Andrographis paniculata: A Review of Pharmacological Activities and Clinical Effects

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Introduction

Andrographis paniculata is a plant that has been effectively used in traditional Asian medicines for centuries. Its perceived "blood purifying" property results in its use in diseases where blood "abnormalities" are considered causes of disease, such as skin eruptions, boils, scabies, and chronic undetermined fevers. The aerial part of the plant, used medicinally, contains a large number of chemical constituents, mainly lactones, diterpenoids, diterpene glycosides, flavonoids, and flavonoid glycosides. Controlled clinical trials report its safe and effective use for reducing symptoms of uncomplicated upper respiratory tract infections. Since many of the disease conditions commonly treated with *A. paniculata* in traditional medical systems are considered self-limiting, its purported benefits need critical evaluation. This review summarizes current scientific findings and suggests further research to verify the therapeutic efficacy of *A. paniculata*.

A. paniculata, known on the Indian subcontinent as Chirayetah and Kalmegh in Urdu and Hindi languages, respectively, is an annual plant, 1-3 ft high, that is one of the most commonly used plants in the traditional systems of Unani and Ayurvedic medicines. It is called Creat in English and is known as the "king of bitters." It grows in hedge rows throughout the plains of India and is also cultivated in gardens.[1,2] It also grows in many other Asian countries and is used as a traditional herbal medicine in China, Hong Kong, the Philippines, Malaysia, Indonesia, and Thailand.

The aerial parts are most commonly used; however, the whole plant or roots are mentioned for certain limited purposes in some manuscripts. Traditionally, the plant was used as an infusion, decoction, or powder, either alone or in combination with other medicinal plants. In modern times, and in many controlled clinical trials, commercial preparations have tended to be standardized extracts of the whole plant.

Since many disease conditions commonly treated with *A. paniculata* in traditional medical systems are considered self-limiting, its purported benefits need critical evaluation. This review summarizes current scientific findings and suggests areas where further research is needed.

Uses in Traditional Medical Systems

A. paniculata has been reported as having antibacterial, antifungal, antiviral, choleretic, hypoglycemic, hypocholesterolemic, and adaptogenic effects.[3] In the Unani system of medicine, it is considered aperient, anti-inflammatory, emollient, astringent, diuretic, emmenagogue, gastric and liver tonic, carminative, antihelmintic, and antipyretic. Due to its "blood purifying" activity it is recommended for use in cases of leprosy, gonorrhea, scabies, boils, skin eruptions, and chronic and seasonal fevers.[1] Juice or an infusion of fresh

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leaves is given to infants to relieve griping, irregular bowel habits, and loss of appetite.[2,4,5] The leaves and root are also used in general debility, during convalescence after fevers, for dyspepsia associated with gaseous distension, and in advanced stages of dysentery.[4,5]

In China, the herb derived from the leaves or aerial parts of *A. paniculata* is known as Chuanxinlian, Yijianxi or Lanhelian. It is described as bitter and cold, is considered to be antipyretic, detoxicant, anti-inflammatory, and detumescent, and is thought to remove "pathogenic heat" from the blood. *A. paniculata* is used for the treatment of pharyngolaryngitis, diarrhea, dysentery, cough with thick sputum, carbuncle, sores, and snake bites.[6] Various preparations and compound formulas of the herb have been used to treat infectious and non-infectious diseases, with significant effective rates reported for conditions such as epidemic encephalitis B, suppurative otitis media, neonatal subcutaneous annular ulcer, vaginitis, cervical erosion, pelvic inflammation, herpes zoster, chicken pox, mumps, neurodermatitis, eczema, and burns.[6]

Modern Uses

A primary modern use of *A. paniculata* is for the prevention and treatment of the common cold. It appears to have antithrombotic actions, suggesting a possible benefit in cardiovascular disease.[7] Pharmacological and clinical studies suggest the potential for beneficial effects in diseases like cancer[8-12] and HIV infections.[13]

Phytoconstituents

A. paniculata contains diterpenes, lactones, and flavonoids. Flavonoids mainly exist in the root, but have also been isolated from the leaves. The aerial parts contain alkanes, ketones, and aldehydes. Although it was initially thought that the bitter substance in the leaves was the lactone andrographolide, later investigations showed that the leaves contained two bitter principles – andrographolide and a compound named kalmeghin. Four lactones – chuanxinlian A (deoxyandrographolide), B (andrographolide), C (neoandrographolide) and D (14-deoxy-11, 12-didehydroandrographolide) – were isolated from the aerial parts in China.[6] A diterpene glucoside (deoxyandrographolide-19beta-D-glucoside) has been detected in the leaves[14] and six diterpenoids of the ent-labdane type, two diterpene glucosides and four diterpene dimers (bis-andrographolides A, B, C, and D) have been isolated from aerial parts.[15] Two flavonoids identified as 5,7,2',3'-tetramethoxyflavanone and 5-hydroxy-7,2',3'-trimethoxyflavone were isolated from the whole plant,[16] while 12 new flavonoids and 14 diterpenoids have been reported from the aerial parts.[17,18] Two new flavonoid glycosides and a new diterpenoid (andrographic acid) were recently reported,[19] and two new ent-labdane diterpenoid glycosides were isolated from the aerial parts.[20]

Mechanisms of Action

Hepatoprotective Effects

A. paniculata is extensively used as a hepatostimulant and hepatoprotective agent in Indian systems of medicine.[21] *A. paniculata* is also an ingredient in several polyherbal preparations used as hepatoprotectants in India,[22] one of which has been reported as efficacious in chronic hepatitis B virus infection.[23] Very few studies on the effects of crude extracts of *A. paniculata* on liver function are available. Most studies for hepatic effects have been conducted on either andrographolide or other purportedly active principles.

