EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 5
819-824
Year: 2018

HEPATO PROTECTIVE EFFECT OF *PHYLLANTHUS NIRURI* IN CCL_4 INDUCED HEPATIC DAMAGE IN MUS MUSCULUS

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Article Received on 13/03/2018 **Article Revised on 03/04/2018** **Article Accepted on 23/04/2018**

ABSTRACT
Phyllanthus niruri is commonly used in Ayurvedic system of medicine for the treatment of various diseases. Oral administration of *Phyllanthus niruri* aqueous extract (PNAE) for 2 weeks (200mg/kg, thrice in a week) produced significant hepatoprotection against carbon tetrachloride (CCl_4) induced hepatotoxicity in mice. The increased hepatic levels of thiobarbituric acid reactive substances (TBARS), total cholesterol (TC), triglycerides (TG) and total lipids (TL) in CCl_4 administered mice were observed to be decreased significantly in PNAE treated group. The PNAE treatment restored the marker enzymes and total protein levels to near normal in serum. Thus *Phyllanthus niruri (P. niruri*) could afford a significant protective effect against CCl_4 induced hepatotoxicity. The results of this study reveal that *P. niruri* can be one of the major therapies to cure liver diseases.

KEYWORDS: *Phyllanthus niruri*, Lipid peroxidation, Total cholesterol, Total protein, Triglycerides, Marker enzymes.

INTRODUCTION
Liver is the largest glandular organ of the body which plays a vital role in metabolizing the carbohydrates, lipids, proteins and detoxifying xenobiotics and drugs.[1] It is the site where waste products of metabolism are detoxified. It is responsible for synthesizing lipoproteins, plasma proteins, bile, etc. It maintains a stable blood glucose level by glycogenesis, glycogenolysis and gluconeogenesis. Liver damage is a widespread disease and it is characterized by a progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis and hepatocellular carcinoma.[2]

Hepatotoxicity is defined as an injury to the liver that is associated with an impaired liver function caused by exposure to a drug or another non-infectious agent.[3] It is caused by many xenobiotics, such as alcohol and other medicines, infection, malnutrition and anaemia.[4] CCl_4 is a xenobiotic introduced into the water mainly as industrial wastes from dry cleaning fluids, chlorofluorocarbons, fire extinguishing agents manufacturing industry and it produce hepatotoxicity in human beings and animals. Centrilobular necrosis followed by hepatic cirrhosis is the common characteristic of CCl_4 mediated liver injury. The metabolism of CCl_4 in the liver leads to hepatotoxicity.[5] CCl_4 increases intercellular Ca^{2+} concentration and activates Kupffer cells, which inturn release harmful cytokines and leads to hepatic cell death.[6]

Asian countries have a long tradition of using herbals as medicine.[7] Ancient systems of medicine have used *Phyllanthus* plants in treating liver diseases. *Phyllanthus* have many biological activities which are due to the presence of flavonoids, terpenes, benzoids, lignins, lipids, vitamin C, and steroids. *P. niruri* is an Indian herb and it belongs to the family Euphorbiacea.[8] Most of the studies have demonstrated the protective effects of *P. niruri* against experimentally induced liver injury. Here, an attempt has been made to investigate hepatoprotective activity of aqueous extract of *P. niruri* (PNAE) against CCl_4 induced liver toxicity in mice.

MATERIALS AND METHODS
Plant material
Phyllanthus niruri whole herb was collected from Angamaly, Ernakulam, Kerala. Plant was collected and kept in sunshade for a week. The shade dried plant material was powdered coarsely and used for further analysis.

Extract Preparation
Phyllanthus niruri aqueous extract (PNAE) was prepared according to the standard procedure.[9] The filtered extracts were dried in a vacuum evaporator. The extract was stored in an airtight container and dissolved in water just before use. 1g of extract was dissolved in 25 ml of distilled water.

Animals

Female Swiss albino mice of average weight of about 30-40 g bred in the animal house of Little Flower Medical Research Centre, Angamaly, Ernakulam, Kerala were used for the experiment. The animals were kept at a room temperature of 26±2 °C and humidity of 56%–58%. They were fed with standard diet and water.

Induction of hepatotoxicity

The animals were divided into 3 groups of 6 animals each. Group I was given saline (10 ml/kg body wt/day, p.o.) serving as the normal control set. Group II received CCl_4 in coconut oil (1:1, 1 ml/kg body wt, p.o.) thrice in a week for 2 weeks. Group III received PNAE (200 mg/kg body wt, p.o.), thrice in a week for 2 weeks, simultaneously with equal mixture of CCl_4 and coconut oil. After 2 weeks of treatment, animals were sacrificed. Blood was collected and serum was separated from clotted blood by centrifugation at 2500 rpm for 15 min and biochemical investigations were done. The liver of the animals from all groups were excised and lipid levels were measured.

