

Selected published scientific reports on *Phyllanthus niruri* / *amarus* (and related species)

J Pharm Pharmacol. 2006 Dec;58(12):1559-70.

Phytochemicals from *Phyllanthus niruri* Linn. and their pharmacological properties: a review.

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This review discusses the medicinal plant *Phyllanthus niruri* Linn. (Euphorbiaceae), its wide variety of phytochemicals and their pharmacological properties. The active phytochemicals, flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, coumarins and saponins, have been identified from various parts of *P. niruri*. Extracts of this herb have been proven to have therapeutic effects in many clinical studies. Some of the most intriguing therapeutic properties include anti-hepatotoxic, anti-lithic, anti-hypertensive, anti-HIV and anti-hepatitis B. Therefore, studies relating to chemical characteristics and structural properties of the bioactive phytochemicals found in *P. niruri* are very useful for further research on this plant as many of the phytochemicals have shown preclinical therapeutic efficacies for a wide range of human diseases, including HIV/AIDS and hepatitis B. PMID: 17331318

Food Chem Toxicol. 2007 May;45(5):817-26. Epub 2006 Nov 11.

Protein isolate from the herb, *Phyllanthus niruri* L. (Euphorbiaceae), plays hepatoprotective role against carbon tetrachloride induced liver damage via its antioxidant properties.

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Department of Chemistry, Bose Institute, West Bengal, India.

Phyllanthus niruri L. (Euphorbiaceae) (*P. niruri*) is a well-known hepatoprotective herbal plant. In the present study, hepatoprotective potential of the protein isolate of *P. niruri* was investigated against carbon tetrachloride (CCl₄) induced liver damage in vivo. Protein isolate of *P. niruri* was intraperitoneally injected in mice either prior to (preventive) or after the induction of toxicity (curative). Levels of different liver marker enzymes in serum and different anti-oxidant enzymes, as well as lipid peroxidation products and glutathione (GSH) in liver homogenates were measured in normal, control (toxicity induced) and protein isolate treated mice. Administration of CCl₄ increased the serum glutamate pyruvate transaminase (GPT) and alkaline phosphatase (ALP) levels of mice sera along with increased lipid peroxidation and reduced levels of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) in the liver. Treatment with the protein isolate of *P. niruri* significantly altered these changes to almost normal. The protein isolate also showed protective properties as was evidenced in histopathological studies. Results suggest that the protein isolate of *P. niruri* protects liver tissues against oxidative damage and somehow helps stimulating repair mechanism present in liver. It could be used as an effective hepatoprotector against CCl₄ induced liver damage. PMID: 17175085

Indian J Biochem Biophys. 2006 Oct;43(5):299-305.

Hepatoprotective effect of aqueous extract of *Phyllanthus niruri* on nimesulide-induced oxidative stress in vivo.

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Nimesulide (NIM), an atypical non-steroidal anti-inflammatory drug (NSAID) is also used as analgesic. In the present study, we evaluated its effect on the prooxidant-antioxidant system of liver and the hepatoprotective potential of aqueous extract of the herb *Phyllanthus niruri* (PN) on NIM-induced oxidative

stress in vivo using a murine model, by determining the activities of hepatic anti-oxidant enzymes superoxide dismutase (SOD) and catalase (CAT), levels of reduced glutathione (GSH) and lipid peroxidation (expressed as malonaldehyde, MDA). Aqueous extract of PN at a dose of 50 or 100 mg/kg body wt was administered either intraperitoneally or orally for 7 days, before NIM administration at a dose of 8 mg/kg body wt twice daily for 7 days in mice. Animals were sacrificed 24 h after administration of final dose of NIM. In another set of experiments, both aqueous extract of PN (at a dose of 50 or 100 mg/kg body wt) and NIM (8 mg/kg body wt) were administered simultaneously for 7 days. Animals were sacrificed 24 h after administration of final dose of the extract and NIM, liver tissues were collected, and the activities of SOD and CAT and levels of GSH and lipid peroxidation end-product (as MDA), were determined from the livers of all the experimental animals. Appropriate NIM control was maintained for all sets of experiments. NIM administration (8 mg/kg body wt) for 7 days caused significant depletion of the levels of SOD, CAT and reduced GSH, along with the increased levels of lipid peroxidation. Intraperitoneal administration of the extract at a dose of 50 mg/kg body wt for 7 days, prior to NIM treatment, significantly restored most of the NIM-induced changes and the effect was comparable to that obtained by administering 100 mg/kg body wt of the extract orally. Thus, results suggested that intraperitoneal administration of the extract could protect liver from NIM-induced hepatic damage more effectively than oral administration. Antioxidant property of the aqueous extract of PN was also compared with that of a known potent antioxidant, vitamin E. The PN extract at a dose of 100 mg/kg body wt along with NIM was more effective in suppressing the oxidative damage than the PN extract at a dose of 50 mg/kg body wt. Results suggested that beneficial effect of the aqueous extract of PN, probably through its antioxidant property, might control the NIM-induced oxidative stress in the liver.

PMID: 17133737

J Ethnopharmacol. 2007 Apr 4;110(3):555-8. Epub 2006 Oct 13.

The in vitro activity of geraniin and 1,3,4,6-tetra-O-galloyl-beta-D-glucose isolated from Phyllanthus urinaria against herpes simplex virus type 1 and type 2 infection.

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Phyllanthus urinaria Linnaea (Euphorbiaceae) is a widely used traditional medicinal plant by oriental countries and has been reported to possess various biological activities. Previously, the acetone extract from *Phyllanthus urinaria* was found to inhibit herpes simplex virus (HSV) infection. In this study, geraniin and 1,3,4,6-tetra-O-galloyl-beta-D-glucose (1346TOGDG), both of which were isolated from the acetone extract of *Phyllanthus urinaria*, were examined for their activity against HSV-1 and HSV-2 in vitro. Results showed that geraniin actively suppressed HSV-2 infection, whereas 1346TOGDG effectively inhibited HSV-1 infection. The 50% inhibitory concentration (IC₅₀) was 18.4±2.0 µM for geraniin against HSV-2 infection, and 19.2±4.0 µM for 1346TOGDG against HSV-1. No toxic effect towards the host cell was observed at the antiviral concentrations. In conclusion, geraniin and 1346TOGDG were found to inhibit HSV-1 and HSV-2 multiplication at different magnitudes of potency. PMID: 17113739

Urol Res. 2006 Aug 1; [Epub ahead of print]

Effect of extract of Phyllanthus niruri on crystal deposition in experimental urolithiasis.

Barros ME, Lima R, Mercuri LP, Matos JR, Schor N, Boim MA.

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Phyllanthus niruri (Pn) is a plant that has been shown to interfere in the growth and aggregation of calcium oxalate (CaOx) crystals. In the present study we evaluated the effect of Pn on the preformed calculus induced by introduction of a CaOx seed into the bladder of male Wistar rats. Pn treatment (5 mg/rat/day) was initiated immediately or 30 days after CaOx seeding and thus in the presence of a preformed calculus. Animals were sacrificed 50 or 70 days after surgery. The resulting calculi were weighed and analyzed by X-ray diffraction, stereomicroscopy and scanning electronic microscopy. Precocious Pn treatment reduced the number (75%, $P < 0.05$) and the weight (65%, $P < 0.05$) of calculi that frequently exhibited a matrix-like material on its surface, compared to the untreated CaOx group. In contrast, Pn treatment in the presence of a preformed calculus did not prevent further calculus growth; rather, it caused an impressive modification in its appearance and texture. Calculi from Pn-treated animals had a smoother, homogeneous surface compared to the spicule shape of calculi found in the untreated CaOx group. XRD analysis revealed the precipitation of struvite crystals over the CaOx seed and Pn did not change the crystalline composition of the calculi. This suggests that Pn interfered with the arrangement of the precipitating crystals, probably by modifying the crystal-crystal and/or crystal-matrix interactions. Results suggest that Pn may have a therapeutic potential, since it was able to modify the shape and texture of calculi to a smoother and probably more fragile form, which could contribute to elimination and/or dissolution of calculi. PMID: 16896689

J Urol. 2006 Sep;176(3):1020-2.

