



## HEPATOPROTECTIVE ACTIVITY OF *PHYLLANTHUS NIRURI* WHOLE PLANT EXTRACT AGAINST *STAPHYLOCOCCUS AUREUS* INTOXICATED ALBINO RATS

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### Abstract

In the present study the effect of alcoholic extract of the *Phyllanthus niruri* whole plant on rat biochemical and serum marker enzymes against *Staphylococcus aureus* induced oxidative stress in the rat liver. Pre and post-treatment with extract showed a dose-dependent reduction of *Staphylococcus aureus* induced rats were elevated levels of enzyme activity with parallel increase in total protein and bilirubin, indicating the extract could preserve the normal functional status of the liver. In alcoholic extracts treated groups there was statistical significant decrease in the levels of serum bilirubin, SGOT, SGPT and ALP as compared to the hepatotoxic group. In significant reduction in the activity levels of superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, and also glutathione reductase content were observed in the liver of *S. aureus* intoxicated rats over controls. Thus, the results suggest the beneficial role of *Phyllanthus niruri* might be due to its antioxidative properties against *S. aureus* infection induced oxidative stress in liver.

**Key words:** Hepatoprotective activity, *Phyllanthus niruri*, hepatotoxicity, *Staphylococcus aureus*, Enzyme activity, Biochemical analysis.

### Introduction

Drug-induced liver injury is a major health problem that challenges not only health care professionals but also the pharmaceutical industry and drug regulatory agencies. Herbal medicines have recently attracted much attention as alternative medicines useful for treating or preventing life style related disorders and relatively very little knowledge is available about their mode of action. There has been a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits. Liver, the key organ of metabolism and excretion has an immense task of detoxification of xenobiotics, environmental pollutants and chemotherapeutic agents. Hence, this organ is subjected to variety of diseases and disorders. Several hundred plants have been examined for use in a wide variety of liver disorders (Saleem, 2008 and Ramamurthy *et al.*, 2014).

During the recent past, there is a perception that, a parallel increase exists between bacterial infections and disorders of human health, posing a serious threat to public. Among different types of bacteria, *Staphylococcus aureus* is one of the hospital-borne pathogen which is implicated in hospital and community-acquired diseases. It is anaerobic opportunistic gram positive pathogen capable of causing pathology in virtually every tissue of the host. *S. aureus* is generally considered as non-invasive extracellular pathogen that damages the host cells at least in part in two ways, firstly either by adhering to the extracellular matrix of the cells and/or secondly by invading and persisting in the cells thereby interrupting signaling mechanisms. The persisting nature of the *S. aureus* suggests that it has ability to overcome host defense mechanisms and more over, colonization properties of bacteria could explain its chronic effects including osteomyelitis and mastitis. Emergence of antibiotic (methicillin and vancomycin) resistant strains even becomes a huge threat to public health. Many studies indicated that *S. aureus* affects almost all mammalian hosts and causes a range of diseases including skin infection, nasal colonization, sepsis, renal failure, arthritis and endocarditis (Hari Prasad *et al.*, 2011).

Liver injury is a common feature of bacterial toxemia during sepsis condition which leads to the development of severe shock and multiple organ failure. Klintman *et al.* (2004) suggests that liver injury caused by *S. aureus* mediates Fas ligand, activated by reactive oxygen intermediates. Earlier it has been suggested that *S. aureus* has ability to induce free radicals in Swiss albino mice (Chakraborty *et al.*, 2011). Nevertheless, it has been suggested that reactive oxygen species is also one of the factors associated with apoptosis of hepatocytes, studies related to the *S. aureus* infection and pro and antioxidant status in the liver tissue is poorly understood.

Liver diseases are serious health problem. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices and in traditional system of medicine in India. There is no satisfactory remedy for serious liver disease; most of the herbal drugs speed up the natural healing process of liver. So the search for effective hepatoprotective drug continues (Murugian *et al.*, 2008). Several Indian medicinal plants have been extensively used in the Indian traditional system of medicine for the management of liver disorder. Some of these plants have already been reported to possess strong antioxidant activity (Achuthan *et al.*, 2003; Aniya, 2002; Gupta *et al.*, 2006; Ramamurthy and Ayyadurai, 2009; Ramamurthy and Raveendran, 2010; Ramamurthy and Sagaya Giri, 2013).

