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Original Article

# Therapeutic Potential of *Phyllanthus niruri* Against D-Galactosamine Induced Hepatitis in Wistar Strain Albino Rats: A Study with Reference to Oxidative Stress Markers and Antioxidant Enzymes

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## ABSTRACT

**Background:** *Phyllanthus niruri* (PN), known as Bhumyamalaki and it has been used in Ayurveda to treat liver, kidney and spleen related disorders. But till date no study was carried on the antihepatitis activity of phenolic fraction of PN. The objective of the current study was to investigate the antihepatitic, antioxidant and hepatoprotective effect of phenolic fraction of *Phyllanthus niruri* against hepatitis in rats. **Materials and Methods:** Wistar strain albino rats were divided in to 5 groups and treatment was administered as per the experimental design. The antioxidant enzymes Catalase (CAT), Superoxide Dismutase (SOD), Glutathione Reductase (GR), Glutathione Peroxidase (GPx), reduced Glutathione (GSH), Glutathione-S-Transferase (GST), Malondialdehyde (MDA) and liver markers Alkaline Phosphatase (ALP), aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) were estimated in all the groups. **Results:** In hepatitis rats the activities of all antioxidant enzymes were decreased. Whereas GST, MDA levels and AST, ALP, ALT activities were elevated in hepatitis rats. Administration of phenolic fraction of PN significantly increased many antioxidant enzymes in hepatitis rats except GST. Also, these results demonstrate the unique capacity of suppression of free radical toxicity by phenolic fraction of PN in hepatitis rats. Furthermore, histopathological examinations also proved that phenolic fraction of PN supplementation protected the liver tissue from oxidative damage. **Conclusion:** Our results reveal that phenolic fraction of *Phyllanthus niruri* has strong anti-hepatitis, hepatoprotective and antioxidant activity.

**Keywords:** *Phyllanthus niruri*, Hepatitis, Antioxidant enzymes, Hepatic markers, Rats.

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## INTRODUCTION

Medicinal plants have been used since the dawn of civilization to treat various diseases.[1] There are many medicinal plants like Ocimum, Phyllanthus, and ginger have been suggested for the treatment of liver related diseases. These plants possess hepatoprotective, antidiabetic, antioxidant, and other beneficial activities.[2,3]

*Phyllanthus niruri* has been widely used in traditional medicine and Ayurvedic system to treat the several diseases. In liver disorders, it is used as therapeutic agent to treat the jaundice, viral hepatitis and liver inflammation.[4] In kidney disorders, it is traditionally prescribed for urinary calculi (kidney stones), diuresis and nephritis.[5] It is also used for gastrointestinal problems such as dyspepsia, ulcers and diarrhea, for spleen-related disorders like splenomegaly.[6] In addition to that, *P. niruri* contains antidiabetic, antimicrobial and anticancer properties.[4,7]

Hepatitis is one of the major metabolic diseases that leads to death after cancer and diabetes in the world. Hepatitis is an inflammation of the liver. Hepatitis leads to fibrosis or cirrhosis or liver cancer. Hepatitis is commonly caused by the viruses, but other infections and toxic chemicals like alcohol, certain drugs like $CCl_4$, D-Galactosamine HCL can cause hepatitis. At present over

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370 million people infected with hepatitis and approximately 1 million people die annually as a direct consequence of infection.[8] During hepatitis, liver is the most important organ that was damaged and leading to liver failure. So, hepatitis is a clinical challenged disease and is of great importance.[9]

The objective of the present study aimed to evaluate the hepatoprotective and antioxidant potential of the phenolic fraction of *Phyllanthus niruri* (PN) against D-Galactosamine (D-GalN) induced hepatitis in Wistar albino rats. Specifically, the study designed to, (1) assess the effect of phenolic fraction of PN on oxidative stress markers such as MDA, GSH and essential endogenous antioxidant enzymes namely SOD, CAT, GPx, GR and GST, (2) analyze serum biochemical markers of liver function such as ALT, AST, ALP, bilirubin and albumin and (3) validate hepatoprotective effect of PN through histopathological examination of liver tissues. This is the first study examining the therapeutic efficacy of the phenolic fraction of PN against D-GalN induced hepatitis in Wistar albino rats.[10]

## MATERIALS AND METHODS

### Chemicals

In this study chemicals are obtained from Merck (Mumbai, India), Sigma (St. Louis, O, USA), Ranbaxy (New Delhi, India), Fisher Scientific (Pittsburg, PA, USA), and Qualigens (Mumbai, India).