Can Phyllanthus niruri affect the efficacy of extracorporeal shock wave lithotripsy for renal stones? A randomized, prospective, long-term study.

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PURPOSE: Phyllanthus niruri is a plant used in Brazilian folk medicine for the treatment of urolithiasis. We assessed the efficacy of P. niruri after extracorporeal shock wave lithotripsy for renal stones. **MATERIALS AND METHODS:** We prospectively evaluated 150 patients with renal stones that were as large as 25 mm and composed of calcium oxalate. All patients received 1 to 3 extracorporeal shock wave lithotripsy sessions by Dornier Lithotripter S. After treatment 78 of 150 patients (52%) underwent therapy with Uriston, a P. niruri extract (2 gm daily) for at least 3 months (group 1). Otherwise 72 of 150 patients (48%) were used as a control group (group 2). No significant difference in stone size between the 2 groups was found. Stone clearance was assessed after 30, 60, 90 and 180 days by abdominal x-ray and ultrasound scan. **RESULTS:** Stone-free rate (stone-free defined as the absence of any stone or residual fragments less than 3 mm) was 93.5% in group 1 and 83.3% in group 2 ($p = 0.48$) at the end point of the followup (180 days). For lower caliceal stones (56 patients) the stone-free rate was 93.7% in the treatment group and 70.8% in the control group ($p = 0.01$). Re-treatment need for group 1 was 39.7% and for group 2 it was 43.3% ($p = 0.2$). No side effects were recorded with extracorporeal shock wave lithotripsy or P. niruri therapy. **CONCLUSIONS:** Regular self-administration of P. niruri after extracorporeal shock wave lithotripsy for renal stones results in an increased stone-free rate that appears statistically significant for lower caliceal location. Its efficacy and the absolute lack of side effects make this therapy suitable to improve overall outcomes after extracorporeal shock wave lithotripsy for lower pole stones. PMID: 16890682

Phytother Res. 2006 Jul;20(7):595-601.

The protein fraction of Phyllanthus niruri plays a protective role against acetaminophen induced hepatic disorder via its antioxidant properties.

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The aim of this study was to investigate the hepatoprotective action of the protein fraction of *Phyllanthus niruri* against acetaminophen (APAP) hepatotoxicity. The partially purified protein fraction of *P. niruri* was injected intraperitoneally in mice either prior to (preventive) or after the induction of toxicity (curative). Levels of different liver marker enzymes in serum and different antioxidant enzymes, as well as lipid peroxidation in total liver homogenates were measured in normal, control (toxicity induced) and *P. niruri* protein fraction-treated mice. *P. niruri* significantly reduced the elevated glutamate pyruvate transaminase (GPT) and alkaline phosphatase (ALP) levels in the sera of toxicity induced mice, compared with the control group. Lipid peroxidation levels were also reduced in mice treated with *P. niruri* protein fraction compared with the APAP treated control group. Among the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) levels were restored to almost normal levels compared with the control group. *P. niruri* treatment also enhanced reduced hepatic glutathione (GSH) levels caused by APAP administration. The results demonstrated that the protein fraction of *P. niruri* protected liver tissues against oxidative stress in mice, probably acting by increasing antioxidative defense. PMID: 16718736

Am J Chin Med. 2006;34(3):471-82.

Hepatoprotective effect of Phyllanthus in Taiwan on acute liver damage induced by carbon tetrachloride.

Lee CY, Peng WH, Cheng HY, Chen FN, Lai MT, Chiu TH.

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The effect of oral administration of *Phyllanthus* methanolic extracts (PME) (i.e. *P. acidus*, *P. emblica*, *P. myrtifolius*, *P. multiflorus*, *P. amarus*, *P. debilis*, *P. embergeri*, *P. hookeri*, *P. tenellus*, *P. urinaria* L.s. *nudicarpus*, *P. urinaria* L.s. *urinaria*) or gallic acid (GA) on the progression of acute liver damage induced by CCl₄ in rats was examined by morphological and biochemical methods. *P. acidus*, *P. urinaria* L.s. *urinaria*, GA at a dose of 0.5 g/kg, and *P. emblica*, *P. urinaria* L.s. *nudicarpus* at a dose of 1.0 g/kg attenuated CCl₄-induced increase in serum glutamate-oxalate-transaminase (GOT). *P. acidus*, *P. urinaria* L.s. *nudicarpus*, *P. urinaria* L.s. *urinaria*, GA at a dose of 0.5 g/kg, and *P. emblica*, *P. amarus*, *P. hookeri*, *P. tenellus* at a dose of 1.0 g/kg attenuated CCl₄-induced increase in serum glutamate-pyruvate-transaminase (GPT). Concurrently, *P. acidus*, *P. multiflorus*, *P. embergeri*, *P. hookeri*, *P. tenellus* and *P. urinaria* L.s. *urinaria* elevated the activity of liver reduced glutathione peroxidase (GSH-Px). Since the protective effects of *P. acidus*, *P. emblica*, *P. myrtifolius*, *P. embergeri*, *P. urinaria* L.s. *nudicarpus*, *P. urinaria* L.s. *urinaria* and GA correlate with a reduction in liver infiltration and focal necrosis observed using histological methods, these data demonstrate that *P. acidus* and *P. urinaria* L.s. *urinaria* are hepatoprotective and antioxidant agents. PMID: 16710896

Int Immunopharmacol. 2006 Jun;6(6):870-9. Epub 2006 Jan 30.

Anti-tumor and anti-angiogenic effects of Phyllanthus urinaria in mice bearing Lewis lung carcinoma.

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Phyllanthus urinaria, a widely used herb medicine in Asia, was tested for its anti-tumor effect in vivo for the first time. The anti-tumor activity in *P. urinaria* extract was evaluated by its effect on tumor developed in C57BL/6J mice with implantation of Lewis lung carcinoma cells. The oral administration of *P. urinaria* to mice caused significant inhibition of tumor development with lower occurrence rate and markedly reduced tumor size. Neither the total body weight of mouse nor the weights of organs including heart, lung, liver, spleen and kidney revealed any difference between two groups, suggesting limited in vivo cytotoxic effect of *P. urinaria* in mice. TUNEL assay demonstrated the increase of apoptosis in tumor

sections prepared from *P. urinaria*-treated mice compared with control mice. It is worth of note that the neovascularization in tumor was inhibited in *P. urinaria*-treated mice, which implicated the potential anti-angiogenic effect of *P. urinaria*. Further study using an in vitro matrix-induced tube formation of HUVECs again confirmed the anti-angiogenic action of *P. urinaria*. *P. urinaria* exerted no inhibitory effect on the growth of HUVECs, however, the migration of HUVECs as analyzed using transwell assay was suppressed markedly by *P. urinaria* in a dose-dependent manner. All together, the present study indicated that *P. urinaria* extract is an anti-tumor and anti-angiogenic agent, which can be used safely in animals. PMID: 16644472

Pathophysiology. 2006 May;13(2):95-102. Epub 2006 Mar 20.

Herbal (*Phyllanthus niruri*) protein isolate protects liver from nimesulide induced oxidative stress.

Chatterjee M, Sarkar K, Sil PC.

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Present study was conducted to evaluate the role of a protein fraction (PI, protein isolate) of the herb, *Phyllanthus niruri* (*P. niruri*) against nimesulide-induced oxidative stress in vivo using a murine model. Mice were intraperitoneally treated with that at a dose of 5mg/kg body weight for 7 days before and separately 1-5 days after nimesulide (at a dose of 10mg/kg body weight for 7 days) administration to evaluate its preventive and curative role. Levels of reduced glutathione (GSH), antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), as well as thiobarbituric acid reactive substances (TBARS) were measured in the liver homogenates of all study groups. Pretreatment with isolated *P. niruri* protein fraction significantly enhanced nimesulide-induced reduced levels of antioxidant enzymes and GSH as well as reduced the enhanced level of lipid peroxidation. Post-treatment studies showed that the recovery after nimesulide induced oxidative stress was more rapid if PI was administered compared to the spontaneous recovery of liver. Histological studies also suggest that this protein fraction could prevent as well as cure liver from nimesulide induced oxidative stress. DPPH radical scavenging assay showed that it could scavenge free radicals. Its antioxidant property was compared with that of a known potent antioxidant, Vitamin E. Besides, the effect of a non-relevant protein, BSA, was also included in the study. Heat treatment and trypsin digestion destroyed the biological activity of this protein fraction. In conclusion, data obtained suggest that the *P. niruri* protein fraction may protect liver from nimesulide-induced oxidative stress probably via promotion of antioxidant defense. PMID: 16542828

J Nat Prod. 2005 Oct;68(10):1479-83.