Shukla et al reported significant choleretic effects of andrographolide in conscious rats and anesthetized guinea pigs. The protection of andrographolide against acetaminophen-induced reduction in volume and contents of bile was better than that produced by silymarin.[24] Multiple-dose pretreatment with arabinogalactan proteins and andrographolide was protective against ethanol-induced hepatotoxicity in mice and was deemed comparable to the efficacy of silymarin.[25] Choudhury and Poddar reported that oral pre- and post-treatment of adult rats with an extract of *A. paniculata* was protective against ethanol-induced increase in serum transaminases. Administration of the extract to normal adult rats in single and multiple doses for seven and 15 consecutive days did not significantly affect serum transaminases.[26]

A comparative study on the effect of leaf extract or andrographolide on carbon tetrachloride (CCl_4)-induced hepatic microsomal lipid peroxidation revealed a protective effect of a single oral dose of the extract and of andrographolide. However, high concentration CCl_4-induced microsomal lipid peroxidation *in vitro* was completely protected by the extract but not by andrographolide, indicating that the hepatoprotective effect is not solely due to the presence of andrographolide.[27] Hepatoprotective effects of the crude alcohol extract of leaves against CCl_4-induced liver damage have also been reported by Rana and Avadhoot.[28]

Handa and Sharma compared andrographolide, methanol extract of the whole plant containing equivalent amounts of andrographolide, and an andrographolide-free methanol extract against CCl_4-induced liver damage in rats. The CCl_4-induced increases in serum transaminases, serum alkaline phosphatase, serum bilirubin, and hepatic triglycerides were inhibited by 48.6-, 32- and 15 percent, for andrographolide, methanol extract, and andrographolide-free methanol extract, respectively. Since all three treatments resulted in improvement in liver histology,[29] a hepatoprotective role of *A. paniculata* constituents other than andrographolide is suggested and corroborates the observation made by Choudhury and Poddar.[27]

The CCl_4-induced increase in pentobarbitone-induced sleep time in mice is also completely normalized by andrographolide. The effects of intraperitoneal (i.p.) pretreatment for three consecutive days with andrographolide on CCl_4- or tert-butyl hydroperoxide-induced hepatotoxicity in mice were compared with two other diterpenes – andrographiside and neoandrographolide. Both compounds showed a greater protective effect than andrographolide. The protection by andrographiside and neoandrographolide was comparable to silymarin, and neoandrographolide normalized glutathione levels.[30]

Trivedi et al observed protection by both the crude extract of *A. paniculata* and andrographolide against reduced activities of hepatic antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase), depletion of hepatic glutathione, and increased activities of hepatic γ-glutamyl transpeptidase, glutathione-S-transferase, and lipid peroxidase caused by hexachlorocyclohexane in mice.[31] Oral or i.p. pretreatment with andrographolide was also protective against galactosamine-induced liver damage in rats and prevented changes in biochemical parameters and liver histology. Similar protection was observed when rats were treated with andrographolide post-acetaminophen challenge,[32] and on an *ex vivo* preparation of isolated rat hepatocytes.[33]

Various extracts and constituents of *A. paniculata* were used in the experiments mentioned in this subsection. All showed hepatoprotective effects. *A. paniculata* also showed benefits against liver damage caused by agents with different hepatotoxic mechanisms, suggesting *A. paniculata* and its constituents are not agent-specific and might have broad-spectrum hepatoprotective effects. More research is needed to establish the identity of the most effective component(s) for hepatoprotection. Large, multicenter, clinical studies are warranted to determine whether *A. paniculata* is efficacious in patients with liver diseases of various origins.

Effects on Hepatic Metabolizing Enzymes

Drug-herb and drug-nutrient interactions can adversely influence the clinical response to treatment. Therefore, the effect of herbal and nutrient compounds on hepatic metabolic enzymes that influence drug pharmacokinetics is an area of interest in modern medicine. Singh et al reported that an 80-percent hydroalcohol extract (50 and 100 mg/kg/day for 14 days) of *A. paniculata* to mice significantly increased the levels of acid-soluble sulfhydryl content, cytochrome P450 (CYP450), cytochrome P450 reductase, cytochrome b5 reductase, glutathione S-transferase, and superoxide dismutase at both doses; while significant increases in the levels of catalase, glutathione peroxidase, and glutathione reductase were observed only at higher doses.[34] Both aqueous and alcoholic extracts of *A. paniculata* are reported to significantly increase the activities of CYP1A1 and CYP2B without affecting the total hepatic CYP450 contents in ICR male mice.[35] Andrographolide significantly induced CYP1A1 and CYP1A2 mRNA expression in cultures of mouse hepatocytes and acted synergistically with CYP1A inducers.[36] *A. paniculata* extract has recently been reported to noncompetitively inhibit CYP1A2 and CYP2C in rat and human liver microsomes and competitively inhibit CYP3A4 in human microsomes; whereas, andrographolide was found to be a weak inhibitor of rat CYP2E1 only.[37] Similar effects of the extract and andrographolide on CYP2C and CYP3A in rat and human hepatocyte cultures have been observed.[38] Existing evidence is not sufficient to draw any conclusions on drug-herb interactions. More extensive studies on hepatic metabolizing enzymes should be conducted in healthy humans and in humans taking medications that are susceptible to pharmacokinetic alterations by these inducible hepatic enzymes.