### Extraction of Phenols from *Phyllanthus niruri*

The extraction of polyphenolic compounds from PN was followed by the method of Magalhaes *et al.* (2010) with slight modifications. Briefly, 200 g of powdered PN plant material was soaked in to 2.5 L of dichloromethane for removal of hydrophobic compounds and then extracted using methanol/water solution (70:30 v/v) under continuous stirring. The extract was filtered and concentrated under reduced pressure using a rotary evaporator. To enrich phenolic compounds, the concentrated PN extract was passed through a Polyvinylpolypyrrolidone (PVPP) (30 mg/mL) column, which selectively adsorbs polyphenols. The phenolic rich fraction was eluted, followed by freeze-dried and stored at -20°C until further use. The total phenolic content of the fraction was confirmed by spectrophotometric method.

### FT-IR analysis of Phenols

The FT-IR spectrum of the phenolic fraction of PN was recorded using a Fourier Transform Infrared (FT-IR) spectrophotometer (BRUKER Tensor-27, Ettlingen, Germany). The spectra were scanned in the range of 4000-500 $cm^{-1}$ and characteristic peaks were recorded for phenolic functional group identification.[11,12]

### Experimental Protocol

Rats aged 3 Months old, weight 180±20 g are purchased from IISc - Indian Institute of Science, Bangalore. All rats are kept under hygiene laboratory conditions and maintained temperature (27 ± 2°C), humidity 55-60%, photoperiod (12-hr light and 12-hr dark). The rats are given pellet diet and provided access to water and *ad libitum*.

Ethical clearance for performing experiments on animals was obtained from Institutional Animal Ethical Committee (IAEC), Department of Zoology, Sri Venkateswara University, Tirupati with Resolution No. 10/08/a/CPCSCA/IAEC/SVU/09-10/ZOOL/KRS/Dt.25.09.2009.

The animals are separated into 5 groups and in each group there will be 6 rats.

**Group I: Normal Control (NC):** saline was given to these rats for 21 days.

**Group II: Phenolic fraction of Phyllanthus niruri treatment (PnPt):** *Phyllanthus niruri* phenolic fraction (100 mg/kg b/w) was given to rats for 21 days.

**Group III: Hepatitis Control (HC):** D-Galactosamine (800 mg/kg b/w) was given only once intraperitoneally for hepatitis 48 hr before sacrifice.

**Group IV: Hepatitis + Phenolic fraction of Phyllanthus niruri treatment (H+PnPt):** *Phyllanthus niruri* phenolic fraction (100 mg/kg b/w) was given for 21 days to hepatitis rats.

**Group V: Hepatitis + Silymarin treatment (H+St):** Silymarin (100 mg/kg b/w) was given for 21 days to hepatitis rats.

Treatment was given to rats for 21 days, later all the rats are sacrificed by decapitation and liver tissue was used for estimation of antioxidant enzymes and histopathological examination. Serum used for analysis of liver markers.

### Estimation of Antioxidant enzymes

SOD, GR, GPx, CAT, GSH, GST are estimated in the hepatic tissue by the methods of Misra and Fridovich,[13] Flohe and Gunzler,[14] Carlberg, and Mannervik,[15] Aebi,[16] Akerboom and Sies,[17] Habig *et al.*,[18] and Bradford[19] method was used for the estimation of protein.

### Estimation of MDA

Malondialdehyde was described by the method Ohkawa *et al.*[20]

### Estimation of Serum markers

Commercially available kits are used for estimation of AST, ALT, ALP, bilirubin, albumin in serum.

### Histopathological Examination

Liver tissues are isolated and it was dehydrated in alcohol, water then it was embedded in paraffin wax. Liver sections (3 µm thick) were prepared, stained with Haematoxylin and Eosin (H&E 10×).

### Statistical analysis

The data are represented as mean±Standard Deviation (SD). Statistical analysis was done by using SPSS, i.e., Duncan's multiple comparison test. (Version 15, USA). Statistical significance was set at $p<0.05$. The $p$-values are showed in the text.

## RESULTS

### FT-IR analysis of *Phyllanthus niruri*

The FT-IR spectrum of PN phenolic fraction shows a broad O-H stretching peak at 3212 $cm^{-1}$, C-H stretching at 2926 $cm^{-1}$, C=O stretching at 1706 $cm^{-1}$, and C=C stretching at 1600 $cm^{-1}$, confirming the presence of hydroxyl, aromatic and carbonyl groups characteristic of phenolic compounds. Accordingly, the FT-IR results confirm that the extracted fraction from PN contains rich amount of phenolic constituents (Figure 1).

### Effect of Phenolic fraction of phyllanthus on liver antioxidant enzymes in hepatitis rats

In the current study, SOD, GR, GPx, CAT, activities are decreased in hepatitic rats. Whereas with phenolic fraction of phyllanthus treatment in hepatic rats, we reported significant elevation in all the antioxidant enzymes activities.

But activity of GST was significantly up regulated in hepatitis rats. However, phenolic fraction of phyllanthus administration down regulated GST in hepatitis rats (Figure 2).

