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HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF WHOLE PLANT OF PHYLLANTHUS NIRURI AGAINST CCl_4 INDUCED LIVER DAMAGE IN WISTAR RATS

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ABSTRACT

The present study received an ethanolic extract of *Phyllanthus Niruri* (EEPN) to Wistar rats induced with carbon tetrachloride (CCl_4) intraperitoneally (i.p.). This study examined the hepatoprotective properties of the whole plant extract to its ability to protect the liver. The administration of Silymarin at 40 mg/kg of b.w. is a standard treatment. The Wistar rats received EEPN, ranging from 200 mg/kg to 400 mg/kg over 20 days. They tested haematological factors such as haemoglobin (HG), red blood cells (RBC), and white blood cells (WBC), as well as biochemical factors such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), total bilirubin (TB), total cholesterol (TC), and triglycerides (TG). *Ex vivo* tests are used to determine the liver's weight and volume. The hepatic parameters of oxidative stress, namely glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT), were evaluated. After the experiment, the animals were euthanized, and their livers were extracted and washed with saline solution. The retrieved liver tissue was preserved in formalin at a concentration of 10% for histopathological analysis. The statistical analysis of the data was performed using a one-way analysis of variance (ANOVA), followed by a Tukey post hoc multiple comparison test.

KEYWORDS: Phyllanthus Niruri, Hepatoprotective activity, CCl4, Silymarin, and Oxidative stress studies.

1. INTRODUCTION

The effect of hepatoprotective has been evaluated against chemicals and drugs that induced hepatotoxicity in rats, like alcohol, carbon tetrachloride (CCl_4), galactosamine, paracetamol, isoniazid, and rifampicin, peroxidized oil, and aflatoxin. The severity of hepatotoxicity increased considerably if the drug continued after the symptoms' onset. The toxicity of CCl4 was attributed to one of several possible mechanisms, covalent metabolite binding and lipid peroxidation.[1]

Liver damage is accompanied by cellular necrosis, increased tissue lipid peroxidation, and tissue glutathione (GSH) depletion. In addition, serum levels of numerous biochemical markers are elevated, including serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT), triglycerides, cholesterol, bilirubin, and alkaline phosphatase.[2]

Despite traditional medicine's achievements, few synthetic liver medicines exist. These non-specific medicines treat liver problems poorly. Thus, systematic research methodology is needed to evaluate the scientific basis for herbal drugs used in liver disorders and develop a new formula that works scientifically.[3]

Phyllanthus niruri (PN) is a medicinal herb. For centuries, this herb has treated jaundice and other liver problems [(4)]. Therefore, the present investigation aims to evaluate the hepatoprotective activity of ethanolic extract of the whole *Phyllanthus niruri* (EEPN) plant in Wistar rats.

2. MATERIALS AND METHODS

2.1. Plant Material

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

2.2. Drugs and Chemicals

Absolute ethanol was bought from Shri Maruti Chem Enterprise Private Ltd, Mumbai. Sri Krishna Chemicals, Hyderabad, Telangana, supplied 10% formalin. Ether provided by Symax Laboratories, Hyderabad. CCl4 was sold from Meghmani Finechem Limited (MFL), and Silymarin from Alchem International Ltd. All other reagents used in the study were analytical grade.

2.3. Collection and extraction of the whole plant of *Phyllanthus niruri*

The plant was collected, washed, chopped into tiny fragments, dried at room temperature for 14 days, and powdered for analysis. Extraction isolates plant secondary metabolites. Using ethanol (80%), the soxhlet instrument extracted plant powder. Dry and chill the ethanolic extract for further usage.[5]

2.4. Animals

Healthy male Wistar rats aged 6–8 weeks weighing 200–220 g were inbred in the animal house for the experiment. The Wistar rats were housed in polypropylene cages (6 Wistar rats per cage) under standard environmental conditions and 12 hrs-12 hrs light-dark cycle. The animals were allowed free access to tap water and laboratory pellets and acclimatized to laboratory conditions for one week before the experiment.[6] The pharmacological studies have been carried out with Institutional Animal Ethical Committee (IAEC) approval.

