

Evaluation of Ethanolic Leaf Extract of *Phyllanthus Niruri* and Its Effect on Carbon Tetrachloride Intoxicated Hepatotoxicity in Albino Rats

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ABSTRACT: Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. Living in a world of inadequately controlled environment, pollution and expanding therapy with potent drugs, it is continuously exposed to variety of xenobiotics and therapeutic agents resulting in its structural or functional damage. In an attempt to establish its pharmacological potential, we studied the hepatoprotective activity of ethanolic extract of *Phyllanthus niruri* obtained via extraction of leaves. The extract exhibited significant ($p < 0.05$) dose-dependent hepatoprotective activity for the CCl_4 induced model. In alcoholic extract of treated groups there was statistical significant decrease in the levels of serum bilirubin, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and serum alkaline phosphatase (SALP) as compared to the hepatotoxic group. In the liver by a significant reduction in the activity levels of superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, glutathione reductase and also reduced glutathione content were observed in the liver of CCl_4 intoxicated rats over the control. The alcoholic extracts of the leaves of *P. niruri* possess hepatoprotective activity, Anti-stress, antioxidant properties that may be due to cytoprotective mechanism. These results support the ethnomedical uses of the plant in the treatment of liver. However, it suggests that a dose adjustment may be necessary to optimize the effects in clinical settings.

KEYWORDS: Hepatoprotective activity, *Phyllanthus niruri*, hepatotoxicity, carbon tetra chloride, enzyme activity, Biochemical studies.

I. INTRODUCTION

Liver diseases are a serious health problem. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play an important 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. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effect. The Liver is the largest organ in the body carrying out most of the biochemical synthesis and secretory functions. It is involved in several vital functions, such as metabolism, secretion and storage. It has great capacity to detoxicate toxic substances and synthesize useful principles [1]. Liver functions as a centre of metabolism of nutrients such as carbohydrates, proteins and lipids and excretion of waste metabolites. Additionally, it also handles the metabolism and excretion of drugs and other xenobiotics from the body thereby providing protection against foreign substances by detoxifying and eliminating those [2].

Liver diseases have become a global problem. The principal causative factor for the liver diseases in developed countries is chronic alcoholism, while in the developing countries the most frequent causes are malnutrition, anemia, infection and availability of hepatotoxic drugs over the counter. Hepatotoxicity manifest as necrosis and cirrhosis.

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Hardly any effective measures are available for the treatment of liver diseases viz, corticosteroids, anti-viral and immunosuppressant agents are sometimes inadequate and may lead to serious adverse effects.

Liver disease is one of the major causes of morbidity and mortality in public, affecting humans of all ages. About 20,000 deaths occur every year due to liver disorders. Some of the commonly known disorders are viral hepatitis, alcohol liver disease, non-alcoholic fatty liver disease, autoimmune liver disease, metabolic liver disease, drug induced liver injury, gallstones, etc. Hepatocellular carcinoma is one of the ten most common tumors in the world with over 250000 new cases each year. According to WHO estimates, globally 170 million people are chronically infected with hepatitis C alone and every year 3–4 millions are newly added into the list. Also, there are more than 2 billion infected with hepatitis B virus (HBV) and over 5 million are getting infected with acute HBV annually [3].

Many compounds including clinically useful drugs can cause cellular damage through metabolic activation of the compound to highly reactive substances such as free radicals. One such toxicant is carbon tetrachloride (CCl_4); the hepatotoxicity of CCl_4 is attributed to the formation of trichloromethyl and trichloromethyl peroxy radicals, initiating lipid peroxidation and resulting in fibrosis and cell necrosis [4]. Long-term administration of CCl_4 causes chronic liver injury [5].

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 [6]. 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 been already reported to strong antioxidant activity [7-12].

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 niruri* 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 and liver disorders and for treatment of Hepatitis B viral infection. Therefore, in the present study was aimed to investigate whether injection of carbon tetrachloride induces oxidative stress and if so, *Phyllanthus niruri* reduces the CCl_4 intoxicated oxidative stress in the liver of rats.