Antimicrobial and Antiparasitic Effects

A. paniculata has been extensively used to treat a variety of conditions of infectious origin in traditional systems of medicine. Modern research has investigated it for activity against various bacteria, viruses, and parasites. Crude powder suspended in water was reported to be devoid of *in vitro* antibacterial activity against Salmonella, Shigella, *Escherichia coli*, gram A Streptococci, and

Staphylococcus aureus, even at a concentration of 25 mg/mL crude powder. Administration of a single oral dose of powder, up to 6 g, to healthy volunteers in a randomized crossover manner or daily administration of 0.12-24 g/kg body weight to rats for six months also failed to show any *ex vivo* antibacterial activity.[39] Singha et al reported significant antibacterial activity of an aqueous extract and attributed it to the combined effect of andrographolides and arabinogalactan proteins.[40] A similar conclusion was reached by Zaidan et al who found crude aqueous extract of leaves exhibit significant antimicrobial activity against gram-positive *S. aureus*, methicillin-resistant *S. aureus* (MRSA), and gram-negative *Pseudomonas aeruginosa*, but had no activity against *Escherichia coli* or *Klebsiella pneumoniae*.[41] The ethanol extract was also devoid of significant activity against enterohemorrhagic strains of *E. coli*.[42]

Andrographolide, neoandrographolide, and 14-deoxy-11,12-didehydroandrographolide are reported to be viricidal against herpes simplex virus 1 (HSV-1) without having any significant cytotoxicity at viricidal concentrations.[43]

The alcohol extract of the rhizome was reported to possess significant *in vitro* activity against *Ascaris lumbricoides*.[44] The chloroform extract completely inhibited malarial parasitic growth within 24 hours of incubation at a concentration of 0.05 mg/mL. The same inhibition was achieved in 48 hours with methanol extract at a concentration of 2.5 mg/mL.[45] Mishra et al found that a methanol extract significantly inhibited *Plasmodium falciparum* at a 50-percent inhibitory concentration (IC_{50}) of 7.2 µg/mL.[46] The four xanthones – 1,8-dihydroxy-3,7-dimethoxyxanthone, 4,8-dihydroxy-2,7-dimethoxyxanthone, 1,2-dihydroxy-6,8-dimethoxyxanthone, and 3,7,8-trimethoxy-1-hydroxy-xanthone – isolated from the roots of the plant, also showed *in vitro* anti-malarial activity against *Plasmodium falciparum* and *in vivo* activity in Swiss albino mice infected with *Plasmodium berghei*.[47] The same xanthones also exhibited antiprotozoal activity against *Trypanosoma brucei*, *Trypanosoma cruzi*, and *Leishmania infantum*.[48] Water decoction of the leaves exhibited filaricidal activity, both *in vitro* and in dogs.[49]

Clinical relevance of these studies is inconclusive since results are predominantly *in vitro* or *ex vivo*. Many of the *in vitro* results obtained were also achieved at concentrations that may not be clinically feasible.

Cardiovascular Effects

Aqueous extract of *A. paniculata* produced a dose-dependent fall in systolic blood pressure of both spontaneously hypertensive rats (SHRs) and normotensive Wistar-Kyoto rats, with a corresponding significant decrease in plasma angiotensin converting enzyme (ACE) activity and lipid peroxidation in kidneys in extract-treated SHRs. The decreases in ACE activity and lipid peroxidation were not significantly altered in normotensive Wistar-Kyoto rats, an indication that suggests its hypotensive effect in hypertensive and normotensive rats is not mediated through identical mechanisms.[50] The hypotensive effect of n-butanol and aqueous fractions of the crude water extract is antagonized or attenuated by phentolamine, hexamethonium, pyrilamine, and cimetidine, but not by propranolol, atropine, or captopril.[51] However, the fall in mean arterial pressure produced by 14-deoxy-11, 12-didehydroandrographolide (DDA), one of the three active diterpenoids, in anesthetized Sprague-Dawley rats was attenuated in the presence of propranolol, hexamethonium, and captopril. DDA also antagonized the positive chronotropic effect of isoproterenol on the isolated rat right atria in a non-competitive and dose-dependent manner.[52] Hypotensive and negative chronotropic effects of DDA have been corroborated by a recent study that suggested that vascular smooth muscle is the major site of hypotensive activity of DDA and high-DDA extracts.[53]

Several studies investigated the effect of water extract and active constituents of *A. paniculata*, both pre- and post-experimental myocardial infarction (MI), in animals. A water extract was administered intravenously one hour after MI in dogs. Treatment restricted the infarct size and produced a milder core ischemic area than in control dogs; similar results were also observed with flavones extracted from the root.[54,55] Experimental myocardial ischemia-reperfusion injury in dogs results in ultrastructural changes in the ischemic region with an increase in Ca^{2+} and reduced superoxide dismutase, Ca^{2+}-ATPase, and Na^{+}-K^{+}-ATPase activities. Treatment with an extract of *A. paniculata* prevented Ca^{2+} overloading of the ischemic region and the fall in enzyme activities.[56,57] A refined extract (API0134) administered intravenously 45 min post-ischemia induction prevented increase in the left ventricle end-diastolic pressure and preserved relatively normal cardiac output and rhythm in dogs with experimental ischemia-reperfusion myocardial

injury.[58] Andrographolide pretreatment of rat cardiomyocytes is reported to protect them against hypoxia/reoxygenation injury in a time-dependent manner. This effect was associated with upregulation of cellular reduced glutathione (GSH) level and antioxidant enzyme activities.[59]

Wang and Zhao studied the effects of *A. paniculata* on restenosis after experimental balloon angioplasty. Pretreatment with *A. paniculata* extract prevented atherosclerotic iliac artery stenosis in rabbits produced by de-endothelialization and high cholesterol diet. Restenosis after experimental angioplasty in the stenosed arteries was also significantly prevented. The extract inhibited cell growth and DNA synthesis in a dose-dependent manner. This is similar to the mechanism by which stents are coated with drugs that inhibit cell division.[60,61]