2.5. Acute oral toxicity studies

The crude 80% ethanol extract and solvent fractions underwent OECD-recommended acute oral toxicity testing. Wistar rats were given a single oral dosage of EEPN at 100, 500, or 1000 g/kg b.w. and provided full access to food and water to assess its acute toxicity. For acute oral toxicity, the animals were monitored for 72 h for urine, salivation, asthenia, and feces [(7)]. Wistar rats showed no acute toxicity at 400 mg/kg b.w. of the extract.

2.6. Grouping and dosing of animals

The Wistar rats were divided into five groups, comprising six animals (n=6) in each group. Group, I served as control, receiving DMSO orally (2ml/kg b.w.). Group II, a positive control group, received CCl_4 (1ml/kg b.w.). Group III was treated as a standard treatment (Silymarin 40 mg/ kg b.w.). Group IV and V were administered EEPN orally at 200 and 400 mg/kg b.w. The duration of the study was 20 days. All the animals were sacrificed on the 21st day to estimate biochemical parameters.[8]

2.7. Estimation of serum biochemical parameters

The blood sample was collected under anesthesia by cardiac puncture. Blood samples were centrifuged at 3500 rpm for 15 mins at room temperature to separate serum. The clear, non-haemolysed sera were separated using a clean, dry disposable plastic syringe and stored at -20ºC for measurements of SGOT, SGPT, alkaline phosphatase, total cholesterol, total bilirubin, and triglycerides by using diagnostic kits.[9]

2.8. Estimation of hepatic oxidative stress parameters

The liver was quickly removed and perfused immediately with ice-cold saline (0.9% NaCl). A portion of the lover was homogenized in chilled Tris-HCl buffer (0.025M, pH 7.4) using a homogenizer, the homogenate obtained was centrifuged at 500 rpm for 10min; the supernatant was collected and used for analysis. The liver and kidney homogenates (10%) have been used to estimate in vivo antioxidant studies, such as GSH, SOD, and CAT.[10]

2.9. Histopathological examination of liver

The liver tissue was fixed in 10% formalin immediately after the autopsy. Histology-grade paraffin wax-soaked the tissues. The paraffin-embedded tissues used comparable wax. The wax blocks were mounted and cut with rotary microtome at 4 to 5-micron thickness, and a light microscope made histological observations.[11]

2.10. Statistical analysis

The study results are expressed as the mean ± standard error of the mean (SEM). Statistical data analysis 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.[12]

3. RESULTS AND DISCUSSIONS

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.

3.1. Acute toxicity studies

The acute toxicity test was performed on the Wistar rats, and no abnormality or mortality was seen with 100, 500, and 1000 g/kg body weight, a dose of test ethanolic extract of *Phyllanthus niruri* (EEPN) given orally. Hence the test dose was fixed as 200 and 400 g/kg body weight.[13]

3.2. Effect of EEPN on hematological parameters

The hematological parameters exhibited that the animals administrated with CCl_4 showed increased WBC and decreased HB and RBC levels. Silymarin standard drug bears normal WBC, HB, and RBC levels [(14)]. However, Wistar rats treated with ethanolic EEPN have shown a more or less similar value of WBC ($P \leq 0.05$) and HB level and increased RBC level ($P \leq 0.05$). Compared to normal group Wistar rats in a dose-dependent manner and results are shown in Fig 1.

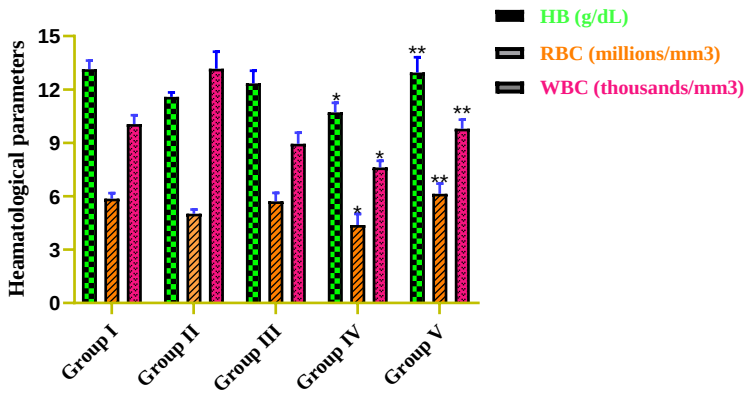

Fig 1: Effect of EEPN on hematological studies

3.3. Effect of EEPN on serum parameters

Liver injury raises serum ALP, SGOT, and SGPT levels. Hepatocyte necrosis releases these enzymes into circulation. SGOT and SGPT, which convert amino acids to keto acids, rise together. EEPN affects Wistar rats' CCl4-induced hepatotoxicity in this study. ALP, SGOT, and SGPT increased significantly ($P < 0.05$) in CCl4-induced Wistar rats.[15] The results are displayed in Fig 2.