Chemical and biological properties of an arabinogalactan from *Phyllanthus niruri*.

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Phyllanthus niruri is a well-known herb widely used medicinally in Asia, Africa, and South America. Aqueous extraction of the intact plant provided an acidic arabinogalactan, which was characterized chemically, and its effects on peritoneal macrophage activation were determined. Methylation analyses and ¹³C NMR spectroscopy showed it to have a complex structure with a (1→4)-linked beta-Galp main chain, substituted by rhamnose, galacturonic acid, arabinose, xylose, galactose, and glucose-containing side chains, with nonreducing end-units of arabinofuranose, xylopyranose, galactopyranose, and glucopyranose. In immunological studies, the arabinogalactan stimulated superoxide anion production, when tested using peritoneal macrophages of mice, but did not interfere with the nitric oxide pathway. Thus, traditional aqueous extraction

methods, such as decoction and infusion, provide a major polysaccharide, which stimulates an intense biological response in macrophages: this could represent an interesting approach in phytotherapeutic treatments. PMID: 16252911

J Cell Biochem. 2006 Mar 1;97(4):795-812.

Antiviral effect of Phyllanthus nanus ethanolic extract against hepatitis B virus (HBV) by expression microarray analysis.

Lam WY, Leung KT, Law PT, Lee SM, Chan HL, Fung KP, Ooi VE, Waye MM.

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Ethanolic extract of *Phyllanthus nanus* (*P. nanus*) treatment exhibited potent antiviral activity against Hepatitis B virus (HBV). The effects of these extracts on HBV in the HBV genome integrated cell lines--Alexander cells and HepG2 2.2.15 cells were examined. Experimental results showed that the ethanolic extract of *P. nanus* produced suppressive effect on HBsAg secretion and HBsAg mRNA expression. The extract also inhibited HBV replication as measured by HBV DNA level in vitro. In addition, using a duck HBV (DHBV) primary culture model, the *P. nanus* ethanolic extract suppressed viral replication of DHBV in DHBV infected primary duck hepatocytes. The gene expression pattern in Alexander cells that had been treated with the ethanolic extract of *P. nanus* was also revealed by microarray techniques. The microarray results indicated that there was up-regulation of expression of several genes, including annexin A7 (Axn7). The subcellular localization of Axn7 and anti-HBV effect of Axn7 over-expression in Alexander cells were also investigated. Results showed that expression of Axn7-GFP fusion protein are localized around the secretory vesicles and could cause a decrease in HBsAg secretion in Alexander cells. Axn7 protein might play an important role in the medicinal effect of the active principle(s) of *P. nanus*. 2005 Wiley-Liss, Inc. PMID: 16237706

J Ethnopharmacol. 2006 Mar 8;104(1-2):79-86. Epub 2005 Oct 19.

Comparative pharmacognostic studies of three Phyllanthus species.

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Different species of *Phyllanthus* are considered to be very effective hepatoprotective agents in the Indian indigenous systems of medicine and are considered bitter, astringent, stomachic, diuretic, febrifuge, deobstruant and antiseptic. Still ayurvedic practitioners prescribed fresh juice of 'Bhuiamliki' for jaundice. Various species of *Phyllanthus* are being sold in India under the trade name 'Bhuiamliki'. During market surveillance of herbal drug, it was observed that almost all the commercial samples, either comprise of *Phyllanthus amarus* Schum & Thonn. or *Phyllanthus maderaspatensis* Linn. or mixture of *Phyllanthus amarus*, *Phyllanthus fraternus* Webster. and *Phyllanthus maderaspatensis*. Therefore, in this context the detailed pharmacognostical evaluation of all the three species has been carried out with the aim to establish the identification markers of this important hepatoprotective agent (effective in hepatitis B too). The study conclude that all the three species can be differentiated on the basis of macro and microscopic characters, physico-chemical values, HPTLC fingerprint profile, and detection of phyllanthin and hypophyllanthin as marker components. Besides, an interesting conclusion can also be drawn that phyllanthin and hypophyllanthin said to protect hepatocytes against carbon tetrachloride and galactosamine induced toxicity, may not be exclusively responsible for hepatoprotective activity as these are present only in *Phyllanthus amarus* while *Phyllanthus fraternus* and *Phyllanthus maderaspatensis* also possess significant hepatoprotective activity. PMID: 16236476

Planta Med. 2005 Aug;71(8):721-6.

Anti-inflammatory properties of extracts, fractions and lignans isolated from *Phyllanthus amarus*.

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This study assessed the anti-inflammatory effect of the extracts and purified lignans obtained from *Phyllanthus amarus*. Given orally, the hexane extract (HE), the lignan-rich fraction (LRF), or the lignans phyltetralin, nirtetralin, niranthin, but not hypophyllanthin or phyllanthin, inhibited carrageenan (Cg)-induced paw oedema and neutrophil influx. The HE, the LRF or nirtetralin also inhibited the increase of IL1-beta tissue levels induced by Cg. Furthermore, bradykinin (BK)-, platelet activating factor (PAF)- and endothelin-1 (ET-1)-induced paw oedema were significantly inhibited by the HE or LRF while histamine- and substance P-induced paw oedema were unaffected. Finally, nirtetralin or phyltetralin caused inhibition of paw oedema induced by PAF or ET-1. These results show that the HE, the LRF and the lignans niranthin, phyltetralin and nirtetralin exhibited marked anti-inflammatory properties and suggest that these lignans seem to be the main active principles responsible for the anti-inflammatory properties reported for the HE of *P. amarus*. PMID: 16142635

Antiviral Res. 2005 Sep;67(3):163-8.

A flavonoid from medicinal plants blocks hepatitis B virus-e antigen secretion in HBV-infected hepatocytes.

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A flavonoid molecule that showed a unique anti-HBV function was isolated from *Phyllanthus urinaria*. The molecular formula was determined as C₁₄H₆O₈ based on FAM-MS analysis and the structure was determined by NMR. The identified flavonoid molecule, ellagic acid, showed unique anti-HBV functions. Ellagic acid did not inhibit either HBV polymerase activity, HBV replication or block HBsAg secretion. Rather, ellagic acid blocks effectively HBeAg secretion in HepG2 2.2.15 cells (IC₅₀=0.07 microg/ml). Since HBeAg is involved in immune tolerance during HBV infection, ellagic acid, a newly identified functional anti-HBV compound, may be a new candidate therapeutic against immune tolerance in HBV-infected individuals.

PMID: 16118024

Phytomedicine. 2005 Jun;12(6-7):494-500.

Chemoprotective activity of an extract of *Phyllanthus amarus* against cyclophosphamide induced toxicity in mice.

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The effect of 75% methanolic extract of the plant *Phyllanthus amarus* (*P. amarus*) was studied against cyclophosphamide (CTX) induced toxicity in mice. Administration of CTX (25 mg/kg b.wt, i.p.) for 14 days produced significant myelosuppression as seen from the decreased WBC count and bone marrow cellularity. Administration of *P. amarus* extract at doses 250 and 750 mg/kg b.wt significantly reduced the myelosuppression and improved the WBC count, bone marrow cellularity as well as the number of maturing monocytes. CTX treatment also reduced the activity of glutathione system and increased the activity of phase I enzyme that metabolize CTX to its toxic side products. *P. amarus* administration was found to decrease the activity of phase I enzyme. Administration of *P. amarus* also increased the cellular glutathione (GSH) and glutathione-S-transferase (GST), thereby decreasing the effect of toxic metabolites of CTX on the cells. Administration of *P. amarus* did not reduce the

tumor reducing activity of CTX. In fact, there was a synergistic action of CTX and *P. amarus* in reducing the solid tumors in mice. Results indicated that administration of *P. amarus* can significantly reduce the toxic side effects of CTX and is not interfering with the antitumor efficiency of CTX.