Aqueous extract, andrographolide, and DDA inhibit thrombin-induced platelet aggregation in time- and concentration-dependent manners. Extracts with a higher DDA concentration have less inhibitory activity than extracts with lower DDA concentration, indicating the presence of other compounds in the water extract with antiplatelet aggregation activity.[62] Andrographolide inhibits platelet-activating factor (PAF)-induced platelet aggregation in a dose-dependent manner without affecting the biosynthesis of eicosanoids.[7]

An extract of *A. paniculata* significantly inhibited *ex vivo* ADP-induced platelet aggregation in 63 patients with cardiac and cerebral vascular diseases three hours after administration. Thirty-three of these patients who were observed for platelet aggregation after one week experienced even more significant effects. Serotonin release from platelets was significantly reduced in 20 extract-treated volunteers, while the plasma serotonin levels remained unchanged.[63]

Reports regarding hypotensive activity of extracts and some constituents are consistent. Further studies are needed to establish the mechanisms of action, constituents with hypotensive actions, constituent interactions with blood pressure-lowering medications, and clinical efficacy in hypertensive humans. The results of cardiovascular and platelet antiaggregation studies require further exploration in clinical situations.

Antioxidant and Anti-inflammatory Activities

Antioxidant and anti-inflammatory activities of *A. paniculata* and its constituents have been reported by various investigators. Das et al reported that nicotine-induced inhibition of mitochondrial electron chain complexes and the resultant increase in nitric oxide (NO) in different parts of rats' brains was prevented by simultaneous treatment with the water and ethanol extracts of *A. paniculata* or andrographolide; the water extract exhibited greater antioxidant activity than the ethanol extract.[64] Phytochemical analysis showed higher flavonoid but lower phenol contents in water extract than in ethanol extract.[65] Verma and Vinayak compared the antioxidant effects of the aqueous extract on liver defense systems in lymphoma-bearing AKR mice. The aqueous extract significantly increased the activities of catalase, superoxide dismutase, and glutathione-S-transferase enzymes and reduced lactate dehydrogenase activity.[66] A methanol extract inhibited formation of reactive oxygen species (ROS) *in vitro* and completely inhibited carrageenan-induced inflammation.[67] Andrographolide pretreatment significantly attenuates accumulation of phorbol-12-myristate-13-acetate (PMA)-induced formation of ROS and N-formyl-methionyl-leucyl-phenylalanine (fMLP)-induced adhesion of rat neutrophils.[68] However, PMA-induced formation of ROS and fMLP-induced adhesion and transmigration of peripheral human neutrophils was only partially reversed by andrographolide. This study suggests that prevention of ROS production was partly mediated by the direct activation of protein kinase C by PMA and partly mediated by down-regulation of surface Mac-1 expression, an essential integrin for neutrophil adhesion and transmigration, respectively.[69]

Excessive amounts of NO and prostaglandin E_2 (PGE_2), due to expression of inducible isoforms of nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) from activated macrophages, play a significant role in inflammatory processes. Lipopolysaccharide (LPS) stimulates and promotes secretion of pro-inflammatory cytokines from macrophages and causes induction of iNOS, resulting in increased production of NO. Incubation of macrophages with methanol extract, andrographolide, and neoandrographolide inhibits LPS-stimulated NO production in a concentration-dependent manner.[70-73] Andrographolide-induced reduction of iNOS activity may be due to reduced expression of iNOS protein.[71,72] Andrographolide also fully restores the maximal contractile response of thoracic aorta to phenylephrine after incubation with LPS, and attenuates the fall in mean arterial blood pressure of anesthetized rats due to LPS.[71] Unlike andrographolide, neoandrographolide was also effective *ex vivo* in suppressing NO production

when macrophages were collected after oral administration of neoandrographolide and subjected to LPS stimulation.[69] Andrographolide inhibited LPS-induced increase in tumor necrosis factor-alpha (TNF-α) and granulocyte-macrophage colony stimulating factor.[74] Neoandrographolide also inhibits PGE_2 synthesis[73] and TNF-α in LPS-stimulated macrophages, and its oral administration to mice significantly suppresses dimethylbenzene-induced ear edema and acetic acid-induced vascular permeability.[75] API0134, a refined extract of *A. paniculata*, also significantly reduces activities of lipid peroxide and endothelin, while the activities of NO, cGMP, and superoxide dismutase are significantly enhanced in experimental atherosclerotic rabbits.[76]

Antihyperglycemic and Hypoglycemic Effects

Water extract of *A. paniculata* significantly prevents orally administered glucose-induced hyperglycemia in nondiabetic rabbits without affecting epinephrine-induced hyperglycemia. Chronic administration of the extract for six weeks also showed no effect on fasting blood glucose level.[77] However, ethanol extract, administered orally twice daily for 14 days to streptozotocin-induced diabetic rats significantly reduced fasting serum glucose and increased body weight in a dose-dependent manner. The extract also significantly lowered levels of thiobarbituric acid-reactive substances in liver and kidney compared to vehicle-treated rats, while significantly increasing the activity of superoxide dismutase and catalase enzymes and hepatic glutathione concentrations in diabetic rats.[78] An ethanol extract at a dose of 400 mg/kg body weight twice daily for two weeks to diabetic rats produced a 49.8-percent reduction in fasting serum triglyceride levels. This was greater than the 27.7-percent decline achieved with 500 mg/kg body weight metformin twice daily for 14 days.[79] An aqueous extract (50 mg/kg body weight) given to streptozotocin-diabetic rats resulted in a 52.9-percent decrease in blood glucose levels. Freeze-dried material decreased blood glucose by 61.8 percent at a lower dose of 6.25 mg/kg body weight.[80] Similar results were obtained by Dandu and Inamdar with oral administration of an aqueous extract of *A. paniculata* leaves. A dose of 400 mg/kg lowered blood glucose level of streptozotocin-induced animals and increased activity of superoxide dismutase and catalase. Oral administration of the decoction also significantly reduced blood glucose levels in alloxan-induced diabetic rats, and reduced food and water intake compared to vehicle-treated diabetic controls.[81] Extended mean estrous cycles (eight days) was reduced to five days in treated diabetic rats.[82]