### Effect of Phenolic fraction of phyllanthus on GSH and MDA levels in Hepatitis rats

GSH a sensitive marker of antioxidant defense system and was decreased in hepatitis condition, but with phenolic fraction of phyllanthus, GSH levels increased in hepatitis condition.

MDA contents in hepatitis rats was significantly elevated, when compared to the normal group. However, with phenolic fraction of phyllanthus treatment in hepatitis rats, MDA level was significantly depleted. So, with phyllanthus administration in hepatitis rats lipid peroxidation levels were decreased (Figure 3).

### Effect on liver markers by phenolic fraction of PN in hepatitis rats

In this investigation, ALP, ALT, AST and bilirubin levels are upregulated in hepatitis rats. Whereas levels of albumin are down regulated in hepatitis rats. This indicates severe liver damage in hepatitis condition. However, with phenolic fraction pretreatment for 21 days significantly modulated all the liver marker enzymes and serum markers in hepatitis rats (Table 1).

### Effect of phyllanthus phenolic fraction on liver tissue in hepatitis rats

Histopathological assessment represented in Figure 4. Hepatitis rat liver showed changes like degeneration of hepatocytes, degeneration of central vein and sinusoids. However phenolic fraction of PN supplementation to hepatitis rats, decreased oxidative stress and oxidative damage hence, all the liver cells like hepatocytes, central vein and sinusoids are regenerated. These observations significantly prove that hepatoprotective effect of phenolic fraction of *Phyllanthus niruri*.

## DISCUSSION

The FT-IR spectrum of phenolic fracion was isolated from PN was analysed between the scan ranges of 500-4000 $cm^{-1}$. The FT-IR spectrum obtained for phenols displays a number of absorption peaks like 3212 $cm^{-1}$ corresponds to O-H stretch of phenols, 2926 $cm^{-1}$ for C-H stretch of alkanes, 2306 $cm^{-1}$ for O-H stretch of carboxylic acids, 1706 $cm^{-1}$ for C=O stretch of carboxylic acids, 1600 $cm^{-1}$ for C=O stretch of α, β-unsaturated aldehydes or ketones, 1514 $cm^{-1}$ for N-O asymmetric stretch of nitro compounds, 1446 $cm^{-1}$ for C-C stretch of aromatics, 1323 $cm^{-1}$ for N-O symmetric stretch of nitro compounds, 1191 $cm^{-1}$ for C-H wag of alkyl halides, 1024 $cm^{-1}$ for C-N stretch of aliphatic amines, 750$cm^{-1}$ for =C-H bend of alkenes, 590 $cm^{-1}$ for C-Cl stretch of alkyl halides. From the FT-IR studies the broad peak obtained at 3212 $cm^{-1}$ corresponds to O-H stretch of phenols compare to other peaks the broadness is due to presence of phenols in a greater quantity (Figure 1).[11,12]

**Table 1: Effect of Phenolic fraction of PN on Serum markers in hepatitis rats.**

| Groups | AST (IU/L) | ALT (IU/L) | ALP (IU/L) | AL (G/dL) | BL (mg/dL) |
|---|---|---|---|---|---|
| Group I (NC) | 42.213 (±4.921) | 41.843 (±1.620) | 151 (±9.826) | 3.1 (±0.492) | 1.0 (±0.2) |
| Group II (Pn Pt) | 42.175 (±4.228) | 42.535 (±3.52) | 142 (±12.264) | 2.7 (±0.38) | 0.8 (±0.14) |
| Group III (HC) | 206.442* (±7.642) | 131.642* (±11.398) | 302* (±12.368) | 1.2* (±0.82) | 2.7* (±0.046) |
| Group IV (H+Pn Pt) | 86.267* (±4.25) | 76.348* (±4.52) | 192* (±12.46) | 2.6* (±0.082) | 1.2* (±0.069) |
| Group V (H+St) | 66.731* (±4.614) | 51.189* (±3.092) | 151* (±8.814) | 2.2* (±0.142) | 0.8* (±0.063) |

All the values are Means±SD of six individual observations.* Significant at $p<0.001$ with respect to normal control.