PMID: 16008127

Biol Pharm Bull. 2005 Jul;28(7):1165-71.

Antioxidative and cardioprotective effects of *Phyllanthus urinaria* L. on doxorubicin-induced cardiotoxicity.

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Cardiac toxicity is a major adverse effect caused by doxorubicin (DOX) therapy. Many recent studies have shown that DOX toxicity involves generation of reactive oxygen species (ROS). Although protection or alleviation of DOX toxicity can be achieved by administration of antioxidant vitamins such as ascorbic acid and vitamin E, their cardioprotective effect remains controversial. Thus alternative naturally occurring antioxidants may potentially be candidates for antioxidant therapy. In this study, we investigated the antioxidative and cytoprotective effects of *Phyllanthus urinaria* (PU) against DOX toxicity using H9c2 cardiac myoblasts. The total antioxidant capacity of PU (1 mg/ml) was 5306.75 \pm 461.62 FRAP value (microM). DOX IC₅₀ values were used to evaluate the cytoprotective effects of PU ethanolic extract (1 or 10 microg/ml) in comparison with those of ascorbic acid (VIT C, 100 microM) and N-acetylcysteine (NAC, 100 microM). PU treatments (1 or 10 microg/ml) dose dependently caused rightward DOX IC₅₀ shifts of 2.8- and 8.5-fold, respectively while treatments with VIT C and NAC increased DOX IC₅₀ by 3.3- and 4.2-fold, respectively. Additionally, lipid peroxidation and caspase-3 activity were parameters used to evaluate cytoprotective effect. All antioxidants completely inhibited cellular lipid peroxidation and caspase-3 activation induced by DOX (1 microM). Endogenous antioxidant defense such as total glutathione (tGSH), catalase and superoxide dismutase (SOD) activity was also modulated by the antioxidants. PU treatment alone dose dependently increased tGSH, and this effect was retained in the presence of DOX. Similar effect was observed in the assessment of catalase and SOD enzyme activity. The nuclear factor kappaB (NFkappaB) transcription factor assay demonstrated that all antioxidants significantly inhibited DOX-induced NFkappaB activation. Our results suggest that PU protection against DOX cardiotoxicity was mediated through multiple pathways and this plant may serve as an alternative source of antioxidants for prevention of DOX cardiotoxicity.

PMID: 15997091

Antiviral Res. 2005 Jul;67(1):24-30.

Acetone, ethanol and methanol extracts of *Phyllanthus urinaria* inhibit HSV-2 infection in vitro.

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Phyllanthus urinaria Linnaea (Euphorbiaceae) is one of the traditional medicinal plants that are widely applied by oriental people, especially by Chinese and Indian, to ameliorate various kinds of ailments. Many biological activities, including anti-hepatitis B virus, anti-Epstein-Barr virus and anti-retroviral reverse transcriptase, of *P. urinaria* have been reported, but not against herpes simplex virus (HSV). In this study, the anti-HSV-1 and HSV-2 activities of different solvents extracted from *P. urinaria* were investigated in vitro by plaque reduction assay. Results showed that acetone, ethanol and methanol extracts of *P. urinaria* inhibited HSV-2 but not HSV-1 infection. The 50% inhibitory concentration

against HSV-2 infection (IC50) of acetone, ethanol and methanol extracts was 4.3 +/- 0.5, 5.0 +/- 0.4 and 4.0 +/- 0.9 mcg/ml, respectively. All three extracts showed no cytotoxic effect against Vero cells at concentrations of 10.0 mcg/ml or below. The time-of-addition study demonstrated that these three extracts were only effective when added during the HSV-2 infection which, therefore, suggested that they disturb the initial stage of HSV-2 infection. Furthermore, they can diminish virus infectivity without significantly affecting incubation time and temperature. Therefore, the acetone, ethanol and methanol extracts of *P. urinaria* were concluded to likely inhibit HSV-2 infection through disturbing the early stage of virus infection and through diminishing the virus infectivity. PMID: 15885815

Rev Cubana Med Trop. 2003 Sep-Dec;55(3):169-73.

[Preliminary evaluation of the antiviral activity of the aqueous extract of *Phyllanthus orbicularis* vs HIV-1 infection] [Article in Spanish]

Garcia SV, del Barrio Alonso G, Gaiten YG, Diaz LM.

Facultad de Biología, Universidad de La Habana Calle Cuba.

The antiviral activity of the aqueous extract of *Phyllanthus orbicularis*, a member of the Euphorbiaceae family, against the simple herpes virus typel was evaluated in cellular culture, specifically in fibroblasts of human prepuceum, and in an animal model, Balb-c mice. The extracellular effect of the extract on HIV-1 proved to be effective. A selective index of 44 was obtained, which shows a possible virucidal action on the particle. In the trial in vivo, the topical administration of the aqueous extract (12 mg/kg) reduced significantly the development of lesions in mice subcutaneously infected with HIV-1 (1 x 10⁶ UFP). These results suggest the consideration of *Phyllanthus orbicularis* as a possible anti-HIV-1 candidate

PMID: 15849921

Antiviral Res. 2004 Nov;64(2):93-102.

Concerted inhibitory activities of *Phyllanthus amarus* on HIV replication in vitro and ex vivo.

Notka F, Meier G, Wagner R.

Institute of Medical Microbiology and Hygiene, University of Regensburg, Franz-Josef-Strauss Allee 11, 93053 Regensburg, Germany

Phyllanthus amarus derived preparations were previously shown to inhibit RT inhibitor-resistant HIV variants as efficiently as wild-type strains. The drugs target different steps of the HIV life cycle, thereby presenting multiple antiviral activities. Here we show that a water/alcohol extract blocks HIV-1 attachment and the HIV-1 enzymes integrase, reverse transcriptase and protease to different degrees. A gallotannin containing fraction and the isolated ellagitannins geraniin and corilagin were shown to be the most potent mediators of these antiviral activities. The *P. amarus* derived preparations blocked the interaction of HIV-1 gp120 with its primary cellular receptor CD4 at 50% inhibitory concentrations of 2.65 (water/alcohol extract) to 0.48 microg/ml (geraniin). Inhibition was also evident for the HIV-1 enzymes integrase (0.48-0.16 microg/ml), reverse transcriptase (8.17-2.53 microg/ml) and protease (21.80-6.28 microg/ml). In order to prove the in vivo relevance of these biological activities, plant material was administered orally to volunteers and a potent anti-HIV activity in blood could be demonstrated. Sera at a final concentration of 5% reduced HIV replication by more than 30%. These results support the conclusion that *P. amarus* has inhibitory effects on HIV not only in vitro but also in vivo.

PMID: 15498604

Indian J Exp Biol. 2003 Nov;41(11):1325-8.

Chemopreventive action of *Phyllanthus urinaria* Linn on DMBA-induced skin carcinogenesis in mice.

Bharali R, Tabassum J, Azad MR.

The inhibition of tumor incidence by hydro-alcoholic extract of the whole plant of *P. urinaria* was evaluated in 6-7 weeks old female albino mice on two-stage process of skin carcinogenesis induced by a single application of 7,12-dimethylbenz(a)anthracene (50 microg/50 microl of acetone), and 2 weeks later, promoted by repeated application of croton oil (1% in acetone/three times a week) till the end of the experiment (15 weeks). Topical application of the extract at a dose of 5 mg/kg body weight/day for 15 weeks at the peri-initiational stage (i.e., 7 days before and 7 days after DMBA application), promotional stage (i.e., from the time of croton oil application) and both peri and post-initiational stages (i.e., 7 days prior to DMBA application and continued till the end of the experiment) on the shaven backs of the mice recorded a significant reduction in tumor incidence to 50, 33.3 and 16.7% respectively in comparison to the control (i.e., the mice treated with DMBA and croton oil only) where tumor incidence was found to be 81.8%. The average number of papillomas per mouse was also significantly reduced. The results suggest a possible chemopreventive property of *P. urinaria* against DMBA-induced skin papillomagenesis in mice. PMID: 15332506

Am J Chin Med. 2004;32(2):175-83.