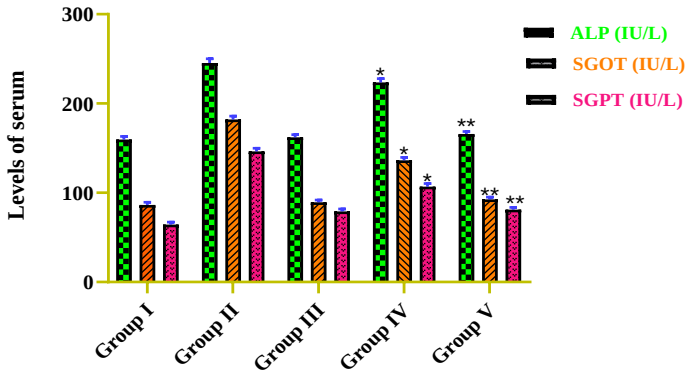

Fig 2: Effect of EEPN on ALP, SGOT and SGPT

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

3.4. Effect of EEPN on biomarkers of TB, TG, and TC

Compared to normal rats, CCl4 also raises TB, TC, and TG levels. EEPN (200 and 400 mg/kg b.w.) reduced the biochemical indicators to near average. EEPN at 400 mg/kg b.w. had a significant hepatoprotective effect; however, it was smaller than Silymarin standard treatment.[16] The results are shown in Fig 3.

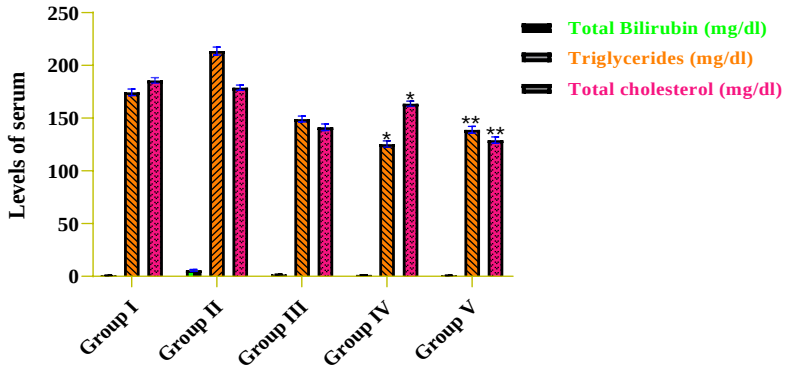

Fig 3: Effect of EEPN on total bilirubin, triglycerides and total cholesterol

3.5. Determination of liver weight and volume.

Liver weights were relative to body weight. Silymarin-induced Wistar rats have a liver weight of 6.23 gm and a volume of 6.6 ml. EEPN lower and higher doses indicate 6.89 gm and 7.5 ml and 5.73 gm and 5.3 ml, respectively.[17] The results are shown in Fig 4.

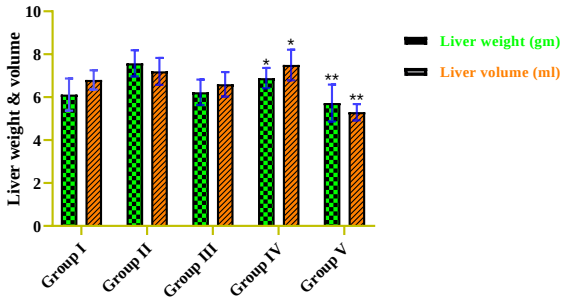

Fig 4: Effect of EEPN on liver weight and liver volume

3.6. Effect of EEPN on the indices of oxidative stress

Compared to Wistar rats in the control group, CCl4-induced rats had significantly lower SOD, CAT, and GSH levels in their liver tissue ($P \leq 0.05$). Notably, higher SOD, CAT, and GSH levels were found in rats treated with EEPN compared to those in the CCl4-administered group. Antioxidant enzyme levels were significantly ($P \leq 0.05$) higher in silymarin-treated rats compared to CCl4-treated rats.[18] The results are displayed in Fig 5.