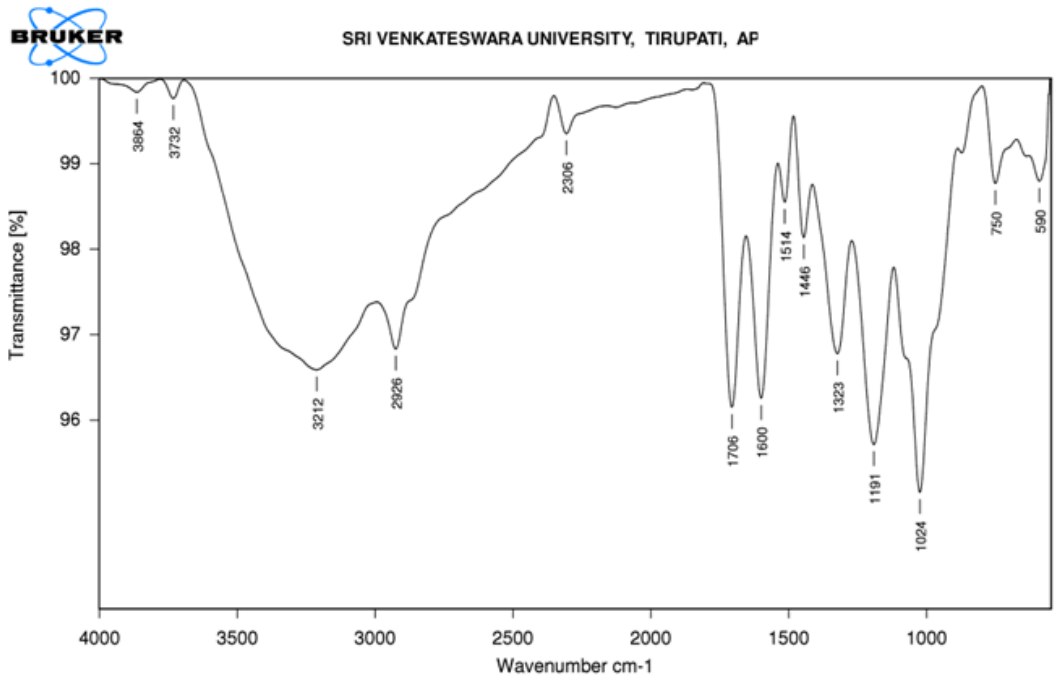
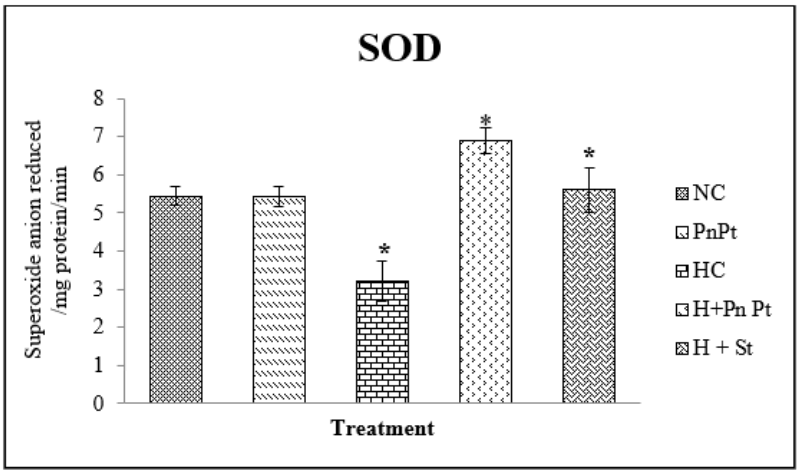
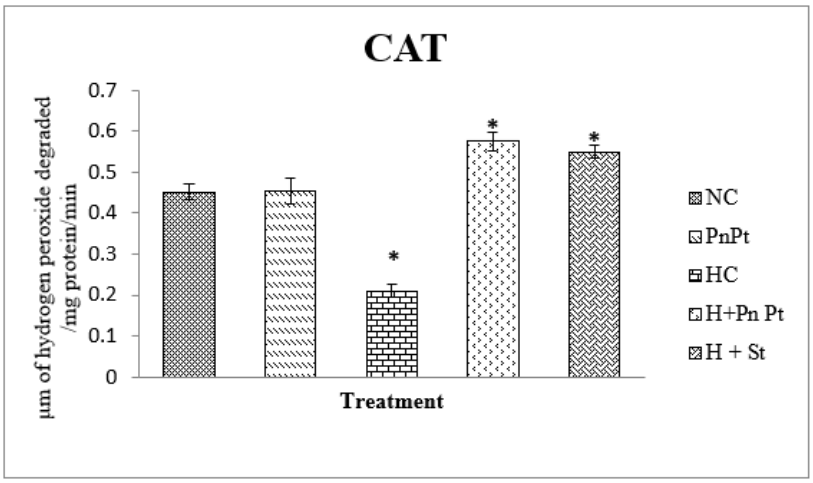
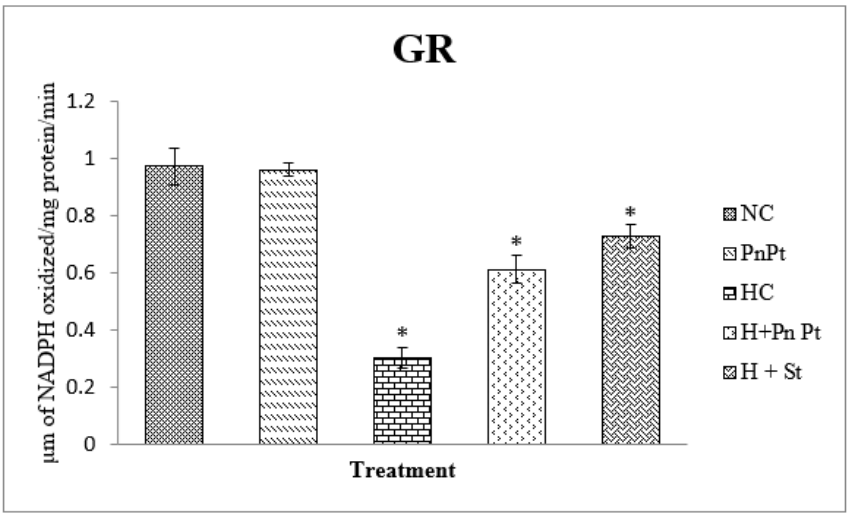
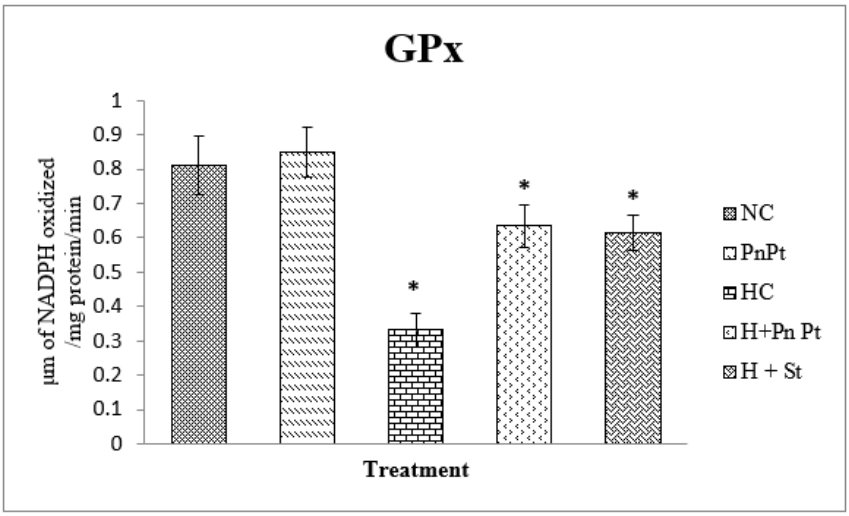