Aqueous extract of *Phyllanthus urinaria* induces apoptosis in human cancer cells.

Huang ST, Yang RC, Pang JH.

Cell apoptosis is now known to play an important role in the maintenance of cellular homeostasis and anti-carcinogenesis. The anticancer effect of aqueous extract prepared from *Phyllanthus urinaria* (*P. urinaria*) was investigated by analyzing its potential to induce apoptosis in human cancer cells. We showed that the aqueous extract of *P. urinaria* could reduce the viability by inducing the apoptosis in human cancer cells derived from several different origins as demonstrated by morphological changes and DNA fragmentation. Yet, *P. urinaria* extract exhibited no cytotoxic effect on normal human cells, including vascular endothelial cells and liver cells under the same conditions. It suggests that the aqueous extract of *P. urinaria* is substantially useful in treating various kinds of human cancer cells without toxic side effect on normal cells. PMID: 15315256

Urol Res. 2004 Oct;32(5):362-6. Epub 2004 Jun 19.

***Phyllanthus niruri* normalizes elevated urinary calcium levels in calcium stone forming (CSF) patients.**

Nishiura JL, Campos AH, Boim MA, Heilberg IP, Schor N.

Phyllanthus niruri is a plant used for years in Brazil to treat urinary calculi. We prospectively evaluated the effect of *P. niruri* intake on 24 h urinary biochemical parameters in an attempt to assess its in vivo effect in calcium stone forming (CSF) patients. A total of 69 CSF patients (39 males and 30 females, 38+/-8 years old) were randomized to take either *P. niruri* (n=33) (450 mg capsules, td) or placebo (n=36) for 3 months. Blood calcium, uric acid, citrate, magnesium, oxalate, sodium and potassium were determined at baseline and at the end of the study. A subset analysis was made in patients classified according to the presence of metabolic abnormalities (hypercalciuria, hyperuricosuria, hyperoxaluria, hypocitraturia and hypomagnesiuria). Overall, there were no significant differences in the mean values of urinary parameters between the urine samples before and after *P. niruri* intake, except for a slight reduction in mean urinary magnesium after *P. niruri*, which was within the normal range. However, in the subset analysis, we observed that *P. niruri* induced a significant reduction in the mean urinary calcium in hypercalciuric patients (4.8+/-1.0 vs 3.4+/-1.1 mg/kg/24 h, P<0.05). In this short-term follow-up, no significant differences in calculi voiding and/or pain relief between the groups taking *P. niruri* or the placebo were detected. Our data suggest that *P. niruri* intake reduces urinary calcium based on the analysis of a subset of patients presenting with hypercalciuria. Larger trials including primary hypercalciuric stone formers should

be performed in order to confirm these findings and to determine the possible clinical consequences of urinary calcium reduction during *P. niruri* administration.
PMID: 15221244

J Radiat Res (Tokyo). 2004 Mar;45(1):133-9.

Protective effect of an extract of *Phyllanthus amarus* against radiation-induced damage in mice.

Kumar KB, Kuttan R.

Amala Cancer Research Centre.

The radioprotective effect of an extract of the plant *Phyllanthus amarus* (*P. amarus*) was investigated in adult BALB/c mice. *P. amarus* extract (750 mg/kg b.wt and 250 mg/kg b.wt) was administered orally to mice for five days prior to whole body radiation (6 Gy) and for one month after radiation. The animals were sacrificed on days 3, 9, 12, and 30 after radiation. *P. amarus* significantly increased the total W.B.C count, bone marrow cellularity, and alpha-esterase activity as compared to untreated radiation-exposed animals. *P. amarus* treatment also increased the activity of various antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GPX), and glutathione reductase (GR), both in blood and tissue, which were reduced by radiation treatment. There was also a significant increase in the glutathione (GSH) levels of blood and tissue. Lipid peroxidation levels, which were increased after radiation, were significantly reduced by *P. amarus* treatment, both in serum and liver. The results collectively indicate that *P. amarus* extract could increase the antioxidant defense mechanism in mice and there by protect the animals from radiation-induced cellular damage.

PMID: 15133301

J Ethnopharmacol. 2004 May;92(1):67-70.

Links

Further studies on the antihepatotoxic activity of *Phyllanthus maderaspatensis* Linn.

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Phyllanthus maderaspatensis (whole plant extracts) was evaluated for its antihepatotoxic and choleric activities in rats. The plant extracts (200 mg/kg, n-hexane, ethyl alcohol or water) showed a remarkable hepatoprotective activity against acetaminophen-induced hepatotoxicity as judged from the serum marker enzymes. The water and ethyl alcohol extracts showed moderate activity compared to the n-hexane extract, which showed activity at a dose as low as 1.5 mg/kg. The antihepatotoxicity of the hexane extract was found to be better than silymarin, a standard hepatoprotective herbal drug. The effect of n-hexane extract was found to be concentration-dependent. This extract also exhibited choleric activity in normal rats, and in vitro hydroxyl radical scavenging activity and inhibition of lipid peroxidation. Copyright 2004 Elsevier Ireland Ltd.

PMID: 15099850

Hum Exp Toxicol. 2003 Dec;22(12):639-45.

Links

Hepatocurative and antioxidant profile of HP-1, a polyherbal phytomedicine.

Tasaduq SA, Singh K, Sethi S, Sharma SC, Bedi KL, Singh J, Jaggi BS, Johri RK.

Biochemistry Lab, Division of Pharmacology, Regional Research Laboratory, Canal Road, Jammu-Tawi 180 001, India.

HP-1 a herbal formulation comprising of *Phyllanthus niruri* and extracts of *Terminalia bellerica*, *Terminalia chebula*, *Phyllanthus emblica* and *Tinospora cordifolia* has been evaluated for hepatoprotective activity against carbon tetrachloride (CCl₄) induced toxicity. Results show that HP-1 reversed the

leakage of lactate dehydrogenase (LDH) and glutamate pyruvate transaminase (GPT) and prevented the depletion of glutathione (GSH) levels in a primary monolayer culture of rat hepatocytes (in vitro). HP-1 attenuated the serum toxicity as manifested in elevated levels of transaminases (glutamate oxaloacetate transaminase (GOT), and GPT) The antioxidative enzymes in liver (catalase and superoxide dismutase (SOD)) were restored to normal values after the oral administration of HP-1. HP-1 suppressed the formation of the superoxide anion radical and reduced CCl₄ mediated lipid peroxidation (LPO). Silymarin and antioxidants (ascorbic acid, beta-carotene and alpha-tocopherol) were used for comparison. The present study showed that HP-1 is a potential hepatoprotective formulation with an additional attribute of being anti-peroxidative.
PMID: 14992325

Indian J Med Sci. 2003 Sep;57(9):387-93.

Effects of alkaloidal extract of Phyllanthus niruri on HIV replication.

Naik AD, Juvekar AR.

Pharmaceutical Division, Institute of Chemical Technology, University of Mumbai, Matunga, Mumbai-400019, India.

Phyllanthus niruri has been found to exhibit marked inhibitory effect on hepatitis B virus evident by its exhaustive utility in cases of chronic jaundice. However, till date, research has not been focused on identification and validation of active pharmacophores of Phyllanthus niruri responsible for the reported inhibitory effect of its aqueous extract on anti-human immunodeficiency virus. The present investigation examines the anti-HIV effects of the alkaloidal extract of Phyllanthus niruri in human cell lines. The inhibitory effect on HIV replication was monitored in terms of inhibition of virus induced cytopathogenicity in MT-4 cells. The alkaloidal extract of Phyllanthus niruri showed suppressing activity on strains of HIV-1 cells cultured on MT-4 cell lines. The CC₅₀ for the extract was found to be 279.85 microg/mL(-1) whereas the EC₅₀ was found to be 20.98 microg/mL(-1). Interestingly the Selectivity Index (SI) was found to be 13.34, which showed a clear selective toxicity of the extract for the viral cells. The alkaloidal extract of Phyllanthus niruri was thus found to exhibit sensitive inhibitory response on cytopathic effects induced by both the strains of human immunodeficiency virus on human MT-4 cells in the tested concentrations.