*Phyllanthus niruri* are originated in India, usually occurring as a winter weed throughout the hotter parts. The *Phyllanthus* genus contains over 600 species of shrubs, trees, and annual or biennial herbs distributed throughout the tropical and subtropical regions of hot hemispheres. Unfortunately, there remains a great deal of confusion among scientists regarding plant identification and many cases, plant misidentification make evaluation of published information

difficult. *P. amarus* and *P. sellowianus* are often considered a variety of *P. niruri*, or no distinction is made among these three species in published clinical research. Often time's one name is indicated tube synonymous with another and, sometimes, both names are used interchangeably as if referring to one plant. It became so confusing that, in the 1990s, a major reorganization of the *Phyllanthus* genus was conducted (which classified *P. amarus* as a type of *P. niruri*). *Phyllanthus niruri* is an herb of Euphorbiaceae family that grows upto 60 cm. *Phyllanthus* means "leaf and flower" because the flower, as well as the fruit, seems to become one with the leaf. *Phyllanthus nirui* is a common kharif (rainy season) weed found in both cultivated fields and wastelands.

*Phyllanthus niruri* is an annual herb belonging to the family Euphorbiaceae grows 50 to 70 centimeters tall and bears ascending herbaceous branches. The bark is smooth and light green. It bears numerous pale green flowers which are often flushed with red. The fruits are tiny, smooth capsules containing seeds. It produces phyllanthid branches with the presence of flowers and fruits at the base of each leaf, one of the identification characteristics of this plant. *Phyllanthus niruri* is used in the treatment of various ailments like jaundice, diabetes, kidney stones, liver disorders and for treatment of Hepatitis B viral infection. Therefore, in the present study was aimed to investigate whether injection of *S. aureus* induces oxidative stress and if so, *Phyllanthus niruri* reduces the *S. aureus* -induced oxidative stress in the liver of rats.

## Materials and Methods

For the present study, the medicinal plant *Phyllanthus niruri* belongs to family Euphorbiaceae was collected from in and around area of Thanjavur, Tamil Nadu, South India. The plant was identified with the help of flora of presidency, Tamil Nadu and Karnatic flora (Gamble, 1967 and Matthew, 1983) and standard references (Kirtikar and Basu, 1993).

### Preparation of plant extracts

The *Phyllanthus niruri* was collected washed, cut into small pieces and dried at room temperature (28°C) for two weeks and made into powder by using mixture for further analysis. Extraction is a process, to separate or isolate the secondary metabolites from plant material. It is basically two types i.e. heat and cold extraction. Heat extraction has some advantage over cold extraction like time consistency and also no contamination by microbes. An apparatus called soxhlet did heat extraction. 100g of the plant leaf powder were packed into the thimble of a soxhlet apparatus. The ratio of the plant powder and solvents were maintained at 1:4.

### Bacterial strain

The test bacterial clinical isolate, *S. aureus* was collected from the Department of Biochemistry, Marudupandiyar College, Thanjavur. Preliminary confirmation and phenotypic studies were performed according to standard protocols by using gram staining and biochemical parameters including coagulate test and screened by growing on Baird-Parker selective Agar (Hi Media, India). After confirmation studies, the bacterial culture was grown in tryptic broth and incubated over night. The bacterial culture was then centrifuged at 10,000 rpm for 20 min and the pellet was resuspended and washed with sterile phosphate buffer saline (PBS). The absorbance was measured at 620 nm using a UV-spectrophotometer (Schimadzu) and the viable bacterial count was adjusted to approximately  $1.0 \times 10^9$  colony forming units (CFU)/mL, which corresponds to an optical density of 1.6. Serial dilution was performed with PBS to get a final concentration  $5 \times 10^6/0.1$  mL of bacterial suspension (Chakraborty *et al.*, 2011).