**Figure 1:** FT-IR spectrum of the phenolic fraction of PN showing characteristic absorption peaks corresponding to hydroxyl, carbonyl and aromatic functional groups.





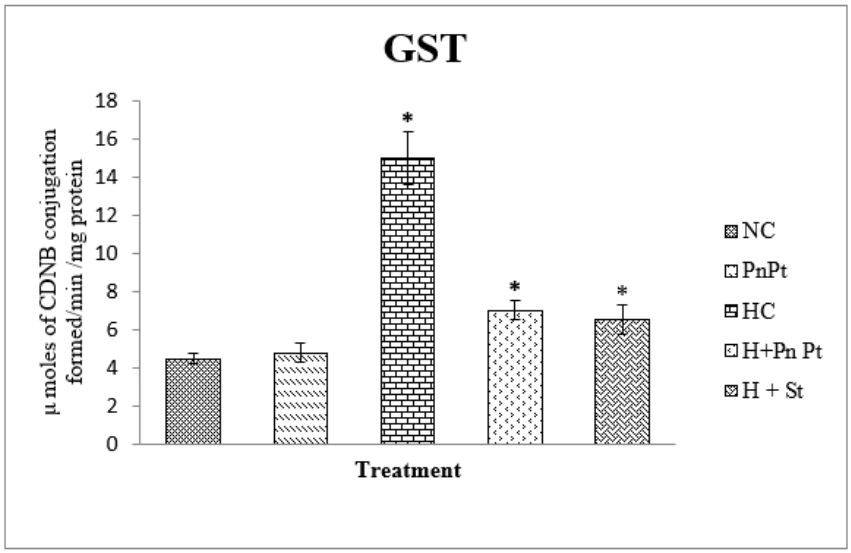
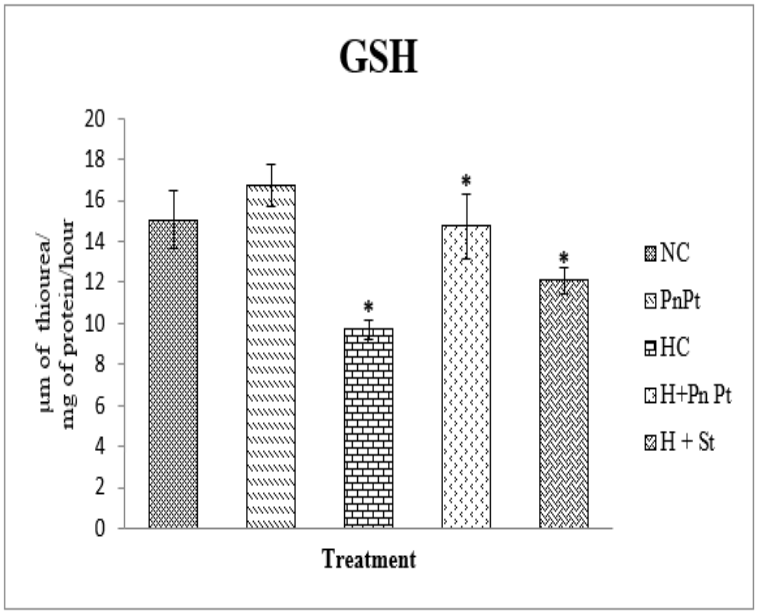