Biochemical parameters

Serum hepatic marker enzymes namely, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were measured by commercial kits (Span Diagnostic, Surat). Determination of protein in serum was done by Biuret method using diagnostic kit supplied by Agappe diagnostics, Maharashtra. Hepatic TC, TG and TL levels were measured using assay kits (Sigma Aldrich, Bangalore, India). LPO (Lipid peroxidation) levels in liver tissues were assayed by colorimetric determination of TBARS according to the method of Ohkawa *et al*., 1979.[10] All other chemicals used in this study were obtained commercially and were of analytical grade.

Histopathological examination

Fresh Liver tissue of each animal were dissected out and then fixed in buffered formalin for 12 hours and processed for histopathological examination. Four µm-thick paraffin sections were stained with hematoxylin and eosin for light microscope examination using conventional protocol.

Statistical analysis

All data are represented as mean ± SD. One-way analysis of variance (ANOVA) followed by Duncan multiple range test was done to determine significant differences in all parameters. $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

Marker Enzymes in Serum

Serum AST, ALT and ALP are the most sensitive markers employed in the diagnosis of hepatic damage, because these are cytoplasmic in location and are released into the circulation after cellular damage.[11] High serum concentrations of transaminases are taken as an index of hepatic injury where elevation of ALT is regarded as a more sensitive indicator.[12] Elevated activity of transaminases in serum might be due to the release of these enzymes from the cytoplasm, into the blood circulation rapidly after rupture of the plasma membrane and cellular damage. The activities of marker enzymes such as AST, ALT and ALP in the serum of control and experimental groups of mice are shown in fig. 1. In the present investigation, CCl_4 induced hepatocellular damage is clearly evidenced by the marked elevation in the activities of AST, ALT and ALP in serum. Administration of PNAE (200 mg/kg b.wt) significantly reduced the CCl_4 induced elevated levels of marker enzymes.

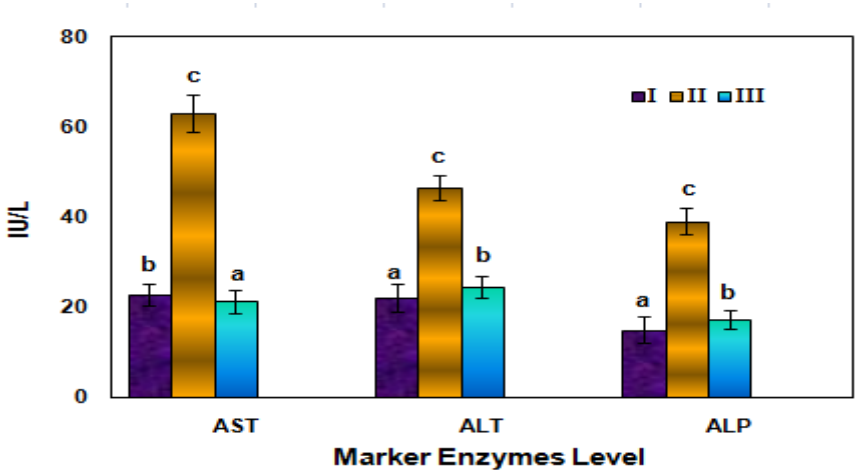


Fig. 1: Effect of PNAE on the restoration of liver function markers in serum in CCl_4-intoxicated mice. Valves are mean ± SD (n=6). Values not sharing a common letter differ significantly at P<0.05 by DMRT.

Serum Total Protein

The total protein levels will be depressed in hepatotoxic conditions due to defective protein biosynthesis in liver.[13] Fig. 2 shows the effect of PNAE on the levels of serum total protein in CCl_4 induced mice. Levels of serum protein decreased significantly ($P<0.05$) in CCl_4

treated mice compared to normal. Decreased serum total protein demonstrates the decreased functional ability of the liver and this is a sign of poor health. Stabilization of serum protein levels through the administration of PNAE is further a clear indication of the improvement of the functional status of the liver cells.[14]

