CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187

Available online at: http://www.iajps.com Research Article

PHARMACOLOGICAL EVALUATION OF *PHYLLANTHUS NIRURI* FOR HEPATOPROTECTIVE ACTIVITY IN CARBON TETRACHLORIDE-INDUCED WISTAR RATS

Gaddam Sushma
Sri Kakatiya Institute of Pharmaceutical Sciences, Unikicherla, Hanamkonda

Article Received: November 2022 **Accepted:** November 2022 **Published:** December 2022

Abstract:
In the current research, CCl4-induced Wistar rats were given an ethanolic extract of the Phyllanthus Niruri (EEPN). This extract was tested for its potential to safeguard the liver. Carbon tetrachloride (CCl4) is an initiating agent through the intraperitoneal route. Silymarin is a standard drug at a dose of 50 mg/kg body weight. The treatment consisted of giving the patient daily dosages of EEPN ranging from 250 mg/kg to 500 mg/kg for twenty days. They utilized diagnostic tools to evaluate various parameters, including some biochemical parameters such as SGOT, SGPT, ALP, TB, TC, and TG. After the experiment, the animals were slaughtered, their livers have removed, rinsed with salt water, and their weight and recorded the volume. After being preserved in formalin at a concentration of 10%, a sample of liver tissue was subsequently used for histopathological examination. A one-way analysis of variance (ANOVA) was used to conduct the statistical analysis of the data, and a Tukey post hoc multiple comparison test followed this.

Keywords: *Phyllanthus Niruri, Hepatoprotective activity, CCl4, and silymarin*

Corresponding author:
Gaddam Sushma,
Sri Kakatiya Institute of Pharmaceutical Sciences.
gaddamsushma13sg@gmail.com

QR code

Please cite this article in press Gaddam Sushma, ***Pharmacological Evaluation Of Phyllanthus Niruri For Hepatoprotective Activity In Carbon Tetrachloride-Induced Wistar Rats.,****Indo Am. J. P. Sci, 2022; 09(12).*

1. INTRODUCTION:

Liver damage caused by drugs and toxins may mimic all acute and chronic liver diseases. The pathogenesis of drug or toxin-induced liver injury usually involves the participation of toxic metabolites that either elicit an immune response or directly affect the biochemistry of the cell [(1)]. Carbon tetrachloride (CCl4) continues to be one of the most commonly used toxins in experimental studies of liver diseases. The toxicity of CCl4 has been attributed to one of several possible mechanisms, covalent metabolite binding, and lipid peroxidation [(2)].

In spite of tremendous advances made in allopathic medicine, very few synthetic drugs are available to treat liver disorders. These drugs are non-specific and often have limited efficacy in treating liver disorders [(3)]. So there is a need to follow systematic research methodology and to evaluate the scientific basis for herbal drugs used in liver disorders and also to develop a new formula that works on a scientific basis; hence a combination of different herbs or active constituents claimed to possess hepatoprotective activity may give better results [(4)].
Phyllanthus Niruri (PN) is a plant possessing several pharmacological properties. This herb has been used since ancient times to treat jaundice and other liver-related disorders. It has hepatoprotective actions against alcohol, CCl4, and thioacetamide-induced toxicity [(5)]. Therefore, the present investigation aims to evaluate the hepatoprotective activity of ethanolic extract of the whole plant of *Phyllanthus niruri* in Wistar rats.

2. MATERIALS AND METHODS:

2.1. Plant Material:

The plant *Phyllanthus niruri* (PN) was collected from surrounding areas of Hyderabad, India. A taxonomist, Dr. E. Narasimha Murthy, Professor, Department of Botany, Hyderabad, Telangana, authenticated the plant specimen.

2.2. Drugs and Chemicals:

Chemicals and reagents used were absolute ethanol (Sigma-Aldrich, Germany), 10% formalin (Novochem Engineering, India), ether (Neutron Drugs & Pharmaceuticals Pvt Ltd, Hyderabad), the standard drug silymarin (Silybon-140, Micro Lab Limited, India), CCl4 (Sigma-Aldrich, Germany), assay kits (Humana, Germany). All reagents used were of analytical grade.