**Figure 2:** Effects of the phenolic fraction of *Phyllanthus niruri* (PN) and silymarin on SOD, CAT, GR, GPx, and GST activities in liver tissues of normal control and D-GalN induced hepatitis rats. Data are expressed as mean±SD (*n*=6). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test. $p<0.001$ vs. normal control; #$p<0.01$ vs. hepatitis control.

Hepatitis is the most important public health problem in the world which is responsible for morbidity and mortality.[21] Oxidative stress causes liver cirrhosis.[22] Liver is the target organ in hepatitis condition. In this study, we observed that SOD and CAT activity was decreased in hepatitis rats. SOD is an important enzyme which helps in the removal of ROS in hepatitis rats. SOD removes superoxide by converting it to $H_2O_2$, which can be converted to water by CAT.[23,24] Whereas with phyllanthus supplementation to hepatitis rats, SOD and CAT activity was increased. This may be efficient protective mechanism offered by phyllanthus in response to ROS and also to toxic free radicals in hepatitis condition (Figure 2).

In this study, GR and GPx activities are down regulated in hepatitis rats. D-GalN intoxication results in depletion of GPx and GR activities.[25] GPx reduces the $H_2O_2$ to water and GR reduces the oxidised GSH into reduced GSH, which is crucial during hepatitis condition, because for protection against toxic free radicals. Whereas, phyllanthus administration elevated GPx and GR activity by suppressing the free radicals during hepatitis rats. Our study reported that the phenolic fraction of phyllanthus



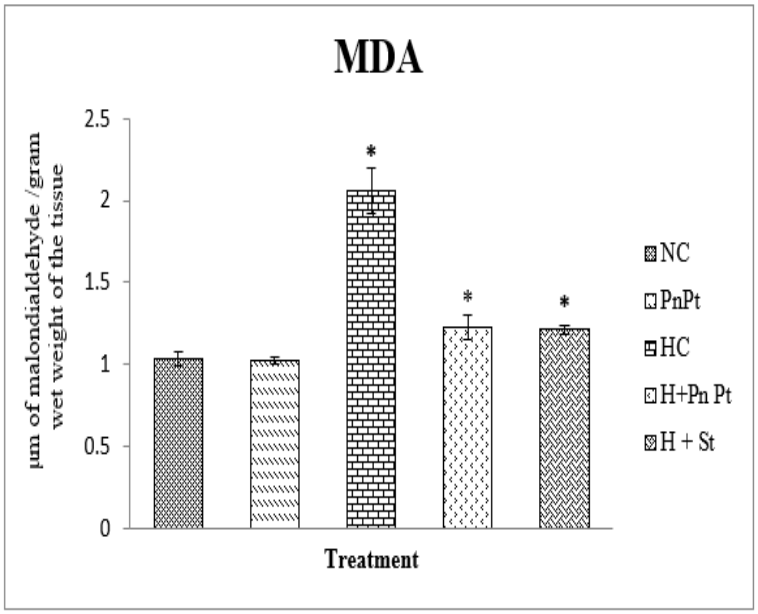




**Figure 3:** Effects of Phenolic fraction of PN and silmyrin on GSH and MDA levels in the liver tissues of normal control and D-GalN induced hepatitis rats. Data are expressed as mean±SD ($n$=6). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test. $p$<0.001 vs. normal control; #$p$<0.01 vs. hepatitis control.