Andrographolide appears to dose-dependently reduce plasma glucose concentration in streptozotocin-induced diabetic rats and normal rats, with a more marked effect in normal rats than in diabetic rats.[83] This is a significant difference from the water extract, which did not show a glucose-lowering effect in one study of normoglycemic rats.[81] Andrographolide also attenuates the increase in plasma glucose in response to an intravenous glucose challenge in normal rats and enhances the uptake of radioactive glucose by isolated soleus muscle of streptozotocin-diabetic rats in a concentration-dependent manner. Repeated intravenous administration of andrographolide in diabetic rats for three days resulted in an increase in mRNA and protein levels of glucose transporter (GLUT4) in the soleus muscle, an indication that the glucose-lowering effect of andrographolide could be due to better glucose utilization by skeletal muscle.[83] However, after *in vitro* experiments, Wibudi et al concluded that the hypoglycemic effect of *A. paniculata* is due to insulin release from pancreatic β-cells through ATP-sensitive potassium channels, similar to other insulinotropic antidiabetic agents.[84] *In vitro* experiments conducted by Subramanian et al suggested that inhibition of alpha-glucosidase and alpha-amylase enzyme could be the mechanism by which the ethanol extract of *A. paniculata* and andrographolide produce hypoglycemic effect.[85]

Available evidence suggests that the hypoglycemic and antihyperglycemic activities of the extract and andrographolide may involve different mechanisms in normal and diabetic conditions. Water extract seems to be a more suitable candidate for further studies as it does not affect fasting blood glucose levels of nondiabetic animals. Identification of blood glucose-lowering constituents in both water and ethanol extracts may be of value.

Effects on Reproductive Systems

A number of animal studies report an effect of *A. paniculata* on male and female reproduction. Early reports of oral administration of powdered stem indicated an antifertility effect in male Wistar mice, but no impact on fertility in female mice.[86,87] In has also been reported that administration of *A. paniculata* resulted in abortion in pregnant rabbits.[6] Intraperitoneal injection of the decoction of aerial parts to female albino mice was reported

to prevent implantation and caused abortion at different gestation periods. Early pregnancy was also terminated by intramuscular, subcutaneous, and intravenous administration. Administration of progesterone or luteinizing hormone-releasing hormone completely or markedly antagonized the abortifacient effects, indicating an interference with progesterone activity as a potential mechanism for this abortifacient effect. In addition, the herb is reported to suppress growth of human placental chorionic trophoblastic cells *in vitro*.[6]

Zoha et al fed female mice sun-dried Andrographis powder at a dose of 2 g/kg body weight/day for six weeks. When they were mated with untreated males of proven fertility, pregnancy was inhibited in 100 percent of the animals. Conversely, more than 95 percent of untreated female mice in the control group became pregnant when mated with males in a similar fashion.[88] Akbarsha et al administered dry leaf powder to male albino rats (20 mg daily for 60 days). They reported inhibition of spermatogenesis, degenerative changes in the seminiferous tubules, regression of Leydig cells, and regressive and/or degenerative changes in the epididymis, seminal vesicle, ventral prostate, and coagulating glands.[89] Andrographolide also produced similar results when orally administered to male Wistar albino rats for 48 days. Sperm count and sperm motility were decreased and sperm abnormalities were noted.[90] However, Burgos et al found no testicular toxicity in male Sprague Dawley rats after treatment with a standardized dried extract in doses of up to 1,000 mg/kg daily for 60 days. Their analysis was based on testicular weight and histology, ultrastructural analysis of Leydig cells, and testosterone levels.[91] Extract of *A. paniculata* also did not affect the progesterone levels in pregnant rats when administered orally in doses of 200, 600, and 2,000 mg/kg daily during the first 19 days of pregnancy.[92] Burgos et al reported that dried extract of *A. paniculata* induces uterine relaxation by blocking voltage-sensitive calcium channels.[93] A phase I clinical study on Kan-Jang (a combination of *A. paniculata* and *Eleutherococcus senticosus*) reported no significant negative effects on sperm quality and fertility of healthy adult males.[94]

Existing evidence is too inconsistent, with some findings directly contradicting others, to reach any definitive conclusion about the reproductive effects of *A. paniculata*. The existing evidence does suggest that *A. paniculata* is unlikely to be an effective form of birth control. Further studies on short- and long-term effects on fertility are warranted.