### Experimental design

The animals were randomly divided in to four groups, each containing three animals. The extract of *Staphylococcus aureus* and saline were given with the help of feeding cannels. Four groups (Group I, Group II, Group III and Group IV) of rats, three rats in each group were taken. Group – I: Served as normal, which received, feed and water only. Group – II : Animals in group 2, received single intraperitoneal injection of bacterial suspension at a dose of  $5 \times 10^6$  CFU /0.1 mL once in every three days. Group – III : Single intraperitoneal injection of bacterial suspension at a dose of  $5 \times 10^6$  CFU /0.1 mL once in every three days. Then the animals were treated with the ethanolic extract of *Phyllanthus niruri* daily for 15 days in physiological saline at concentration of 100mg / kg of body weight. Group – IV: Single intraperitoneal injection of bacterial suspension at a dose of  $5 \times 10^6$  CFU /0.1 mL once in every three days. Then the animals were treated with Silymerin for 15 days at concentration of 25 mg/kg of body weight. The injection dose of bacteria and plant extract dose was based on earlier reports (Hari Prasad *et al.*, 2011). The experimental period for the present study was 15 days. After 15<sup>th</sup> days of herbal treatments, the animals were fasted for 12hours after the last dose of drug treatment and were scarified cervical decapitation under mild chloroform anesthesia. The blood was collected for serum separation. The organs were excised and they were washed in ice-cold saline until homogenized. Liver 10%, homogenate was prepared in 0.1ml Tris HCl buffer P<sup>H</sup> 7.4. The serum separated by the centrifugation process and was used for following estimation.

Biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvate transaminase (SGPT) by the methods of Reitman and Frankel (1957), alkaline phosphatase (Kind and King, 1954), total bilirubin (Mallay and Evelyn, 1937) and protein (Lowry *et al.*, 1951) were analyzed. Reduced glutathione (GSH) was estimated using DTNB (Sedlak and Lindasy, 1968). The blood glutathione was estimated by the method of Beutler *et al.* (1963). The catalase was determined by the methods of Aebi (1974). The activity of superoxide dismutase was assayed by the method of Woolliams *et al.* (1983). The concentration of Thiobarbutiric acid reactive substances (TBARS) was measured in liver using the method of Ohkawa *et al.* (1979).

## Results

The treatment with the extract did not decrease water and food consumption. The body weight of the rats treated with alcoholic extract once a day during 15 days (sub-acute treatment) did not show any significant change when compared with the control group, although had a tendency to decrease body weight (500 mg/kg). This decrease can be

associated with the decrease of liver weight at the dose of 500 mg/kg in comparison with the control group without any concomitant alteration in the activity of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase. Estimation of the serum activity of total bilirubin, protein, reduced glutathione, TBARS, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase is one of the most widely used means of measuring hepatocellular injury (Table 1).

Antioxidant activity of *Phyllanthus niruri* leaves extract was studied by LPO method and dose 500mg/kg produced significant antioxidant activity as shown in Table 1. The maximum antioxidant activity was exhibited at dose 500mg/kg in alcoholic extract of *Phyllanthus niruri*. Among the two extracts, the alcoholic extracts exhibited more antioxidant activity. The results of the present study suggests that co-administration of aqueous extract of *Phyllanthus niruri* ameliorates antioxidant status in *S. aureus* induced oxidative stress in the liver of rats as evidenced by decrease in the lipid peroxidation products and increase in the activity levels of antioxidant enzyme and reduced glutathione levels. There are changes in the level of GSH and TBARS in the liver homogenate of normal and experimental rats. The activity of GSH was observed to changes significantly in *S. aureus* intoxicated rats. This indicated that *Phyllanthus niruri* improved the enzymatic antioxidant status in rat liver since it is known that a marked increase in SOD and CAT activity can offer first line protection against the damaging effects of superoxide radicals in the liver.

**Table 1. Effect of *Phyllanthus niruri* extracts on some biochemical and serum marker enzyme parameters in *Staphylococcus aureus* intoxicated albino rats**

S. No	Parameters	Control	<i>Staphylococcus aureus</i> treated group	<i>Phyllanthus niruri</i> treated group	Silymarin treated group
1	Bilirubin (mg/dl)	0.92 ± 0.12	3.10 ± 0.14	1.15 ± 0.44	0.96 ± 0.24
2	Protein (g/dl)	6.48 ± 0.17	5.21 ± 0.42	5.75 ± 0.12	6.12 ± 0.15
3	TBARS (n moles/ml)	2.87 ± 0.14	5.14 ± 0.15	3.41 ± 0.51	3.11 ± 0.22
4	GSH (μ mole/g of tissue)	8.41 ± 0.12	4.84 ± 0.18	7.57 ± 0.24	8.15 ± 0.54
5	SGOT (IU/L)	138 ± 0.19	191 ± 0.21	151 ± 0.17	145 ± 0.12
6	SGPT (IU/L)	42 ± 0.51	92 ± 0.24	65 ± 0.36	49 ± 0.17
7	ALP (IU/L)	131 ± 0.12	262 ± 0.19	155 ± 0.18	142 ± 0.24

Results are mean of six observations ± S.E.M.