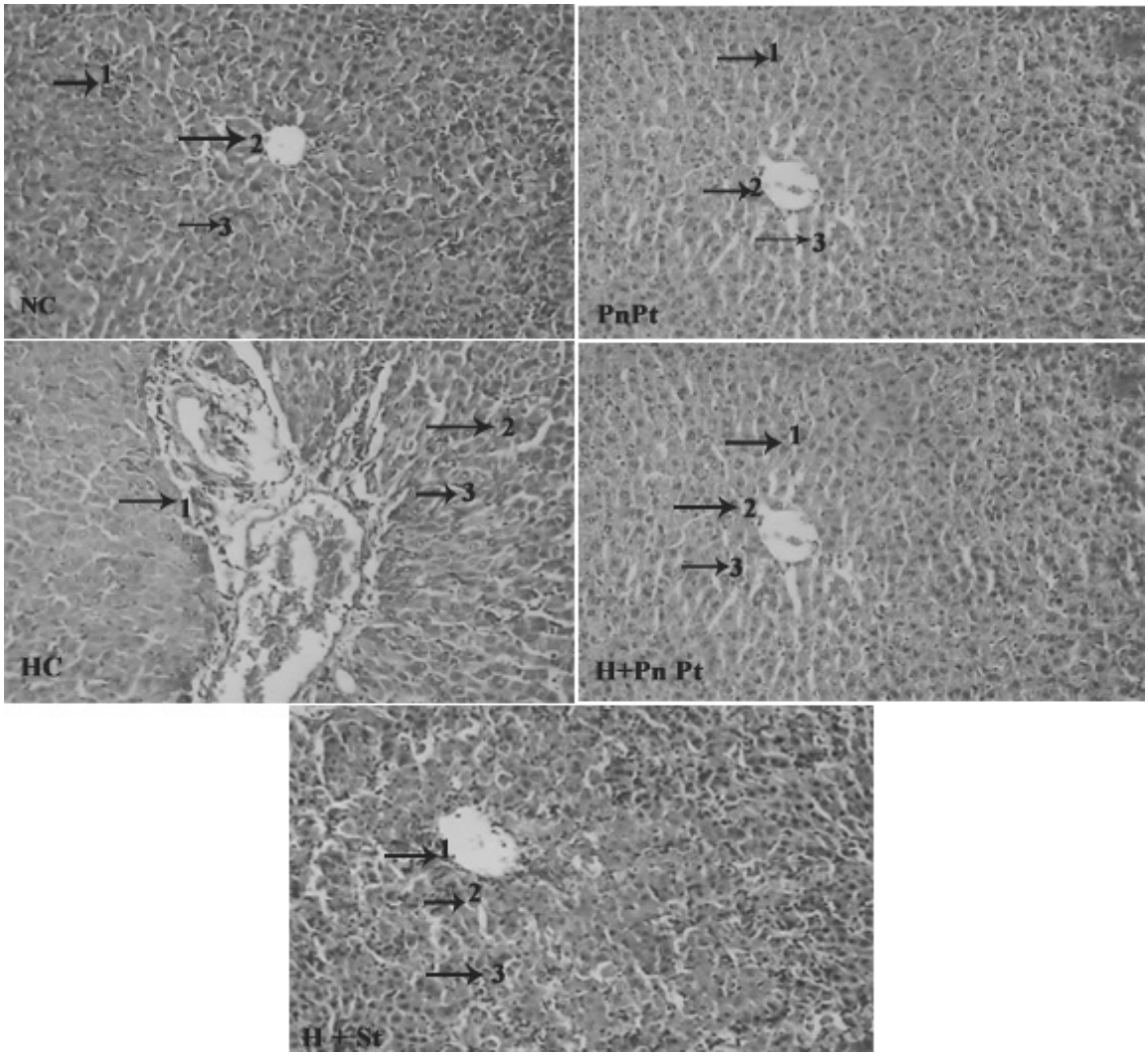



**Figure 4:** Impact of Phenolic fraction of PN on liver tissue during hepatitis condition. Photomicrographof Normal Control (NC) liver depicts: 1. Normal hepatocytes, 2. Normal central vein, 3. Normal sinusoids. Photomicrograph of Phenolic fraction of Phyllanthus treatment (PnPt) liver, showing 1. Normal hepatocytes, 2.Normal central vein, 3. Normal sinusoids. Photomicrograph of Hepatitis Control (HC) liver, showing 1. Degeneration of central vein 2. Degeneration of hepatocytes, 3. Degeneration of sinusoids. Hepatitis + PnPt (H + PnPt) liver, showing 1. Regeneration of hepatocytes 2. Regeneration of central vein, 3. Regeneration of sinusoids. Photomicrograph of Hepatitis + Silmyrin (H + St) liver,showing 1. Regeneration of central vein, 2. Regeneration regeneration of hepatocytes, 3. Regeneration of sinusoids (H and E 10X).

may reduce oxidative stress by scavenging reactive oxygen species and enhancing antioxidant enzymes (Figure 2).