2.3. Preparation of plant extracts

The *Phyllanthus niruri* was collected, washed, cut into small pieces, dried at room temperature (37^{o}C) for 14 days, and made into powder for further analysis. Extraction is a process to separate or isolate the secondary metabolites from plant material. The powdered parts of the plant were extracted with the help of the soxhlet apparatus with ethanol (75%) as a solvent. The ethanolic extract was dried and refrigerated at 4^{0}C for further usage. The ratio of the plant powder and solvents was maintained at 1:4 [(6)].

2.4. Experimental animals

Wistar rats (180-200 g) maintained under standard husbandry conditions (temperature 25±1°C, relative humidity 55 ± 10%, and 12-h light and 12-h dark cycle) were used for all experiments. Animals were allowed to be fed standard animal feed and tap water. All the pharmacological investigations were carried out only after obtaining Institutional Animal Ethical Committee (IAEC) approval.

2.5. Experimental design

Wistar rats were divided into five groups containing 6 rats (n=6). Group, I served as control, receiving dimethyl sulphoxide (DMSO) (2mL/kg b.w.) for 20 days. Other group animals were administered CCl4 intra peritoneal region 1mL/kg b.w. (1:1, v/v, CCl4 in olive oil) for the initial two days. Group II served as CCl4 treated group. Group III was given a reference drug (silymarin 50 mg/ kg b.w.) for 20 days. Group IV and V were administered PNEE (Phyllanthus niruri ethanolic extract) orally at 250 and 500 mg/kg b.w. for 20 days [(7)].

2.6. Estimation of biochemical parameters

The blood serum biochemical parameters were estimated following standard protocols. Glutamic oxaloacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) are estimated by Reitman and Frankel (1957) method, alkaline phosphatase (ALP) by King and King (1954) method, total bilirubin by Malloy and Evelyn, (1937) method. Total cholesterol (TC), total bilirubin (TB), and triglycerides (TG) were carried out using standard kits [(8)].

2.7. *Ex vivo* studies

Determination of liver weight and volume

Animals were sacrificed, and livers were isolated and washed with saline, and significance was determined using an electronic balance. The liver weights were expressed concerning its body weight. After recording the weight, all the livers were dropped individually in a measuring cylinder containing a fixed volume of distilled water or saline, and the volume displaced was recorded [(9)].

2.8. Histopathological studies

A fraction of the tissues (liver) was fixed in 10% formalin immediately after autopsy. The mixed tissues were placed in saline 10% for 60 min to resolve shrinkage owing to a higher concentration of formalin. They were left overnight in running water securing the mouths of the vessels with the cotton gauze. In ascending grades of isopropanol, the tissues were dehydrated. The dehydrated tissues were cleared in 2 changes of xylene, one hour each. Then the tissues were impregnated with histology-grade paraffin wax. The wax-permeated tissues were embedded in paraffin blocks and utilized similar quality wax. The wax blocks were mounted and incised with rotary microtome at 3-micron breadth. Tissue sections were incubated at 60ºC and cooled for five minutes [10].

2.9. Statistical analysis

The study results are expressed as the mean ± standard error of the mean (SEM). Statistical analysis of the data was performed with a one-way analysis of variance (ANOVA) followed by a Tukey post hoc multiple comparison test. Significant differences were set at p-values lower than 0.05 [11].

3. RESULTS AND DISCUSSIONS:

Various plant constituents are detected in the extract by preliminary phytochemical screening. The qualitative phytochemical investigation of the 75% ethanol extract was carried out using standardized tests to identify the presence of secondary metabolites like polyphenols, saponins, flavonoids, terpenoids, and alkaloids.

3.1. Effect of EEPN on ALP, SGOT and SGPT

The serum levels of alkaline phosphatase (ALP), serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic pyruvate transaminase (SGPT) are present in high concentration when the liver is under damage. Due to hepatocyte necrosis, these enzymes are released from the hepatic cells, and their levels in the blood increase. The rise in the SGOT is usually accompanied by an elevation in the ranks of SGPT, which play a vital role in converting amino acids to keto acids. Our present study presents the effects of EENP on the CCl4-induced hepatotoxicity of rats. There was a significant increase ($P \leq 0.05$) in serum biomarkers such as ALP, SGOT, and SGPT in CCl4 induced Wistar rats group. The results are presented in Fig 1.