**Table 2. Effect of *Phyllanthus niruri* on Antioxidant status in the liver of *S. aureus* intoxicated rats**

S. No	Treatment group	GSH (μ mole/g of tissue)	TBARS (n moles/ml)	LPO (μg/mg/protein)	SOD (μ mole/g of tissue)	Catalase (μ mole/g of tissue)
1	Control	8.84 ± 0.22	2.15 ± 2.04	0.44 ± 0.05	22.7 ± 0.04	11.7 ± 0.25
2	<i>Staphylococcus aureus</i> treated groups	5.45 ± 0.27	4.50 ± 2.06	0.89 ± 0.08	15.5 ± 0.09	6.25 ± 0.14
3	<i>Phyllanthus niruri</i> treated groups	7.55 ± 0.18	3.15 ± 2.07	0.58 ± 0.02	20.2 ± 0.08	9.12 ± 0.66
4	Silymarin treated groups	8.12 ± 0.12	2.52 ± 2.05	0.51 ± 0.06	21.5 ± 0.03	10.45 ± 0.45

Results are mean of six observations ± S.E.M.

## Discussion

The present study was carried out to evaluate the hepatoprotective activity of *Phyllanthus niruri* against paracetamol induced hepatocellular degenerative in albino rats. The effectiveness of this medicinal plant was screened by assessing biochemical changes of different groups of experimental animals. *Phyllanthus niruri* possessed very high levels of alkaloids and flavonoids and are employed in medicinal uses. The plants studied here can be seen as a potential source of useful drugs.

The results of biochemical parameters revealed the elevation of enzyme level in *S. aureus* treated group, indicating that *S. aureus* induces damage to the liver. Liver tissue rich in both transaminases increased in patients with acute hepatic diseases, SGPT, which is slightly elevated by cardiac necrosis, is a more specific indicator of liver disease (Murugaian *et al.* 2008 and Sukumaran *et al.*, 2008). A significant reduction ( $P < 0.005$ ) was observed in SGPT, SGOT, ALP, total bilirubin and protein levels in the groups treated with silymarin and alcoholic extract of *S. brevistigma*. The enzyme levels were almost restored to the normal (Ayyadurai and Ramamurthy, 2009; Ramamurthy and Sagaya Giri, 2013).

The present study was observed that *Phyllanthus niruri* has a significant hepatoprotective effect in *S. aureus* administrated rats that hepatocellular degenerative and necrotic changes are slight without advanced fibrosis and cirrhotic process in *Phyllanthus niruri* treated group. However, Ramamurthy and Raveendran (2010); Ramamurthy and Sagaya Giri (2013) found that *Nigella sativa* L can prevent liver fibrosis and cirrhosis, suggesting that *Nigella sativa* L protects liver against fibrosis possibly through immunomodulator and antioxidant activities.

Liver is the most important and main part of the animal body. It is highly affected primarily by toxic agents and that is why the above-mentioned parameters have been found to be of great importance in the assessment of liver damage. The abnormal high level of serum ALT, AST, ALP and bilirubin observed in our study (Table 1) are the consequence of *Staphylococcus aureus* induced liver dysfunction and denotes the damage to the hepatic cells. Treatment with *Phyllanthus niruri* reduced the enhanced level of serum ALT, AST, ALP and bilirubin, which seem to offer the protection and maintain the functional integrity of hepatic cells.

Liver plays an important role in the protein synthesis. It is considerably affected when there is a disturbance in protein metabolism. The site-specific oxidative damage of some of the susceptible amino acids of proteins is now recorded as the major cause of metabolic dysfunctions during pathogenesis (Uday Bandyopandhyay *et al.*, 1999). Decrease in serum bilirubin after treatment with the extract in liver damage indicated the effectiveness of the extract in normal functional status of the liver. In the present study the lowered level of total proteins and bilirubin recorded in blood sample of *Staphylococcus aureus* treated rats reveals the severity of hepatopathy. In the *Phyllanthus niruri* treated group, the protein and bilirubin level of animal was almost normal. This result is supported by stimulations of protein synthesis have been advanced as a contributory hepatoprotective mechanism, which accelerates the regeneration process and the protection of liver cell (Ramamurthy *et al.*, 2014).