II. MATERIALS AND METHODS

For the present study, the mature green leaves of *Phyllanthus niruri* belongs to family Euphorbiaceae were collected from in and around area of Thanjavur District, Tamil Nadu, South India. The plant was identified with the help of manual of Tamil Nadu and Karnatic flora [13-14] with standard references [15].

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Preparation of plant extract: The *Phyllanthus niruri* was collected, washed, cut into small pieces and dried at room temperature ($28\pm 1^\circ\text{C}$) for two weeks and made into powder for further analysis. The aerial parts were washed under tap water, air dried, homogenized to fine powder and stored in airtight bottles. Ten grams of dried powder was first defatted with petroleum ether and then extracted with ethanol by using Soxhlet apparatus. The solvent was evaporated to dryness and the dried crude extract was stored in air tight bottle at 4°C . The percentage yield of ethanol extract was 36%. The ethanol extract of *Phyllanthus niruri* was used for the entire study.

Experimental Animals: Adult Wistar albino rats weighing of 200 - 220 gm breed in the Central Animal House, Department of Pharmacology, Periyar College of Pharmaceutical Sciences, Trichy - 21, were used in this study. They were housed in Tarson's polypropylene cages with metal grill tops and provided with food and water *ad libitum*. They were maintained in a controlled environment under standard conditions of temperature and humidity with alternating light/dark (LD 12:12) cycle. In the laboratory, rats were fed with standard rat pellet diet. The animals used in the present study were approved by the ethical committee, Department of Pharmacology, Periyar College of Pharmaceutical Sciences, Trichy - 21, India. The Registration Number is CPCSEA/265 and was in accordance with the guidelines of the National Institute of Nutrition (NIN), Indian Council of Medical Research (ICMR), Hyderabad, India.

Acute Toxicity Study: Acute oral toxicity study was performed as per OECD-404 guidelines (1987). 10 rats/group (5 males and 5 females) were used for the study. Group 1 was control group and other three groups were that of WFM at different doses (500, 2000, and 4000 mg/kg body weight). Single dose of the extract was administered orally to each animal. Signs of toxicity, body weight, feed and water consumption of each animal was observed every day for 14 days.

Experimental design: The animals were randomly divided in to four groups, each containing three animals. 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 liver damage was induced by oral dose of CCl_4 (0.2ml/ 100g of body weight) once in every three days along with liquid paraffin as vehicle. Group – III: CCl_4 treated at a dose of 0.2ml/100mg of body weight once in every three days along with the liquid paraffin. Then the animals were treated with the ethonolic extract of *Phyllanthus niruri* daily for 15 days in physiological saline at concentration of 500mg / kg of body weight. Group – IV: CCl_4 treated as a dose of 0.2ml/100mg of body weight once in every three days along with the liquid paraffin. Then the animals were treated with Silymerin for 15 days at concentration of 2.5 mg/kg of body weight. After 15th days of treated 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^{H} 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 & Franke [116], alkaline phosphatase [17], total bilirubin [18] and protein [19] were analyzed. Reduced glutathione (GSH) was estimated using DTNB [20]. The blood glutathione was estimated by the method of Beutler et al. [21]. The catalase was determined by the methods of Aebi [22]. The activity of superoxide dismutase was assayed by the method of Woolliams [23]. The concentration of Thiobarbituric acid reactive substances (TBARS) was measured in liver using the method of Ohkawa et al. [24].

III. RESULTS AND DISCUSSION

The treatment with the extract did not decrease water and food consumption rats. 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 (100 mg/kg). This decrease can be associated with the decrease of liver weight at the dose of 100 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).

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A lot of medicinal plants, traditionally used for thousands of years, by the Indian traditional health care system (ayurvedic) named 'Rasayana' for their antioxidative properties. *Phyllanthus niruri* was a very good antioxidant and hepatoprotective agent [2, 25]. *P. niruri* (100-200mg/kg) increased cell viability of rat hepatocytes being treated with CCl₄ intoxicated rats. The present study was carried out to evaluate the hepatoprotective activity of *P. niruri* against CCl₄ induced hepatocellular degenerative in albino rats. The effectiveness of this medicinal plant was screened by assessing biochemical changes of different groups of experimental animals. *P. 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 CCl₄ treated group, indicating that CCl₄ induces damage to the liver (Table 1). Liver tissue rich in both transaminases increased in acute hepatic diseases SGPT, which is slightly elevated by cardiac necrosis is a more specific indicator of liver disease [6, 26]. A significant reduction ($P < 0.005$) was observed in SGPT, SGOT, ALP, total bilirubin and protein levels in the groups treated with silymarin and extract of *P. niruri*. The results confirmed that the enzyme levels were almost restored to the normal levels [10, 12].