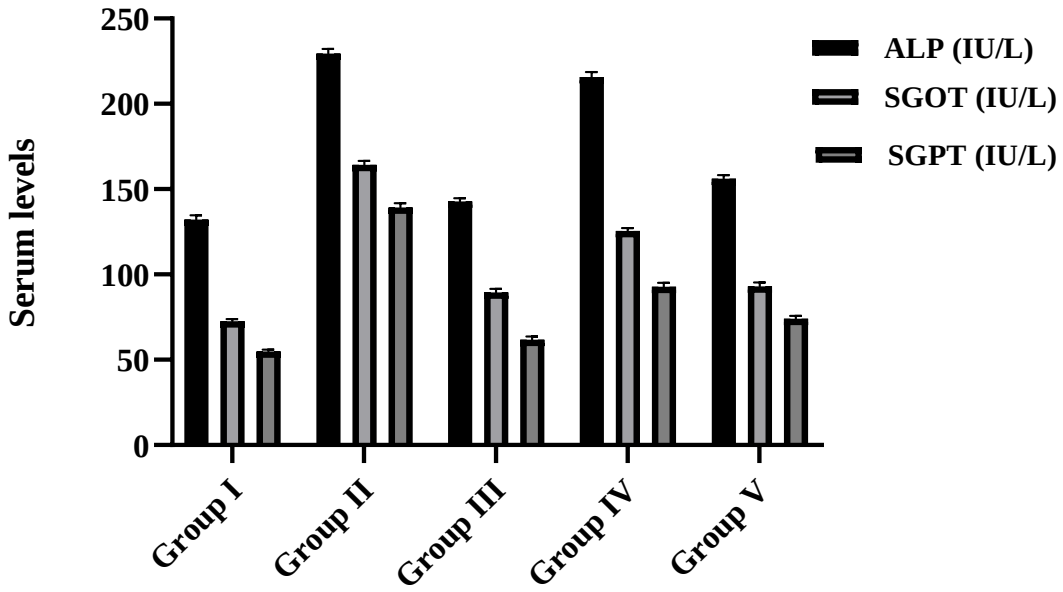

Fig 1: Effect of EE of PN on ALP, SGOT and SGPT

Data are expressed as mean ± SD (n = 6). Mean values with differences are significantly different, as revealed by the Tukey post hoc test ($P \leq 0.05$).

3.2. Effect of EEPN on TB, TG and TC

The effect of CCl4 induced on other biomarkers such as total bilirubin (TB), total cholesterol (TC), and triglycerides (TG) also got increased compared to the normal rat group. After treatment with ethanolic extract of *Phyllanthus niruri* (EEPN) (250 and 500 mg/kg b.w.), the above-mentioned biochemical markers level was brought down to near average. Treatment with a high dose of EEPN (500 mg/kg b.w.) showed a considerable hepatoprotective effect, but its impact was less than the standard drug silymarin (50 mg/kg b.w.) treated group [46]. The results are shown in Fig 2.

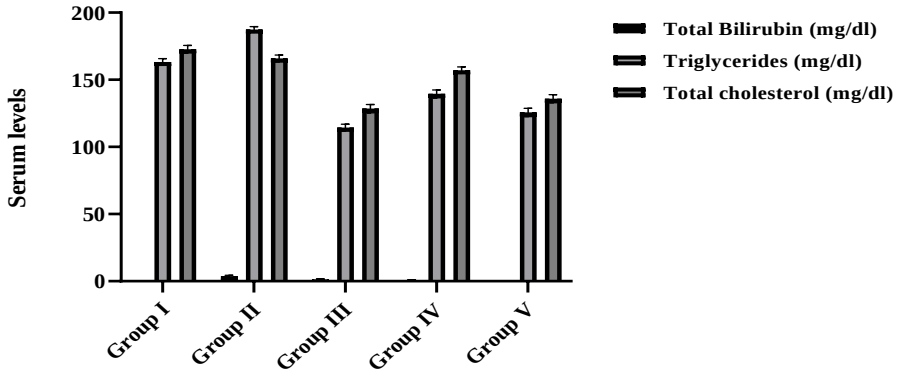

Fig 2: Effect of EE of PN on TB, TG and TC

3.3. *Ex vivo* Studies of physical parameters

Determination of liver weight and volume

The liver weights were expressed concerning its body weight. The CCl4-induced group of Wistar rats shows a liver weight of 6.37 gm & volume of 6.1 ml; silymarin shows 5.24 gm & 5.8 ml. EEPN lower dose and higher dose shows 5.71 gm & 6.0 ml and 4.73 gm & 4.8ml respectively. The results are shown in Fig 3.