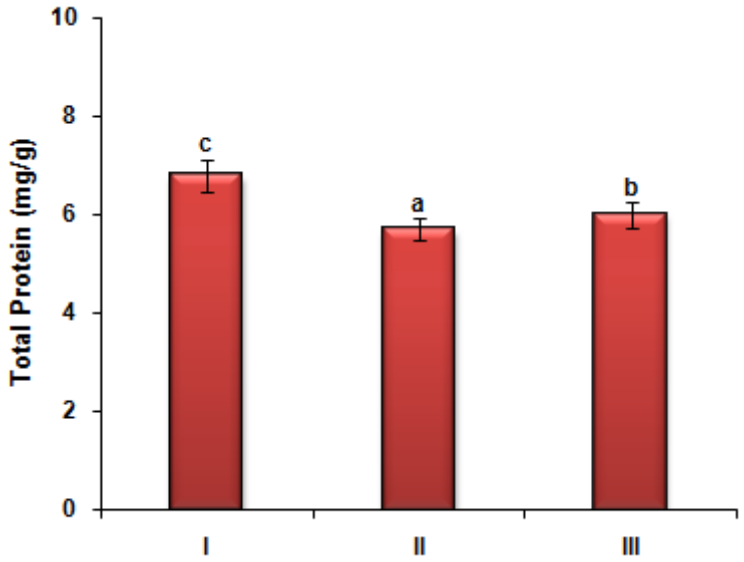


Fig. 2: Effect of PNAE on the level of total protein in serum in CCl_4-intoxicated mice. Values not sharing a common letter differ significantly at P<0.05 by DMRT.

Hepatic Lipid Profile

The liver is the major site for the synthesis and metabolism of cholesterol, bile acids and phospholipids.[15] Fig. 3 demonstrates the effect of PNAE on the levels of hepatic TC and TG in CCl_4 induced mice and fig. 4 demonstrates the levels of hepatic TL in all experimental groups. The above altered lipid profiles were significantly (P<0.05) reverted back to near normal levels in PNAE treated groups (200 mg/kg b.wt). The increase in cholesterol level increases the membrane fluidity, regulates membrane permeability and alters internal viscosity and also the internal chemical composition.[16]

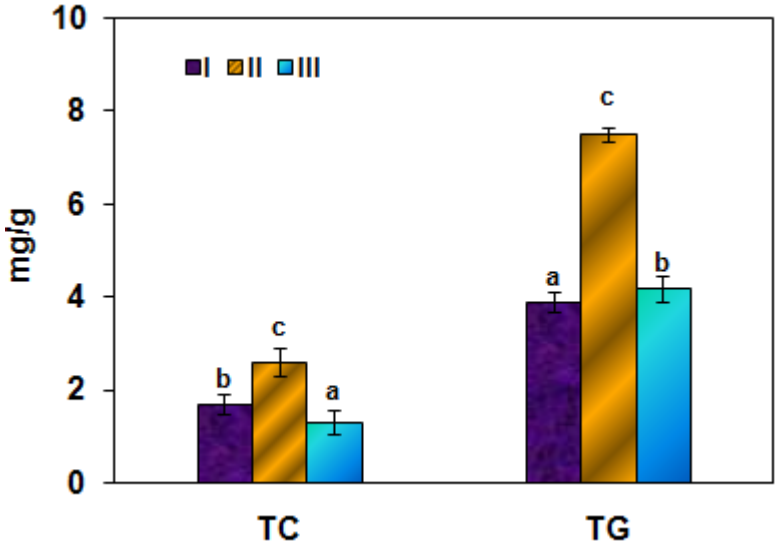


Fig. 3: Effect of PNAE on the hepatic TC and TG level in CCl_4-intoxicated mice. Values not sharing a common letter differ significantly at P<0.05 by DMRT.

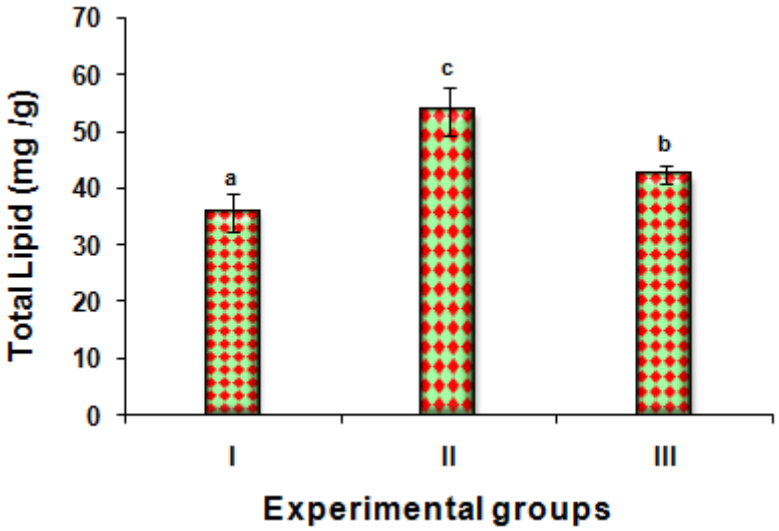


Fig. 4: Effect of PNAE on the hepatic total lipid in CCl_4-intoxicated mice. Values not sharing a common letter differ significantly at P<0.05 by DMRT.