The results of the present study suggest that co-administration of aqueous extract of *Phyllanthus niruri* ameliorates antioxidant status in *S. aureus* induced oxidative stress in the liver of rats as evidenced by decrease in the lipid peroxidation products and increase in the activity levels of antioxidant enzyme and reduced glutathione levels. Liver sepsis is a serious ongoing problem all over the world and *S. aureus* is also one of the major contributors of liver sepsis and consequently liver injury. It is well known that *S. aureus* produces a broad array of virulence determinants which may be structural components of the bacterial cell envelope and adhesins, or toxins and enzymes, which are secreted extracellularly (Larkin *et al.*, 2009). These virulence determinants are believed to mediate pathogenesis of bacteria including sepsis (Klintman *et al.*, 2004). Studies of Klintman *et al.* (2004) also reported that Fas ligand is one of the virulence factors responsible for liver injury caused by *S. aureus*. Further, studies of Weglarczyk *et al.* (2004) also suggested that *S. aureus* infection leads to release of reactive oxygen free radicals which is important for the activation of CD95 (Fas) - Fas ligand interactions thereby leads to apoptosis of monocytes. Thus, it is apparent that *S. aureus* induced effects are complex and at least in part mediate oxidative stress (Baran *et al.*, 2001).

In general a balance exists between the generation of lipid peroxidation products viz., reactive oxygen species (ROS) and the level of endogenous antioxidants during physiological conditions which serve to protect tissue from oxidative damage. Disruption of this balance, either through increased production of ROS or decreased levels of antioxidants, results in a condition referred to as "oxidative stress". Thus, evaluation of lipid peroxidation, and antioxidant enzyme status and reduced glutathione content in biological tissue has been always used as markers for tissue injury and oxidative stress. Lipid peroxidation can cause changes in membrane fluidity and permeability and increase the rate of protein degradation, which eventually lead to cell lysis. It is well acknowledged that free radical scavengers such as SOD, CAT and GSH and metabolism regulatory enzymes such as GSH-Px, GR and GST can protect the cellular system from deleterious effect of free radicals (Olga Blokhina *et al.*, 2003). SOD, as a first line defense antioxidant enzyme plays an important role in the dismutation of superoxide and thereby leads to hydrogen peroxide which is eventually neutralized by GPx and catalase. In the present study, there was a significant increase in the lipid peroxidation products with a significant decrease in the activity levels of antioxidant enzymes such as SOD and catalase, antioxidant content reduced glutathione in the liver of rats intoxicated with *S. aureus*. The decrease in the activity levels of SOD and catalase indicates accumulation of superoxide ions and also hydrogen peroxide ions, which might lead to an observed increase in the lipid peroxidation products in the liver of rats intoxicated with *S. aureus*.

ALT and AST are the specific markers to assess hepatocellular damage leading to liver cell necrosis. In present study ALT and AST activities were assessed as it is the more specific index of liver cell damage. High level of SGOT indicates liver damage such as due to cellular damage. SGPT catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore SGPT is more specific to the liver and a better parameter for detecting liver damage (Ramamurthy *et al.*, 2014). In the present study *Staphylococcus aureus* injection significantly increased serum ALT and AST indicating induction of hepatic damage. Alcoholic extracts of *Phyllanthus niruri* at the dose of 500 mg/kg decreased the levels of both SGOT and SGPT. In the present investigation, it was observed that serum SGOT, SGPT and ALT levels were significantly reduced in animals receiving *P. niruri* and *Staphylococcus aureus* than those given *S. aureus* alone indicating that the degree of hepatic cell damage was lesser magnitude in treated groups. In conclusion, the results of present study demonstrate that extracts of *P. niruri* has potent hepatoprotective activity against *Staphylococcus aureus* induced liver damage in rats. Hence our present investigation reveals that the *P. niruri* species possess the hepatoprotective activity. The extract is non-toxic even at relatively high concentrations. The hepatoprotective activity is probably due to the presence of flavanoids. Further studies are being carried out to characterize and explore the biological activity of the compounds present in the extract.

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