The study was taken up with an aim to determine the efficacy of marketed hepatoprotective agents. The present work dealt with the comparative study of two best selling herbal hepatoprotective formulations, silymarin and *Phyllanthus niruri*. A herbal standardized extract of *P. niruri* was also evaluated for hepatoprotective activity. The herbal extract of *P. niruri* was used to determine whether addition of many plants in a formulation is superior to the use of one single herb for hepatoprotection. From the results, it is evident *P. niruri* produced better effect compared to the other herbal products. Among the herbal formulations, silymarin was more effective than other plant extract. In spite of the tremendous advances made in allopathic medicine, no effective hepatoprotective medicine is available. Plant drugs are known to play a vital role in the management of liver diseases. There are numerous plants and polyherbal formulations claimed to have hepatoprotective activities. Nearly 150 phytoconstituents from 101 plants have been claimed to possess liver protecting activity [27-30].

In India, more than 87 medicinal plants are used in different combinations in the preparation of 33 patented herbal formulations [28-30]. Some of the polyherbal formulations are verified for their hepatoprotective action against chemical induced liver damage in experimental animals [31-32]. In most of these studies, marginal or moderated levels of hepatoprotective activities were observed. It is believed that efficacy is not sufficient enough to use these agents as effective drugs [33].

Besides, most of the reported studies described the beneficial effects of the drugs against few hepatotoxic chemical-induced subclinical level of hepatotoxicity. It is not known whether or not these drugs exhibit any beneficial effects against severe liver damage. Further, the herbal products manufactured in India have been criticized by regulatory agencies in both India and abroad. Dhiman and Chawla [34] also reported that Liv 52 is not effective for treatment of alcohol induced liver damage. However, *P. niruri* was reported to be effective. In the present study the lowered level of some clinical parameters were recorded in blood sample of CCl₄ treated rats reveals the severity of hepatotoxicity. In the *P. niruri* treated group, the some clinical parameters level of animal was almost normal. This difference could probably be due to the different mechanisms by which hepatotoxicity is induced. In acute CCl₄ model, injury produced by CCl₄ is mediated by a selective metabolite like trichloromethyl free radicle (CCl₃-) formed by the haemolytic cleavage of CCl₄ or by an even more reactive species like trichloro peroxy free radicle (Cl₃COO-) formed by the reaction of CCl₃ and oxygen [35].

In case of CCl₄ (0.2 ml/kg b.wt) intoxicated rats in acute model, the extract of *Phyllanthus niruri* (100 mg/kg b.wt) and silymarin (2.5 mg/kg b.wt) prevented the increase of ALT levels and but did not prevent the increase in other biochemical marker levels and liver weight. In chronic CCl₄ model, *P. niruri* and silymarin decreased the ALT and AST levels significantly. The liver weight of *P. niruri* and silymarin pretreated animals decreased significantly.

The present study was observed that *P. niruri* has a significant hepatoprotective effect in CCl₄ intoxicated rats that hepatocellular degenerative and necrotic changes are slight without advanced fibrosis and cirrhotic process in treated group. However, Ramamurthy and Raveendran [11] found that plants can prevent liver fibrosis and cirrhosis, suggesting that the medicinal plants protect liver against fibrosis possibly through immunomodulator and antioxidant activities. Similar studies Ramamurthy and Abarna [2] observed that *P. niruri* and Ramamurthy and Gowri [25]

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observed that *Aegle marmelos* has a significant hepatoprotective effect in *S. aureus* intoxicated rats that hepatocellular degenerative and necrotic changes.