Clinical Studies

Dysentery/Gastroenteritis

Studies conducted in China have reported therapeutic value in acute bacillary dysentery and gastroenteritis. Ethanol extract tablets reportedly cured 88.3 percent of acute bacillary dysentery and 91.3 percent of acute gastroenteritis cases.[6] Andrographolide administration was reported to cure 91 percent of acute bacillary dysentery cases.[6] The same cure rate (91.1%) was achieved with a compound tablet containing andrographolide and neoandrographolide (at a ratio of 7:3) in cases of bacillary dysentery. This was reported to be higher than cure rates obtained with furazolidine or chloramphenicol.[6]

Infectious Diseases

A. paniculata or its constituents have been used to treat cases of leptospirosis, pulmonary tuberculosis (especially the exudative type), tuberculous meningitis, and acute pyelonephritis.[6] In acute pyelonephritis, the results were reported to be similar to those obtained with nitrofurantoin, but with fewer adverse effects.[6] Intra-arterial or retrograde intravenous injections of the herb were reportedly effective in thromboangiitis obliterans, especially of "heat toxic type."[6] Ten cases of viper bites were reportedly cured in 3-5 days by a compound formula that had *A. paniculata* as the chief constituent.[6]

A phase I, dose-escalating clinical trial of andrographolide was conducted in 13 HIV-positive patients and five HIV-negative healthy volunteers. The planned protocol was to start with a dose of 5 mg/kg body weight for the first three weeks, increase to 10 mg/kg body weight for three weeks, and then to 20 mg/kg body weight for a final three weeks. Andrographolide administration significantly improved the CD4+ lymphocyte count from a baseline mean of 405 cells/mm^3 to 501 cells/mm^3 in HIV-positive patients. There was no statistically significant change in mean plasma HIV-1 RNA levels. This trial was stopped after six weeks because of adverse events.[13]

A. paniculata has been used for uncomplicated upper respiratory tract infections (URTIs). There appears to be differences in the degree of therapeutic effect based upon the kind of preparation used and duration of treatment. Pills (made from the whole powdered plant with water) and tablets (made from the water extract of the herb) produced aggregate effective rates of 88 percent and 61 percent in URTI, respectively.[6] In a randomized, double-blind, controlled study, Thamlikitkul et al

gave *A. paniculata* at a dose of 6 g/day for seven days to 152 Thai adults suffering from pharyngotonsillitis. Efficacy was comparable to acetaminophen in relieving symptoms of fever and sore throat.[95] A study of 158 adult patients suffering from common cold used a standardized *A. paniculata* dried extract SHA-10 (1,200 mg/day) for five days. The extract significantly decreased the intensity of the symptoms of tiredness, sleeplessness, sore throat, and nasal secretion, starting from the second day of treatment.[96] Self-limiting side effects were reported in 20 percent of patients from both groups in the former study and no significant adverse effects were reported in the latter study.

A commercial preparation consisting of a standardized extract of *A. paniculata* in a fixed combination with *Eleutherococcus senticosus* (Kan Jang) has been tested in uncomplicated URTIs. Two randomized, double-blind, placebo-controlled parallel group trials were conducted in Sweden. One was a pilot study that involved 46 patients who were treated with Kan Jang three times daily for a minimum of three days and a maximum of eight days; the other was a phase III study of 179 patients for three days. Patients' self-evaluation in regard to muscle aches, cough, sore throat, headache, nasal discharge, eye symptoms, and fever served as the primary outcome measures. Throat symptom relief was highly significant in treated groups compared to placebo-treated groups in both studies.[97] In a similar Armenian study, 95 patients with acute URTIs, including sinusitis, were treated with Kan Jang for five days, while a group of 90 patients served as control. A highly significant improvement in headache, nasal and throat symptoms, and general malaise was reported in the treated groups, including the sinusitis subgroups, while cough and eye symptoms did not differ significantly from the placebo group.[98]

Effects of Kan Jang on uncomplicated respiratory disease have also been studied in children (ages 4-11 years). In a three-arm study, a group treated with standard common cold treatment served as a control. In two groups, Kan Jang or an Echinacea preparation was added as an adjunct to standard treatment for 10 days. Patients receiving Kan Jang as adjunct to standard therapy at an early stage of the common cold showed less severe symptoms, especially nasal congestion and secretions, faster recovery, and a significantly lower need for standard medication.[99] Two systemic reviews of randomized, controlled trials concluded that *A. paniculata* was a safe and efficacious alternative treatment for uncomplicated URTIs compared to placebo.[100,101]

The results from these Kan Jang trials, in both adults and children, cannot be attributed exclusively to the effects of *A. paniculata*, since it was given in combination with *E. senticosus*. The majority of other clinical trials have been published in Chinese journals and lack sufficient details to determine actual efficacy, especially since many conditions studied are self-limiting. More clinical research is required before *A. paniculata* can be deemed efficacious for conditions other than URTI.

Toxicity and Dosing

The LD_{50} of the alcohol extract, obtained by cold maceration, is 1.8 g/kg.[28] The LD_{50} of andrographolide (yield 0.78% w/w from whole plant) in male mice through intraperitoneal route is 11.46 g/kg.[29]

In the study on HIV-positive patients a dose of 1,500-2,000 mg of andrographolides was given daily for six weeks. Side effects were common and the study was discontinued early despite some improvements in CD4+ counts.[13]

Until definitive information on *A. paniculata* and its constituents on reproduction is available, it would be prudent for both men and women to avoid this herb during desired conception and for women during pregnancy.

The majority of studies for common colds and URTI used a patented product – Kan Jang – which combines *A. paniculata* and *E. senticosus*. Andrographis used in this product is standardized to contain 4-6 percent andrographolides and the dose used provided anywhere from 60-72 mg per day (low range) up to about 300 mg per day (highest dose). Existing evidence suggests that best results might be obtained if taken within the first 24 hours of URTI symptoms.

Conclusions and Other Potential Uses

The hepatoprotective effects of pretreatment with various extracts and constituents of *A. paniculata* are very consistent. Moreover, its inclusion in effective polyherbal formulations for liver ailments not amenable to any modern intervention lends support to its potential effectiveness. Before definitive conclusions can be drawn, it would be prudent to study the effects of *A. paniculata* or its constituents under experimental conditions of post-hepatic damage to determine if and how they reverse the pathological changes and which form is the most effective. More research is needed to determine the effects of this

herb on liver-metabolizing enzymes and drug interactions.