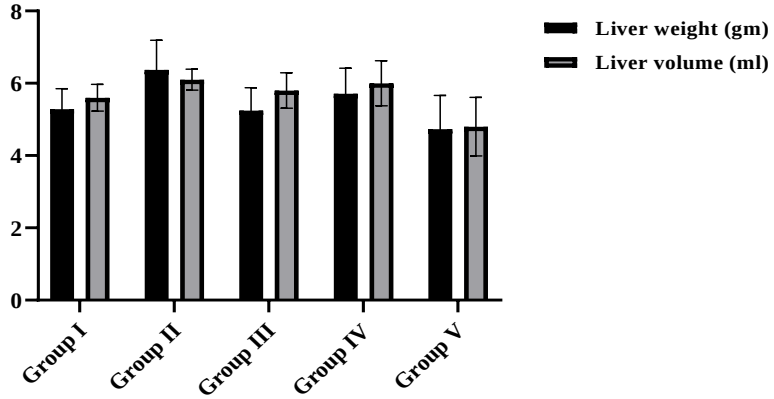

Fig 3: Effect of EE of PN on liver weight and liver volume

3.4. Histopathological studies

The hepatoprotective effect of EEPN was confirmed by histopathological assessment of the liver tissue of group I control and extracts treated Wistar rats. A liver segment of group I hold rats showed normal liver lobular architecture with a prominent nucleus and well-brought-out central vein and nucleolus. Toxic control CCl4 (2ml /kg b. w) Treated Wistar rats liver (Group II) showing severe toxicity with congested blood vessels with inflammatory cell collection and endothelial cell swelling. EEPN (250 mg/kg) treated rats (Group IV) showed only moderate inflammation. EEPN (500 mg/kg) treated rats (Group V) showed only mild inflammatory cells around the portal tract. Standard drug (Silymarin 50mg/kg) treated rats (Group III) indicate normal cellular boundaries and absence of necrosis. The results are displayed in Fig 4.