PMID: 14515028

J Ethnopharmacol. 2003 Aug;87(2-3):193-7.

Inhibition of experimental gastric lesion and inflammation by Phyllanthus amarus extract.

Raphael KR, Kuttan R.

Amala Cancer Research Centre, Thrissur, Kerala 680 553, India.

Methanolic extract of Phyllanthus amarus Shum & Thonn (Euphorbiaceae) 50, 200, and 1000 mg/kg body weight significantly inhibited gastric lesions, induced by intragastric administration of absolute ethanol (8 ml/kg). Mortality, increased stomach weight, ulcer index, and intraluminal bleeding were reduced significantly by Phyllanthus amarus. Biochemical analysis indicated that reduced glutathione (GSH) of gastric mucosa produced by ethanol administration was significantly elevated by treatment with Phyllanthus amarus extract. Aqueous and methanol extracts of Phyllanthus amarus produced an inhibition of rat paw edema up to 42% compared to control in 3h and continued up to 8h. Anti-oxidant activity of the extract as well as presence of tannins in the extract may be responsible for these observed activities.

PMID: 12860307

Phytother Res. 2003 May;17(5):449-53.

Screening of 25 compounds isolated from Phyllanthus species for anti-human hepatitis B virus in vitro.

Huang RL, Huang YL, Ou JC, Chen CC, Hsu FL, Chang C.
National Research Institute of Chinese Medicine, Taiwan
Using an HBV-producing cell line and inhibition of the expression of the HBsAg and HBeAg as antiviral indicators, a study was conducted on 25 compounds isolated from four *Phyllanthus* (Euphorbiaceae) plants, including *P. amarus* Schum. & Thonn., *P. multi florus* Willd., *P. tenellus* Roxb. and *P. virgatus* Forst. f. It was found that niranthin (1), nirtetralin (3), hinokinin (5) and geraniin (13) at the non-cytotoxic concentration of 50 micro m, suppressed effectively both HBsAg and HBeAg expression, with the highest inhibition at 74.3%, 45.3%; 69.6%, 33.9%; 68.1%, 52.3%; 32.1%, 46.6%, respectively. Of these, niranthin (1) showed the best anti-HBsAg activity, while the most potent anti-HBeAg activity was observed with hinokinin (5). PMID: 12748977

Antiviral Res. 2003 Apr;58(2):175-86.

Inhibition of wild-type human immunodeficiency virus and reverse transcriptase inhibitor-resistant variants by *Phyllanthus amarus*.

Notka F, Meier GR, Wagner R.

CMI-Centers for Medical Innovation AG, Germany.

Substantial progress has been made in research on natural products which effectively inhibit HIV-1 replication. Many active compounds were isolated from traditionally used medicinal plants including *Phyllanthus* species. This study shows that aqueous as well as alcohol-based *Phyllanthus amarus* extracts potently inhibit HIV-1 replication in HeLa CD4(+) cells with 50% effective concentration (EC(50)) values ranging from 0.9 to 7.6 microg/ml. A gallotannin enriched fraction showed enhanced activity (0.4 microg/ml), and the purified gallotannins geraniin and corilagin were most active (0.24 microg/ml). HIV-1 replication was also blocked in CD4(+) lymphoid cells with comparable EC(50) values. Applying a cell-based internalization assay, we could demonstrate 70-75% inhibition of virus uptake at concentrations of 2.5 microg/ml for the water/alcohol extract and geraniin. In addition, a concentration-dependent inhibition of HIV-1 reverse transcriptase (RT) could be demonstrated in vitro. The 50% inhibitory concentration (IC(50)) values varied from 1.8 to 14.6 microg/ml. The ability to inhibit replication of a variety of RT inhibitor-resistant HIV-1 strains points to the potential of *P. amarus* extracts, as natural products, in the chemotherapy of HIV infections. PMID: 12742578

Indian J Exp Biol. 2002 Aug;40(8):905-9.

Hypoglycemic effect of methanol extract of *Phyllanthus amarus* Schum & Thonn on alloxan induced diabetes mellitus in rats and its relation with antioxidant potential.

Raphael KR, Sabu MC, Kuttan R.

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Methanolic extract of *P. amarus* was found to have potential anti-oxidant activity as it could inhibit lipid peroxidation, and scavenge hydroxyl and superoxide radicals in vitro. The amount required for 50% inhibition of lipid peroxide formation was 104 microg/ml and the concentrations needed to scavenge hydroxyl and superoxide radicals were 117 and 19 microg/ml respectively. The extract was found to reduce the blood sugar in alloxan diabetic rats at 4th hr by 6% at a dose level of 200 mg/kg body wt and 18.7% at a concentration of 1000 mg/kg body wt. Continued administration of the extract for 15 days produced significant ($P < 0.001$) reduction in blood sugar. On 18th day after alloxan administration values were almost similar to normal in the group taking 1000 mg/kg body wt. PMID: 12597020

J Hepatol. 2003 Mar;38(3):289-97.

***Phyllanthus amarus* has anti-inflammatory potential by inhibition of iNOS, COX-2, and cytokines via the NF-kappaB pathway.**

Kiemer AK, Hartung T, Huber C, Vollmar AM.

Department of Pharmacy, Center of Drug Research, University of Munich

BACKGROUND/AIMS: *Phyllanthus amarus* is a herbal medicine traditionally applied in the treatment of viral hepatitis. Aim of this study was to investigate potential anti-inflammatory properties of standardized *P. amarus* extracts concerning a potential influence of *P. amarus* on endotoxin-induced nitric oxide synthase (iNOS), cyclooxygenase (COX-2), and cytokine production in vivo and in vitro. **METHODS:** Investigations were performed in rat Kupffer cells (KC), in RAW264.7 macrophages, in human whole blood, and in mice. Cells were stimulated with lipopolysaccharides (LPS) in the presence or absence of *P. amarus* extracts (hexane, EtOH/H₂O), mice were treated with galactosamine/LPS as a model for acute toxic hepatitis. Nitrite was measured by Griess assay, prostaglandin E₂ (PGE₂) by radioimmunoassay, and cytokines by enzyme-linked immunosorbent assay. iNOS and COX-2 were determined by Western blot, activation of NF-kappaB and AP-1 by EMSA. **RESULTS:** *P. amarus* EtOH/H₂O and hexane extracts showed an inhibition of LPS-induced production of NO and PGE₂ in KC and in RAW264.7. The extracts also attenuated the LPS-induced secretion of tumor necrosis factor (TNF-alpha) in RAW264.7 as well as in human whole blood. Both extracts reduced expression of iNOS and COX-2 and inhibited activation of NF-kappaB, but not of AP-1. *P. amarus* inhibited induction of interleukin (IL)-1beta, IL-10, and interferon-gamma in human whole blood and reduced TNF-alpha production in vivo. **CONCLUSIONS:** This work shows that standardized extracts of *P. amarus* inhibit the induction of iNOS, COX-2, and TNF-alpha. Therefore, we report for the first time an anti-inflammatory potential of this traditionally employed herbal medicine both in vitro and in vivo.

PMID: 12586294

Life Sci. 2003 Feb 28;72(15):1705-16.

Phyllanthus urinaria triggers the apoptosis and Bcl-2 down-regulation in Lewis lung carcinoma cells.

Huang ST, Yang RC, Yang LJ, Lee PN, Pang JH.

Graduate Institute of Clinical Medical Sciences, Chang Gung University, Taiwan

Phyllanthus urinaria (*P. urinaria*), a widely used herb medicine, was tested for the anticancer effect in its water extract for the first time. The water extract of *P. urinaria* significantly decreased the number of Lewis lung carcinoma cells in a dose- and time-dependent manner as determined by MTT assay. However, the water extract of *P. urinaria* did not exert any cytotoxic effect on normal cells such as endothelial cells and liver cells. Result from flow cytometry revealed a dose-dependent increase of dead cells 24 hours after treating Lewis lung carcinoma cells with *P. urinaria* extract. The anticancer activity of *P. urinaria* extract was due to the apoptosis induced in Lewis lung carcinoma cells, which was demonstrated by DNA fragmentation analysis and increased caspase-3 activity. The apoptosis triggered by *P. urinaria* extract in Lewis lung carcinoma cells was associated with the down-regulation of Bcl-2 gene expression, but not with p53, p21 and Bax. Furthermore, the partial inhibition of *P. urinaria*-induced apoptosis in Lewis lung carcinoma cells by pretreatment with cyclosporin A, a mitochondria permeability transition pore inhibitor, suggesting that *P. urinaria* extract induced the apoptosis of Lewis lung carcinoma cells, at least in part, through a mitochondria-associated intrinsic pathway. Copyright 2002 Elsevier Science Inc.