Inconsistency in *in vitro* antibacterial effects could be due to several factors, the variation of the constituents in the material tested being the prime suspect. The negative antibacterial results have been reported from Thailand by Leelarasamee et al[39] and Voravuthikunchai and Limsuwan,[42] while the results reported from India[40] and Malaysia[41] have been positive. Place and timing of collection of the plant, storage, and extraction conditions may all affect the constituents both qualitatively and quantitatively. It is imperative to establish the relationship of the activity with the presence of constituents in cases where a crude preparation is tested for it to be declared as possessing any antimicrobial activity.

The plant has shown some significant effects on blood pressure. Before *A. paniculata* can be used clinically in hypertensive conditions, further research must be conducted to expand the understanding of this plant and its constituents on blood pressure and its regulation. The same is true in other cardiovascular conditions where pharmacological studies have suggested potential effectiveness, such as in restricting the infarct size,[54,55] maintaining cardiac function under experimental cardiac ischemic conditions,[58] preventing platelet aggregation,[63] and preventing restenosis after balloon angioplasty.[60,61]

Significant antihyperglycemic activity in diabetic rats has been observed with both water and alcohol extracts. The alcohol extract reduced the serum triglyceride levels highly significantly, and better than metformin treatment (an extensively used antidiabetic drug).[79] Both extracts increased activities of antioxidant enzymes, a mechanism suggested as a potential glucose lowering mechanism.[78,81] Better glucose utilization via upregulation of GLUT4[83] and increased insulin release[84] have also been proposed as mechanisms for the antihyperglycemic effect. This activity requires further exploration.

Existing evidence supports *A. paniculata*'s role in the treatment of URTI. It might also have a role in accelerating the course of other self-limited infections.

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Kutkins- A Review of Chemistry and Pharmacology

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Abstract

Kutkins are group of pharmacologically active compounds present in Picrorhiza kurroa Royle (Scrophulariaceae). Picrorhiza kurroa is traditionally known as kutki and has intense bitter taste. In Ayurveda Picrorhiza kurroa is a reputed remedy for the treatment of liver diseases. The chemical composition of the Picrorhiza has been studied and active constituents are group of iridoid glycosides known as picrosides and kutkosides. The mechanism of action of kutkins appears to the same as that of silymarin (active constituent and hepatoprotective constituent of Silybum marianum). Studies have shown that kutkins are more potent than silymarin as far as hepatoprotective activity is concerned.

Keywords: Hepatoprotective/Picrorhiza kurroa/Kutkin**.**

Introduction:

Picrorhiza kurroa Royle is a distinguished medicinal herb of Ayurveda. It has been described under the group of bitter drugs. Picrorhiza kurroa is a small perennial herb that grows in hilly parts of India particular in Himalayas between 3000 and 5000 meters. It is an established herbal remedy for variety of disease ranging from indigestion to hepatitis. Modern clinical studies have confirmed the efficacy and safety of Picrorhiza kurroa for the treatment of liver disease. The roots and rhizomes are used in medicinally important parts. Powder, decoction, infusion, confection, and alcoholic extract of the drug are prescribed in Ayurveda and Homeopathy.

Botany:

Picrorhiza kurroa has a long, creeping rootstock that is bitter in taste, and grows in rock crevices and moist, sandy soil. The leaves of the plant are flat, oval, and sharply serrated. The flowers, which appear June through August, are white or pale purple and borne on a tall spike; manual harvesting of the plant takes place October through December.

Chemistry:

The chemistry of Picrorhiza kurroa is complex. The active constituent is known kutkin and is a mixture of:

A. Kutkoside

B. Picroside.

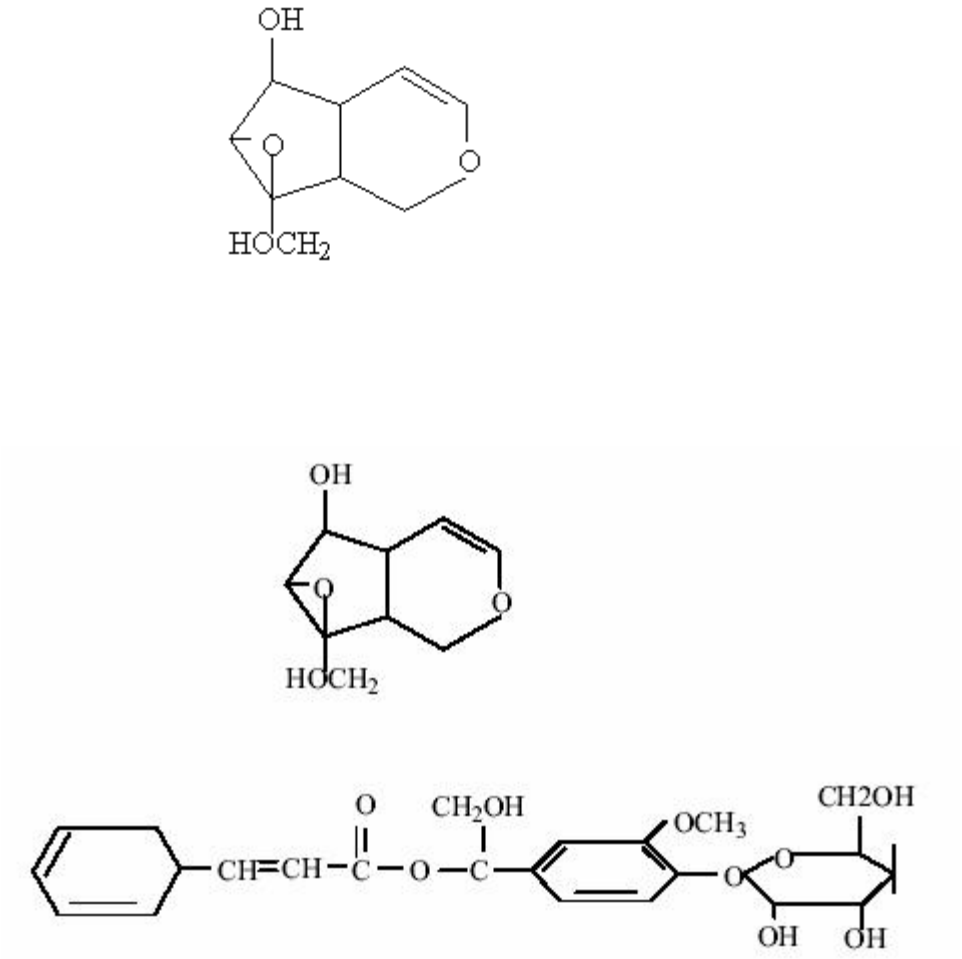
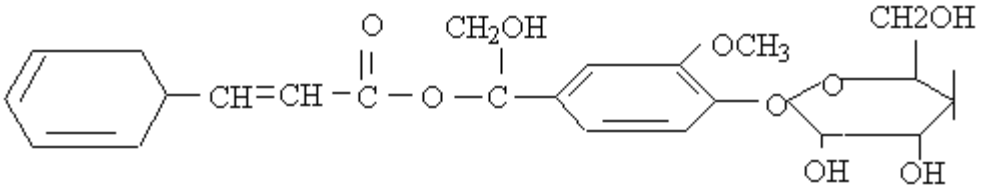