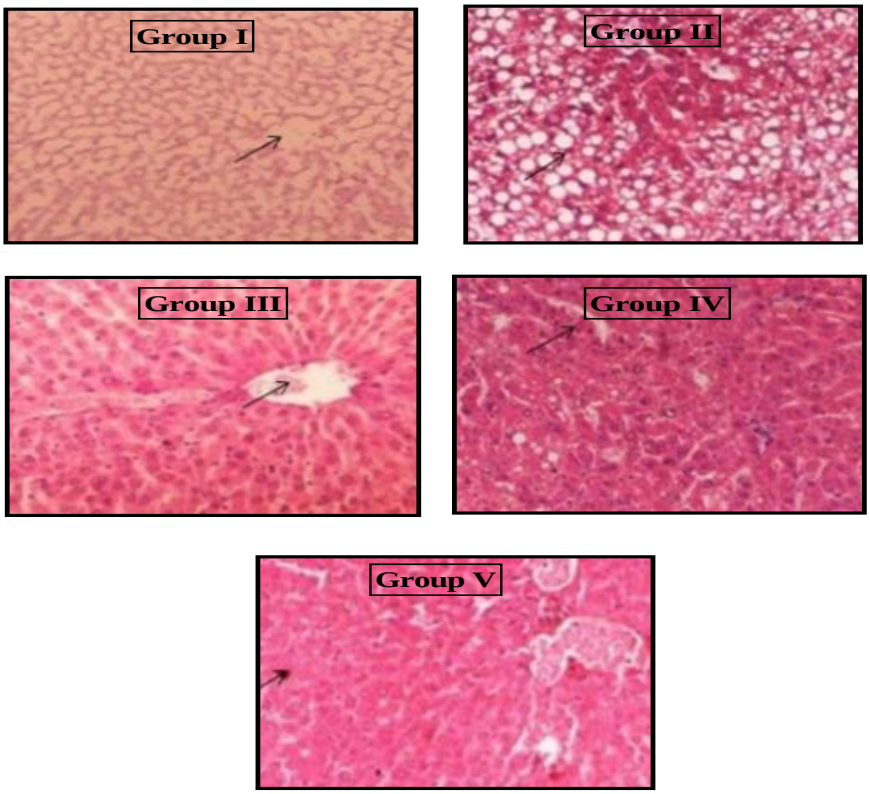

Fig 4: Histopathological analysis of Wistar rat's liver tissue

4. CONCLUSION:

The result of the present study indicates that the EEPN of the plant possesses hepatoprotective activity against CCl4-induced hepatotoxicity. SGOT, SGPT, ALP levels, and histopathological observations of test groups, compared with the CCl4-induced group. The EEPN showed good hepatoprotective activity on both doses of 250 and 500 mg/kg orally. Treatment with EEPN showed a curative effect on histological alterations in CCl4-administered rats. Hence, the presence of phytoconstituents such as flavonoids, alkaloids, and glycosides, which are present in the ethanolic extract, could be responsible for the significant hepatoprotective activity.

5. REFERENCES:

1. Chattopadhyay RR, Bandyopadhyay M. Effect of *Azadirachta indica* on serum lipid profile changes in normal and streptozotocin induced diabetic rats, *African Journal of Biomedical Research.* 2005; 8, 101-104.
2. Mohan GK, Pallavi E, Ravi Kumar B, Ramesh M, Venkatesh S. Hepatoprotective activity of Ficus caricaLinn leaf extract against carbon tetrachloride-induced hepatotoxicity in rats. *Daru.* 2007; 15:162–166.
3. Johnson DE, Kroening C. Mechanism of early carbon tetrachloride toxicity in cultured rat hepatocytes. *Pharmacol. Toxicol.* 1998; 83:231–239.
4. Saraswat B, Visen PK, Patnaik GK, Dhawan BN. Anticholestastic effect of Picroliv, active hepatoprotective principle of Picrorhiza kurroa against CCl4 induced cholestasis. *Indian J. Exp. Biol.* 1993; 31:316–318.
5. Meyer SA, Kulkarni AP. Hepatotoxicity. In: Hodgson E, Smart RC, editors. *Introduction to Biochemical Toxicology.* 3rd ed. New York: John Wiley and sons; 2001. pp. 487–490.
6. Guntupalli M, Chandana V, Pushpangadan P, Shirwaiker AI. Hepatoprotective effects of rubiadin, a major constituent of Rubia cordifolia Linn. *J. Ethnopharmacol.* 2006; 103:484–490.
7. Wu J, Danielsson A, Zern MA. Toxicity of hepatotoxins, new insights into mechanisms and therapy. Expert Opi. Investig Drugs. 1999; 8(1), 585–607.
8. Sutha D, Jegathambigai R, Kumar P, Sudhakaran S. A study on the hepatoprotective effect of Andrographis paniculata (burm.f) nees on mice. J Phytol. 2010; 2(11), 25-30.

9. Arun K, Balasubramanian U. Comparative study on hepatoprotective activity of Phyllanthus amarus and Eclipta prostrata against alcohol induced in albino rats. Inter J Environ Sci. 2011; 2(1), 361 – 379.
10. Unander DW, Webster GL, Blumberg BS. Usage and bioassays in Phyllanthus (Euphorbiaceae) IV, Clustering of antiviral uses and other effects. J Ethnopharmacol. 1995; 45, 1–18.
11. Ramamurthy V, Raveendran S. Anil Kumar HV. Hepatoprotective activity of the methanolic extract of Aegle marmelos Leaves in Paracetamol Intoxicated Albino Rats. Int. J. Universal Pharmacy and Bio Sciences. 2014; 3 (2): 1 – 10.
12. Sukumaran M, Ramamurthy V, Raveendran S, Sathick O, Akberhussain A, Boominathan M, Nethaji S, Sridharan G. Hepatoprotective activity of Wedelia Chinese on rats. J. Ecotoxicol. Environ. Monit. 2008; 18 (4): 325 - 330.