PMID: 12559392

J Gastroenterol Hepatol. 2002 Dec;17 Suppl 3:S370-S376.

Links

Herbal medicines for liver diseases in India.

Thyagarajan S, Jayaram S, Gopalakrishnan V, Hari R, Jeyakumar P, Sripathi M.

The use of natural remedies for the treatment of liver diseases has a long history, starting with the Ayurvedhic treatment, and extending to the Chinese, European

and other systems of traditional medicines. The 21st century has seen a paradigm shift towards therapeutic evaluation of herbal products in liver diseases by carefully synergizing the strengths of the traditional systems of medicine with that of the modern concept of evidence-based medicinal evaluation, standardization of herbal products and randomized placebo controlled clinical trials to support clinical efficacy. The present review provides the status report on the scientific approaches made to herbal preparations used in Indian systems of medicine for the treatment of liver diseases. In spite of the availability of more than 300 preparations for the treatment of jaundice and chronic liver diseases in Indian systems of medicine using more than 87 Indian medicinal plants, only four terrestrial plants have been scientifically elucidated while adhering to the internationally acceptable scientific protocols. In-depth studies have proved *Sylibum marianum* to be anti-oxidative, antilipidperoxidative, antifibrotic, anti-inflammatory, immunomodulating and liver regenerative. *Glycyrrhiza glabra* has been shown to be hepatoprotective and capable of inducing an indigenous interferon. *Picrorhiza kurroa* is proved to be anti-inflammatory, hepatoprotective and immunomodulatory. Extensive studies on *Phyllanthus amarus* have confirmed this plant preparation as being anti-viral against hepatitis B and C viruses, hepatoprotective and immunomodulating, as well as possessing anti-inflammatory properties. For the first time in the Indian systems of medicine, a chemo-biological fingerprinting methodology for standardization of *P. amarus* preparation has been patented. PMID: 12472966

J Ethnopharmacol. 2002 Sep;82(1):19-22.

Lipid lowering activity of *Phyllanthus niruri* in hyperlipemic rats.

Khanna AK, Rizvi F, Chander R.

Division of Biochemistry, Central Drug Research Institute, Lucknow 226001, India.

The lipid lowering activity (LLA) of *Phyllanthus niruri* has been studied in triton and cholesterol fed hyperlipemic rats. Serum lipids were lowered by *P. niruri* extract orally fed (250 mg/kg b.w.) to the triton WR-1339 induced hyperlipemic rats. Chronic feeding of this drugs (100 mg/kg b.w.) in animals simultaneously fed with cholesterol (25 mg/kg b.w.) for 30 days caused lowering in the lipids and apoprotein levels of VLDL and LDL in experimental animals. The LLA of this drug is mediated through inhibition of hepatic cholesterol biosynthesis, increased faecal bile acids excretion and enhanced plasma lecithin: cholesterol acyltransferase activity. Copyright 2002 Elsevier Science Ireland Ltd. PMID: 12169400

J Ethnopharmacol. 2002 Jun;81(1):17-22.

Antitumour and anticarcinogenic activity of *Phyllanthus amarus* extract.

Rajeshkumar NV, Joy KL, Kuttan G, Ramsewak RS, Nair MG, Kuttan R.

Amala Cancer Research Centre, Thrissur, Kerala, 680-553, India.

Aqueous extract of *Phyllanthus amarus* (*P. amarus*) treatment exhibited potent anticarcinogenic activity against 20-methylcholanthrene (20-MC) induced sarcoma development and increased the survival of tumour harboring mice. The extract administration (p.o) was also found to prolong the life span of Dalton's Lymphoma Ascites (DLA) and Ehrlich Ascites Carcinoma (EAC) bearing mice and reduced the volume of transplanted solid tumours. The extract inhibited aniline hydroxylase, a P-450 enzyme. The concentration required for 50% inhibition (IC(50)) was found to be 540 microg/ml. The extract was found to inhibit DNA topoisomerase II of *Saccharomyces cerevisiae* mutant cell cultures and inhibited cell cycle regulatory enzyme cdc25 tyrosine phosphatase (IC(50-25) microg/ml). Antitumour and anticancer activity of *P. amarus* may be related with the inhibition of metabolic activation of carcinogen as well as the inhibition of cell cycle regulators and DNA repair. PMID: 12020923

BJU Int. 2002 Jun;89(9):829-34.

The effect of *Phyllanthus niruri* on urinary inhibitors of calcium oxalate crystallization and other factors associated with renal stone formation.

Freitas AM, Schor N, Boim MA.

Nephrology Division, Universidade Federal de Sao Paulo, Brazil.

OBJECTIVE: To evaluate the effect of an aqueous extract of *Phyllanthus niruri* (Pn), a plant used in folk medicine to treat lithiasis, on the urinary excretion of endogenous inhibitors of lithogenesis, citrate, magnesium and glycosaminoglycans (GAGs). **MATERIALS AND METHODS:** The effect of chronic (42 days) administration of Pn (1.25 mg/mL/day, orally) was evaluated in a rat model of urolithiasis induced by the introduction of a calcium oxalate (CaOx) seed into the bladder of adult male Wistar rats. The animals were divided into four groups: a sham control (16 rats); a control+Pn (six); CaOx+water instead of Pn (14); and CaOx+Pn (22). Plasma and urine were collected after 42 days of treatment for biochemical analysis and the determination of urinary excretion of citrate, magnesium and GAGs. The animals were then killed and the calculi analysed. **RESULTS:** The creatinine clearance or urinary and plasma concentrations of Na⁺, K⁺, Ca²⁺, oxalate, phosphate and uric acid were unaffected by Pn or the induction of lithiasis. Treatment with Pn strongly inhibited the growth of the matrix calculus and reduced the number of stone satellites compared with the group receiving water. The calculi were eliminated or dissolved in some treated animals (three of 22). The urinary excretion of citrate and magnesium was unaffected by Pn treatment. However, the mean (sd) urinary concentration of GAGs was significantly lower in rats treated with CaOx+Pn, at 5.64 (0.86) mg/g creatinine, than when treated with CaOx + water, at 11.78 (2.21) mg/g creatinine. In contrast, the content of GAGs in the calculi was higher in the CaOx + Pn rats, at 48.0 (10.4) g/g calculus, than in the CaOx + water group, at 16.6 (9.6) g/g calculus. **CONCLUSION:** These results show that Pn has an inhibitory effect on crystal growth, which is independent of changes in the urinary excretion of citrate and Mg, but might be related to the higher incorporation of GAGs into the calculi. PMID: 12010223

Phytomedicine. 2002 Jan;9(1):26-32.

Antimutagenic and anticarcinogenic effects of *Phyllanthus amarus*.

Sripanidkulchai B, Tattawasart U, Laupatarakasem P, Vinitketkumneun U et al
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This study aimed to examine the antimutagenic and anticarcinogenic potential of *Phyllanthus amarus* Schum. et Thonn. using the bacterial preincubation mutation assay and an in-vivo alkaline elution method for DNA single-strand breaks in hamster liver cells. The aqueous extract of the entire plant showed an antimutagenic effect against induction by 2-aminofluorene (AF2), 2-aminoanthracene (2AA) and 4-nitroquinolone-1-oxide (4-NQO) in *Salmonella typhimurium* strains TA98 and TA100, and in *Escherichia coli* WP2 uvrA/pKM101. All the results were dose-dependent; however, inhibition of N-ethyl-N-nitrosoguanidine (ENNG)-induced mutagenesis was observed only with *S. typhimurium* TA100. The extract also exhibited activity against 2-nitrofluorene (2NF) and sodium azide-induced mutagenesis with *S. typhimurium* TA98 and TA100, respectively. Based on the alkaline elution method, the plant extract prevented in vivo DNA single-strand breaks caused by dimethylnitrosamine (DMN) in hamster liver cells. When the extract was administered 30 min prior to the administration of DMN, the elution rate constant decreased more than 2.5 times, compared to that of control. These results indicate that *P. amarus* possesses antimutagenic and antigenotoxic properties. PMID: 11924760

Zhongguo Zhong Yao Za Zhi. 1998 Jun;23(6):363-4, 384.