Lipid Peroxidation

Lipid peroxidation in the tissue homogenate was measured by estimating the formation of TBARS.[17] CCl_4 increased the hepatic TBARS concentration significantly which was inhibited by PNAE treatment. PNAE was most effective in inhibiting the hepatic lipid peroxidation (Fig. 5).

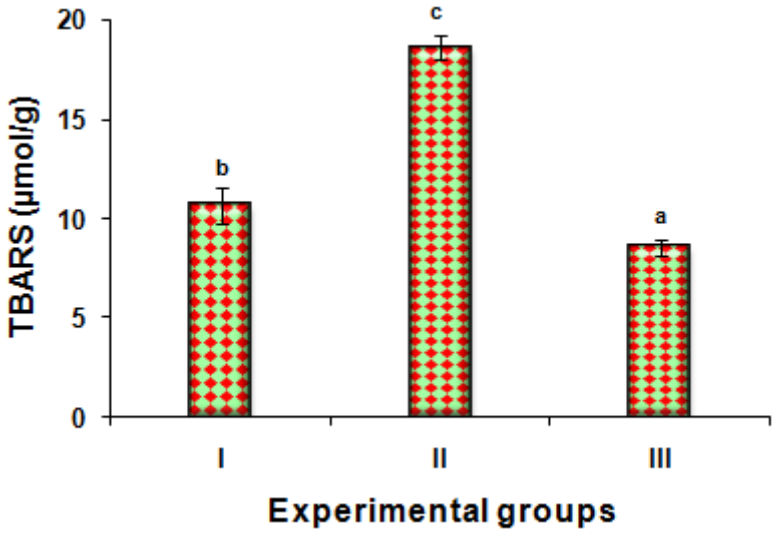
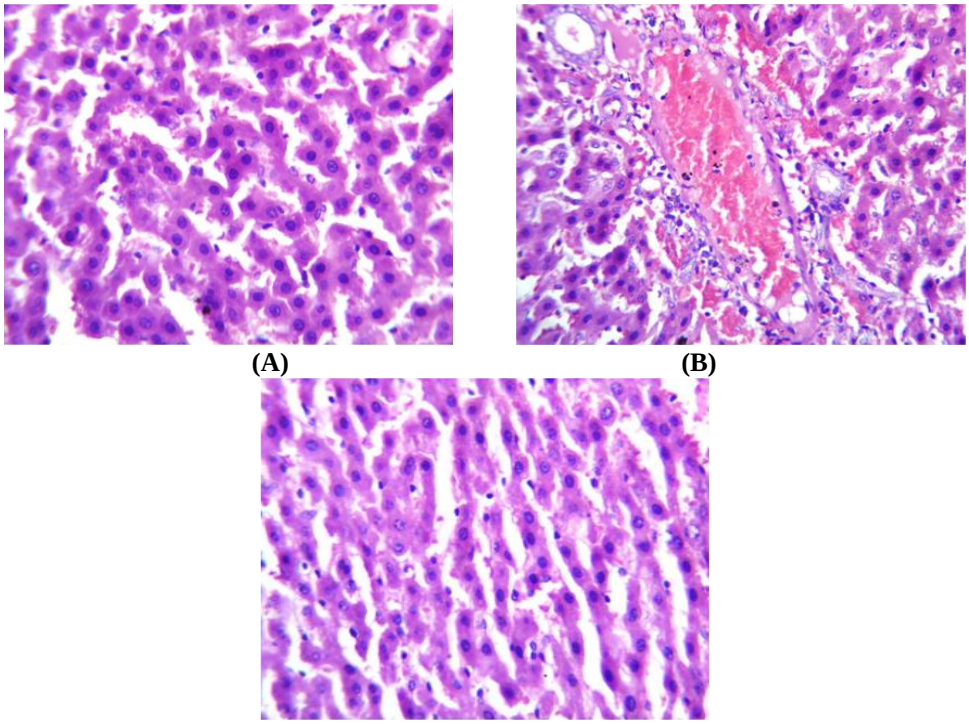


Fig. 5: Effect of PNAE on the hepatic TBARS level in CCl_4-intoxicated mice. Values not sharing a common letter differ significantly at P<0.05 by DMRT.