In the present investigation, GST activity was elevated in hepatitis rats. GST plays an important role in the elimination of free radicals which are produced during hepatitis. Whereas phenolic fraction of PN supplementation depleted GST activity in hepatitis rats. Our study also proves that PN suppressed the toxic free radicals in hepatitis condition (Figure 2).

In this study, GSH levels are down regulated in hepatitis condition.[26] Treatment with the phenolic fraction of PN restored the depleted GSH levels in hepatitis rats. Our study proved that *P. niruri* may decrease oxidative stress and free radical-mediated toxicity and also helps in modulation of antioxidant enzymes In this investigation, we observed that lipid peroxidation levels are enhanced in hepatitis rats.[27] Oxidative stress caused by D-galactosamine leads to liver toxicity and lipid peroxidation due to ROS. Hence, the cellular defense against reactive oxygen species become insufficient, so MDA levels are elevated in hepatitis animals.[28,29] Whereas phenolic fraction of PN treatment, MDA levels are depleted in hepatitis rats. These changes may be due to suppression of free radicals and depletion of oxidative stress by PN in hepatitis rats (Figure 3).

D-GalN induces free radical toxicity in liver tissue of albino rats. It also alters the liver marker enzymes during hepatitis condition. Liver damage induced by D-GalN generally reflects disturbances of liver markers. ALP AST and ALT are markers of liver and their elevated levels are indicative of damage of liver tissue and loss of functional integrity of cell membranes. In the present study, we noticed upregulation in AST, ALT and ALP activities in hepatitis rats.[30] Our study shows that during hepatitis condition, damage

of hepatic tissue thus result in the elevation of the liver markers. Whereas with phenolic fraction of phyllanthus administration in hepatitis rats these liver markers enzyme activities are decreased. Our study reported that hepatoprotective activity of PN. Phyllanthus supplementation could diminish the production of toxic radicals in hepatitis condition and improve the activities of enzymes (Table 1).

In this study, we observed elevated levels of bilirubin and depleted albumin levels. These observations are due to alterations in catabolism and hormonal factors during hepatitis condition. However with phenolic fraction of PN decreased the levels of albumin and increased the level of bilirubin. This may be due to pharmacological compounds in phyllanthus in hepatitis rats. Our study suggests that phenolic fraction of PN possess hepatoprotective activity against D-galactosamine in hepatitis rats.

Figure 4 depicts histopathology of liver tissue. In the present study, liver pictograph of rats treated with phenolic fraction of PN showed normal morphology without any histological abnormalities. Histopathological examination of hepatitis rat liver showed loss of architecture, cell necrosis, degeneration of hepatocytes, degeneration of sinusoids and central vein. However, with phenolic fraction of PN supplementation in hepatitis rats, we reported, regeneration of hepatocytes, regeneration of central vein and regeneration of sinusoids. Our study reported that PN supplementation possesses a hepatoprotective role in hepatitis condition (Figure 4).

## CONCLUSION

This study demonstrates that the phenolic fraction of PN have significant hepatoprotective effects against D-GalN induced hepatitis in rats. It restored antioxidant enzyme activities, reduced lipid peroxidation and improved serum liver markers and liver histological cytoarchitecture. These protective effects are likely due to its rich phenolic content, which enhances antioxidant defense. The findings highlight the potential of phenolic fraction of PN as a natural hepatoprotective agent, further studies, are recommended to confirm its efficacy and elucidate the molecular mechanisms involved.

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## ABBREVIATIONS

**ALP:** Alkaline Phatase; **ALT:** Alanine Aminotransferase; **AST:** Aspartate Aminotransferase; **b/w:** Body Weight; **C=O:** Carbonyl Group; **C-H:** Carbon-Hydrogen; **CAT:** Catalase; **$CCl_4$:** Carbon Tetrachloride; **D-GalN:** D-Galactosamine; **FT-IR:** Fourier Transform Infrared Spectroscopy; **GPx:** Glutathione Peroxidase; **GR:** Glutathione Reductase; **GSH:** Reduced Glutathione; **GST:** Glutathione-S-Transferase; **H&E:** Hematoxylin and Eosin; **HC:** Hepatitis Control; **H+PnPt:** Hepatitis + *Phyllanthus niruri* Phenolic Fraction Treatment; **H+St:** Hepatitis + Silymarin Treatment; **IAEC:** Institutional Animal Ethical Committee; **MDA:** Malondialdehyde; **NC:** Normal Control; **O-H:** Hydroxyl Group; **P. niruri / PN:** *Phyllanthus niruri*; **PnPt:** *Phyllanthus niruri* Phenolic Fraction Treatment; **PVPP:** Polyvinylpolypyrrolidone; **ROS:** Reactive Oxygen Species; **SD:** Standard Deviation; **SOD:** Superoxide Dismutase; **SPSS:** Statistical Package for the Social Sciences.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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