[Chemical constituents of Phyllanthus urinaria L. and its antiviral activity against hepatitis B virus] [Article in Chinese]

Zhong Y, Zuo C, Li F, Ding X, Yao Q, Wu K, Zhang Q et al

Institute of Materia Medica, Shangdong Academy of Medical Sciences, China

Studies on the chemical constituents of Phyllanthus urinaria and its antiviral activity against hepatitis B virus were completed. Eleven compounds have been isolated. Two of them are new compounds methyl ester dehydrochebulic acid and methyl brevifolin carboxylate. Antiviral experiments on HBsAg in vitro and liver damage caused by CCl₄ have shown that. Phyllanthus urinaria possesses antiviral activities against HBV. PMID: 11601301

J Viral Hepat. 2001 Sep;8(5):358-66.

Genus Phyllanthus for chronic hepatitis B virus infection: a systematic review.

Liu J, Lin H, McIntosh H.

The Cochrane Hepato-Biliary Group, The Copenhagen Trial Unit, Centre for Clinical Intervention Research, Copenhagen University Hospital, Copenhagen, Denmark.

To evaluate the efficacy and safety of genus Phyllanthus for chronic hepatitis B virus (HBV) infection we performed a systematic review of randomized clinical trials. Randomized trials comparing genus Phyllanthus vs. placebo, no intervention, general nonspecific treatment, other herbal medicine, or interferon treatment for chronic HBV infection were identified by electronic and manual searches. Trials of Phyllanthus herb plus interferon (IFN) vs. IFN alone were also included. No blinding and language limitations were applied. The methodological quality of trials was assessed by the Jadad scale plus allocation concealment. Twenty-two randomized trials (n=1947) were identified. The methodological quality was high in five double-blind trials and low in the 17 remaining trials. The combined results showed that Phyllanthus species had positive effect on clearance of serum HBsAg (relative risk 5.64, 95% CI 1.85-17.21) compared with placebo or no intervention. There was no significant difference on clearance of serum HBsAg, HBeAg and HBV DNA between Phyllanthus and IFN. Phyllanthus species were better than nonspecific treatment or other herbal medicines for the clearance of serum HBsAg, HBeAg, HBV DNA, and liver enzyme normalization. Analyses showed a better effect of the Phyllanthus plus IFN combination on clearance of serum HBeAg (1.56, 1.06-2.32) and HBV DNA (1.52, 1.05-2.21) than IFN alone. No serious adverse event was reported. Based on this review Phyllanthus species may have positive effect on antiviral activity and liver biochemistry in chronic HBV infection. However, the evidence is not strong due to the general low methodological quality and the variations of the herb. Further large trials are needed. PMID: 11555193

Southeast Asian J Trop Med Public Health. 2001 Mar;32(1):140-2.

A comparative study of Phyllanthus amarus compound and interferon in the treatment of chronic viral hepatitis B.

Xin-Hua W, Chang-Qing L, Xing-Bo G, Lin-Chun F.

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Fifty-five patients with chronic viral hepatitis B were randomly divided into two groups. Thirty patients were treated with Phyllanthus amarus compound (PA Co) for three months in the treatment group, another 25 patients were treated with domestic recombinant human interferon alpha-1b (IFN-alpha 1b) for three months as controls. The total effective rate in the treatment group was 83.3%, showing no significant difference from the control (p>0.05). The normalization rates of ALT, A/G and SB in the treatment group were 73.3%, 80.0% and 78.2% respectively, which were significantly higher than that in the control (p<0.05). The negative conversion rates of HBeAg and HBV-DNA in the treatment group

were 42.3% and 47.8%, showing no significant difference from the control ($p > 0.005$). It is indicated that PA Co has remarkable effect for chronic viral hepatitis B in recovery of liver function and inhibition of the replication of HBV. PMID: 11485076

J Ethnopharmacol. 2000 Nov;73(1-2):215-9.

Phyllanthus amarus extract administration increases the life span of rats with hepatocellular carcinoma.

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The effect of *Phyllanthus amarus* extract administration after induction of hepatocellular carcinoma (HCC) by N-nitrosodiethylamine (NDEA) was studied in Wistar rats. Administration of an aqueous extract of *P. amarus* was found to significantly increase the survival of hepatocellular carcinoma harboring animals. All the untreated rats died of tumour burden by 33.7 ± 1.6 weeks. Administration of *P. amarus* extract (150 mg/kg b.w.) after tumour development increased the survival of animals to an average of 52.2 ± 2.3 weeks. Serum gamma-glutamyl transpeptidase activity which was elevated to 182 ± 23 U/l by NDEA administration was lowered to 112 ± 19 U/l by the administration of *P. amarus* extract. Similarly elevated glutathione S-transferase activity (1534 ± 116 nmol/min per mg protein) and glutathione (20.5 ± 2.4 nmol/mg protein) levels in the NDEA administered group were found to be lowered to 1112 ± 89 nmol/min per mg protein and 14.2 ± 2.2 nmol/mg protein respectively. *P. amarus* administration was found to be ineffective in controlling the liver weight, elevation of tissue gamma-glutamyl transpeptidase, serum alkaline phosphatase and serum glutamate pyruvate transaminase of HCC harboring animals. PMID: 11025159

Eur J Clin Invest. 1997 Nov;27(11):908-15.

Phyllanthus amarus suppresses hepatitis B virus by interrupting interactions between HBV enhancer I and cellular transcription factors.

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The *Phyllanthus amarus* plant suppresses HBV mRNA transcription in vitro and exhibits therapeutic potential in chronic HBV carriers, although further work is necessary to define its mechanism of action. Analysis in HuH-7 cells with transfected plasmids using a luciferase reporter showed that *P. amarus* specifically inhibited HBV enhancer I activity. To identify the mechanism of this HBV enhancer I inhibition, liver-enriched cellular transcription factors were co-expressed in HuH-7 cells. The C/EBP alpha and beta, as well as HNF-3 alpha and beta transcription factors, significantly up-regulated the HBV enhancer I activity. In contrast, co-transfection of HNF-I alpha or beta had no effect upon the HBV enhancer I activity. Exposure to *P. amarus* inhibited C/EBP alpha- and beta-mediated up-regulation of HBV enhancer I activity in a dose-dependent manner, whereas HNF-3 alpha- and beta-mediated up-regulation of HBV enhancer I was unaffected. In vitro gel shifts showed that *P. amarus* inhibited complexing of C/EBP transcription factors to a consensus oligonucleotide sequence, whereas DNA binding of AP-1 and SP-1 transcription factors was unaffected. As *P. amarus* down-regulates HBV mRNA transcription by a specific mechanism involving interactions between HBV enhancer I and C/EBP transcription factors, purification and further analysis of the active *P. amarus* component will advance insights into its antiviral activity. PMID: 9395786

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[Mechanism of protective action of Phyllanthus urinaria L. against injuries of liver cells][Article in Chinese]

Zhou S, Xu C, Zhou N, Huang Y, Huang L, Chen X, Hu Y, Liao Y.

It has been found out that the carbon tetrachloride (CCl₄)-induced increase of serum glutamic-pyruvic transaminase (ALT) and elevation of MDA in liver of mice are significantly lowered by *Phyllanthus urinaria* in vivo, and the coincubation of isolated rat hepatocytes with *Phyllanthus urinaria* in vitro significantly inhibits CCl₄-induced decrease of mobility of membrane of liver cells and increase of intracellular free Ca²⁺ ([Ca²⁺]_i) concentrations of liver cells. These results suggest that the anti-lipid peroxidation effect and protective action of membrane of *Phyllanthus urinaria* may be related to its protective action against CCl₄-induced liver injuries.

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