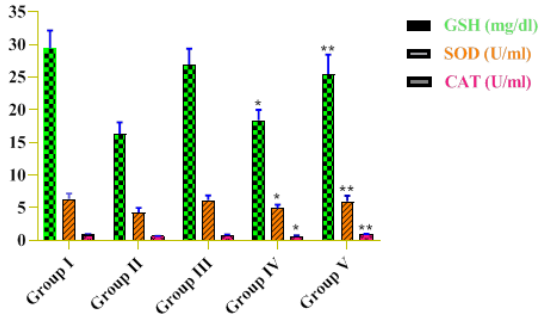

Fig 5: Effect of EEPN on hepatic oxidative stress studies

3.7. Histopathological studies

Histopathology of group I control and extract-treated Wistar rats' liver tissue confirmed EEPN hepatoprotection. Group, I rats exhibited a prominent nucleus, central vein, and nucleolus in their liver lobules. Toxic control CCl4-induced liver damage in group II Wistar rats caused occluded blood vessels, inflammatory cell collection, and endothelial cell edema. EEPN (200 mg/kg)-treated rats (Group IV) developed moderate inflammation. EEPN (400 mg/kg)-treated rats (Group V) demonstrated minimal portal tract inflammation.[19] Silymarin 40mg/kg-treated rats (Group III) exhibited normal cellular boundaries and no necrosis, and results are shown in Fig 6.

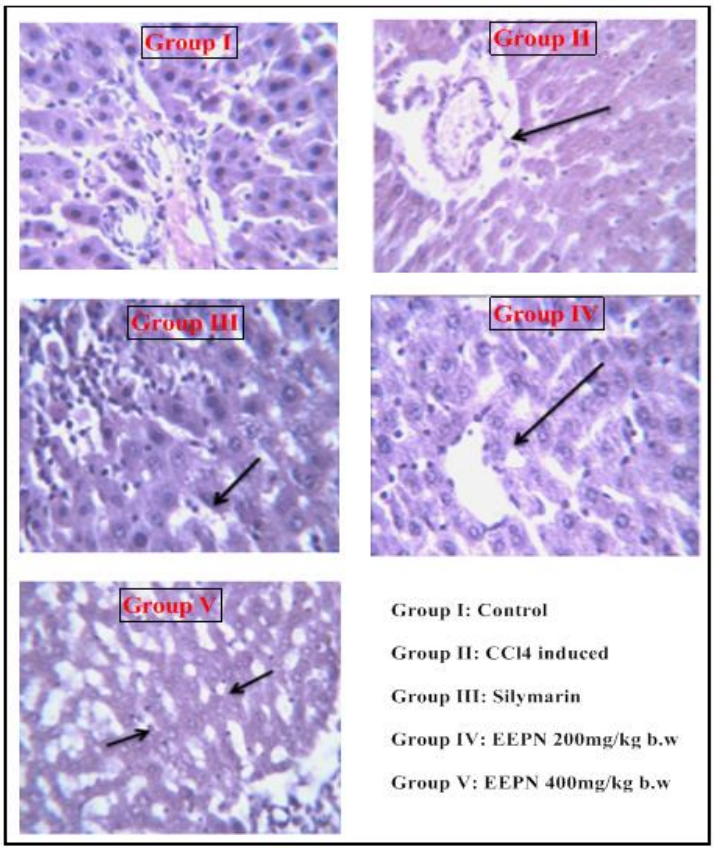

Fig. 6: Effect of EEPN on histopathology of Wistar rat's liver tissue.

4. CONCLUSION

The present study's findings suggest that the hepatoprotective effect of the whole-plant EEPN can mitigate CCl4-induced hepatotoxicity histopathological findings and SGOT, SGPT, and ALP measurements in the control groups against the CCl4-induced group. Oral administration of 200 and 400 mg/kg body weight of EEPN resulted in hepatoprotective effects. The hepatoprotective efficacy of *Phyllanthus niruri* ethanolic extract was noteworthy and on par with that of Silymarin at higher doses. The results of this research show that the EEPN plant as a whole has potent hepatoprotective action. More studies are required to prove the hepatoprotective effect.

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