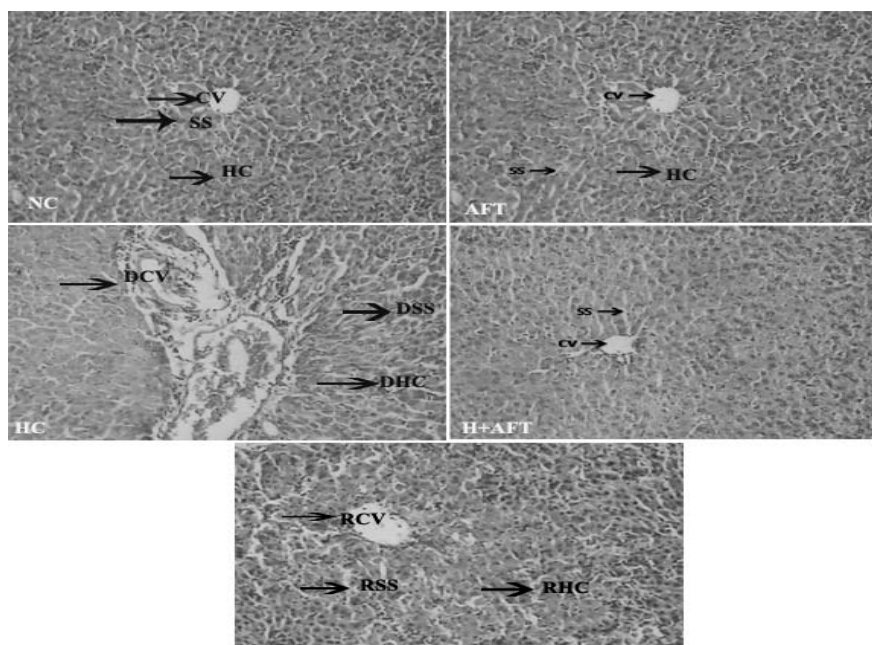


Fig. 3: Effect of PN on liver tissue during hepatitis condition

## DISCUSSION

In the present study, we reported that AST and ALT activities are increased in hepatitis rats. The increase in serum AST and ALT activities is in agreement with the findings [7] who found that AST and ALT activities of rats were significantly increased in hepatitis [7]. The marked rises in serum levels of ALT, AST, due to the damage to the plasma and disturbance in the transport functions of hepatocytes, are circulating markers of lipid peroxidation substances (LPS) induced hepatocyte injury. However, the alkaloid fraction of PN supplementation reduced AST & ALT activities significantly ( $P < 0.01$ ) in hepatitis rats. This may be due to the hepatoprotective activity of PN (fig. 1).

Bilirubin is one of the important stress marker levels. Serum bilirubin serves as an index for the assessment of hepatic function, and any abnormal increase in the levels of bilirubin in the serum indicate hepatobiliary disease and severe disturbance of hepatocellular function. In this study, we reported that BL levels increased in hepatitis rats. The increase in the levels of bilirubin in the serum indicates hepatitis tissue damage and disturbance of hepatocellular function. The increased levels of bilirubin could result from an impairment of uptake or conjugation coupled with decreased excretion of bilirubin. Increased levels of bilirubin in this study are in agreement with the reports of the previous studies that D-GalN-induced hepatitis is characterized by increased levels of bilirubin in serum [8]. However, PN supplementation suppressed the increased bilirubin levels significantly ( $P < 0.01$ ) in hepatitis rats (fig. 2).

Creatinine is one of the most important stress markers. Elevated levels of serum creatinine can be attributed to the increased breakdown of creatine phosphate for energy and conversion of creatine to creatinine for excretion. [9] The restoration of the above parameters to near normal in alkaloid fraction of PN pretreated group could be due to the antioxidant defense of PN that stabilizes the liver function and contributes to its hepatoprotective effect [3] (fig. 2).

In our study, we observed low levels of UR in hepatitis rats. During hepatitis condition serum urea declines significantly due to the failure of the liver to convert amino acids and ammonia to urea. Several studies have reported decreased levels of urea during d-GalN-induced hepatitis in rats [9,10]. The results of our study were similar with the above findings. Urea production is related to metabolic pathways for disposal of ammonia, which is the toxic end

product of nitrogen metabolism [11], have suggested that stoichiometric amounts of aspartate and carbamoyl phosphate are required for proper functioning of the urea cycle. Thus, the reduction in the level of urea may be due to the interference of d-GalN/IPS with the control mechanisms, which regulate the stoichiometric formation of these compounds. Alteration in the level urea is potential markers of free radical damage to protein.

There is an increased catabolism of proteins coupled with the diminished ability of kidneys to excrete nitrogenous waste. This could possibly account for the lowered urea levels in the serum of d-GalN-induced rats. However with an alkaloid fraction of PN, urea levels increased significantly ( $P < 0.01$ ) in hepatitis rats. This may be due to the hepatoprotective activity of PN (fig. 2).

In the present study, we observed normal central vein, normal hepatocytes and normal sinusoids in the liver of normal control rats. Whereas in Hepatitis rats, we observed degeneration of central vein (DCV), degeneration hepatocytes (DHC) and degeneration sinusoids (DSS). However, with PN treatment in hepatitis rats, central vein (RCV), hepatocytes (RHC) and sinusoids (RSS) regenerated. This shows the hepatoprotective activity of *Phyllanthus Niruri* in hepatitis rats. The bioactive and pharmacological compounds of PN in alkaloid fraction may protect the hepatic tissue from hepatitis rats. (fig. 3).

## CONCLUSION

This is first reported data on the hepatoprotective effect of an alkaloid fraction of PN during hepatitis condition. In the current study, we reported that alkaloid fraction isolated from *Phyllanthus niruri* shows a hepatoprotective effect against hepatitis in rats. But, the mechanism of its protective action is not clearly known. Further studies are, therefore, needed to know the mechanism and other related metabolic pathways alterations by an alkaloid fraction of PN in hepatitis condition.

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## CONFLICT OF INTERESTS

Declared none

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