Table 1. Effect of *Phyllanthus niruri* extracts on some biochemical and serum marker enzyme parameters in CCl₄ intoxicated albino rats

Parameters	Control	CCl ₄ treated group	<i>P. niruri</i> treated group	Silymarin (2.5 mg/kg) treated group
Bilirubin (mg/dl)	0.91 ± 0.02	2.85 ± 0.06	1.75 ± 0.07	1.11 ± 0.09
Protein (g/dl)	8.10 ± 0.33	5.20 ± 0.22	7.14 ± 0.08	7.55 ± 0.04
TBARS (n moles/ml)	2.80 ± 0.22	5.52 ± 0.05	4.11 ± 0.12	4.51 ± 0.07
GSH (μ mole/g of tissue)	7.16 ± 0.21	3.15 ± 0.06	6.42 ± 0.33	6.94 ± 0.04
SGOT (IU/L)	141 ± 0.11	205 ± 0.04	166 ± 0.21	154 ± 0.08
SGPT (IU/L)	41.5 ± 0.21	97.2 ± 0.02	53.5 ± 0.41	51.2 ± 0.06
ALP (IU/L)	132 ± 0.19	222 ± 0.06	146 ± 0.16	148 ± 0.03

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 are the consequences of CCl₄ induced liver dysfunction and denotes the damage to the hepatic cells. Treatment with *P. 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. This result is support by stimulations of protein synthesis have been advanced as a contributory hepatoprotective mechanism, which accelerates the regeneration process and the protection of liver cell [1, 25].

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 is released in a similar manner. Therefore SGPT is more specific to the liver and a better parameter for detecting liver damage [1]. In the present study CCl₄ injection significantly increased serum ALT and AST indicating induction of hepatic damage. The extracts of *P. niruri* at the dose of 100 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 Silymarin than those given CCl₄ alone indicating that the degree of hepatic cell damage was lesser magnitude in treated groups.

Antioxidant activity of *Phyllanthus niruri* leaves extract was studied by LPO method and dose 100mg/kg produced significant antioxidant activity as shown in Table 2. The maximum antioxidant activity was exhibited at dose 100mg/kg in alcoholic extract of *Phyllanthus niruri*. The results of the present study suggests that co-administration of extract of *Phyllanthus niruri* ameliorates antioxidant status in CCl₄ 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 CCl₄ 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.

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, antioxidant enzyme status of 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, permeability and increase the rate of protein

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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 [34]. SOD, as a first line defence 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 CCl₄. 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 CCl₄.

Table 2. Effect of *Phyllanthus niruri* on Antioxidant status in the liver of CCl₄ intoxicated rats

S. No	Treatment group	GSH (μ mole/g of tissue)	TBARS (nmoles/ml)	LPO (μg/mg/protein)	SOD (μ mole/g of tissue)	Catalase (μ mole/g of tissue)
1	Control	8.74 ± 0.12	2.15 ± 2.51	0.55 ± 0.36	22.3 ± 1.22	9.85 ± 0.61
2	CCl ₄ treated groups	4.75 ± 0.17	4.61 ± 2.37	0.97 ± 0.17	15.5 ± 1.02	4.55 ± 0.11
3	<i>P. niruri</i> treated groups	7.15 ± 0.25	3.92 ± 2.19	0.85 ± 0.64	21.4 ± 1.07	8.25 ± 0.33
4	Silymarin (2.5 mg/kg)	7.81 ± 0.12	3.98 ± 2.21	0.91 ± 0.22	22.0 ± 1.01	8.88 ± 0.25

IV. CONCLUSION

In rats with damaged liver the treatment with *P. niruri* herb shows decrease in liver enzymes and biochemical level with extremely significant, while there is an improvement in haemoglobin level. The powder of *P. niruri* shows potent hepatoprotective activity. The *P. niruri* could become helpful for patients with damaged liver possibly by reducing liver enzymes and biochemical parameters. It increases haemoglobin level and possibly improves in life style of such patients. These finding shows that *P. niruri* extract have the ability to rectify hepatic damage or toxicity. Hence it is advised that if one happens to take any liver toxic drugs in overdose they can consume *P. niruri* extract as a hepatoprotective agent. Thus always have in mind that "Prevention is better than cure".

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