Histopathological Studies

Fig. 6 shows photomicrographs of hematoxylin eosin stained liver tissues. Liver section of normal control mice showed normal architecture (Fig. 6-A) with well-preserved cytoplasm, a prominent nucleus, and a compact arrangement of hepatocytes with no fatty infiltration. In contrast, we observed the massive presence of necrotic lesions, fatty infiltration and fibrosis in liver sections from CCl_4 treated mice (Fig. 6-B). The presence of liver injury of CCl_4 treated mice was revealed by histopathological examinations. Liver section of mice treated with CCl_4 and PNAE showing an absence of necrosis and inflammatory cells, a hepatic cell with prominent cytoplasm, nucleus and visible central veins (Fig. 6-C).



(A) **(B)**

(C)

Fig. 6: Photomicrographs of histopathological changes in the liver of CCl_4 intoxicated mice. A) Normal control B) CCl_4 induced C) CCl_4 + 200 mg/kg b.wt PNAE treated.

CONCLUSION

In conclusion, this study demonstrated that PNAE possesses hepatoprotective properties and protect the liver from hepatic damage induced by CCl_4. The hepatoprotective effects of PNAE may be due to its antioxidant and anti-inflammatory nature.

REFERENCES

1. Vitaglione P, Morisco F, Caporaso N, Fogliano V. Dietary antioxidant compounds and liver health. Crit Rev Food Sci Nutr, 2004; 44: 575-86.
2. Ferreira EA, Gris EF, Felipe KB, Correia JFG, Ferreira EC, Filho DW, Pedrosa RC. Potent hepatoprotective effect in CCl_4-induced hepatic injury in mice of phloroacetophenone from *Myrcia multiflora*. Libyan J Med, 2010; 5: 1-10.
3. Yang J, Li Y, Wang F, Wu C. Hepatoprotective effects of apple polyphenols on CCl_4-induced acute liver damage in mice. J Agri Food Chem, 2010; 58: 6525-31.
4. Muriel P, Rivera-Espinoza Y. Beneficial drugs for liver disease. J Appl Toxicol, 2008; 28: 93-103.
5. Amat N, Upur H, Blazekovic B. *In vivo* hepatoprotective activity of the aqueous extract of *Artemisia absinthium L.* against chemically and immunologically induced liver injuries in mice. J Ethnopharm, 2010; 131: 478-84.
6. Domitrovic R, Jakovac H, Blagojevic G. Hepatoprotective activity of berberine is mediated by inhibition of TNF-α, COX-2 and iNOS expression in CCl_4 intoxicated mice. Toxicology, 2011; 280: 33-43.
7. Hemannt D, Chandewar AV. A review on pharmacognostical, phytochemical, pharmacological properties of *Phyllanthu amarus*. Intern J Biomed Adv Res, 2013; 4(5): 280-8.
8. Gami B and Kotharil. Antioxidants and antimicrobial activity of *in vivo* and *in vitro* growth of *Phyllanthus nirruri linn*. Intern J Pharma Biosci, 2011; 2(2): 78-89.
9. Fabian C, Amaechina, Eric K. Omogbai. Hypotensive effect of aqueous extract of the leaves of *Phyllanthus amarus Schum* and *Thonn* (Euphorbiaceae). Acta Poloniae Pharmaceutica-Drug Research, 2007; 64(6): 547-52.
10. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem, 1979; 95: 351-8.
11. Sallie R, Tredger JM, Willam R. Drugs and the liver. Biopharm Drug Dispos. 1991; 12: 251-9.
12. Zimmerman HJ. Hepatotoxicity. Disease-a-Month, 1993; 39: 675-787.
13. Clawson GA. Mechanisms of carbon tetrachloride hepatotoxicity. Pathol Immunopathol Res, 1989; 8: 104-12.
14. Friedman RB, Anderson RE, Entine SM, Hirshberg SB. Effects of diseases on clinical laboratory tests. Clin Chem, 1980; 26: 471-6.
15. Taniguchi H, Yomota E, Nogi K, Onoda Y. Effects of antiulcer agents on ethanol induced gastric mucosal lesion in D-Gal induced hepatitis rats. Drug Res, 2004; 52: 600-4.
16. Sathish AM, Pari L, Sathish CY. Antioxidants effects of *Boerhavia diffusa Linn* in tissues of

alloxan induced rats. Ind J Exp Biol, 2003; 42: 989-92.

17. Okhawa H, Ohishi N, Yagi K. Assay of lipid peroxides in animal tissue by thiobarbituric acid reaction. Anal Biochem, 1979; 95: 351-8.