Fig 1. Structure of Kutkins(Kutkosides and Picrosides).



Picrosides are iridoid glycosides and have been further divided into picrosides I, II, and III.

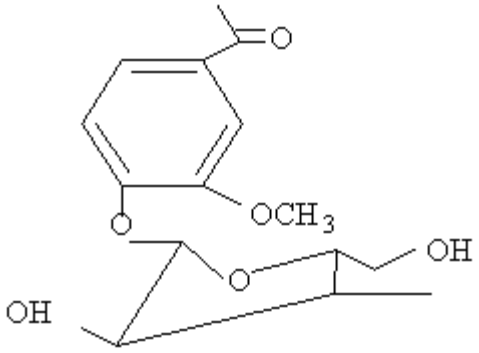


Fig 3. Structure of Androsin

Other constituents are apocynin, andorsin, and cucurbitacin glycosides.

Pharmacologically, Kutkin (Picrosides and kutkosides) has hepatoproptective activity. Apocynin is a potent NADPH oxidase inhibitor and has anti-oxidant and anti-inflammatory activity. Androsin has anti-asthmatic effect.

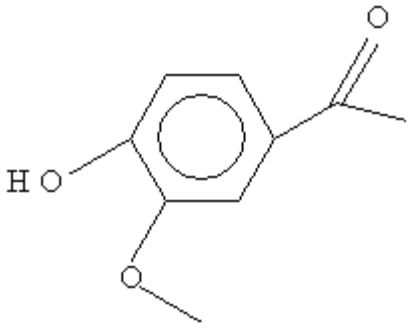


Fig 2: Structure of Apocynin

A colorimetric method has been developed for the analysis of the total iridoid content of the rhizomes of Picrorhiza Kurrooa in terms of catalpol.

Pharmacology:

Some herbalists have described Picrorhiza kurroa as liver herb. Today we have estimated active constituents of the drug, which may be responsible for the hepatoprotective activity of the drug. Most of the studies have shown Picrorhiza kurroa extract (standardized to kutkin content) has potential hepatoprotective activity as compared to placebo.

- Kutkin from Picrorhiza kurrooa has shown significant curative activity in vitro in primary cultured rat hepatocytes against toxicity induced by thioacetamide, galactosamine, and carbon tetrachloride.

- Liver injury was induced in 16 mice by thrice-a-week injection of carbon tetrachloride (CCl4) for nine weeks. Eight of them were given daily feeding of Picrorhiza kurrooa extract (12 mg/Kg) 10 days prior to CCl4 injection. Control mice (n = 6) were injected with olive oil for the same period. Serum markers of liver injury and histology of liver tissues were studied. Hepatic glutathione, total thiol, glucose 6-phosphate dehydrogenase, catalase, lipid peroxidation and plasma membrane-bound Na+/K+ ATPase were also determined. The extract of Picrorhiza kurrooa appears to offer significant protection against liver damage by CCl4.

- In another study, the active constituent of Picrorhiza. kurrooa, showed a dose dependent hepatoprotective activity against oxytetracycline induced hepatic damage in rats.

- In a randomised, double-blind placebo controlled trial in patients diagnosed to have acute viral hepatitis, Picrorhiza kurrooa root powder 375 mg three times a day was given for 2 weeks or a matching placebo was given. Difference in values of bilirubin, SGOT and SGPT was significant between placebo and Pk groups.

Kutkin vs. silymarin:

Silymarin is a well-known hepatoprotective agent. Silymarin is a flavonol- lignan mixture obtained from seeds of Silybum marianum. Silymarin is a mixture of silybin, isosilybin, silychristin and silydianin. Silybin A and B are collectively known as silibinin. Randomized, controlled trials have proved efficacy of silymarin in liver diseases.

Picrorhiza kurroa, when compared with silymarin, the hepatoprotective effect of Picrorhiza kurroa was found to be similar, or in many cases, superior to the effect of Silybum marianum.

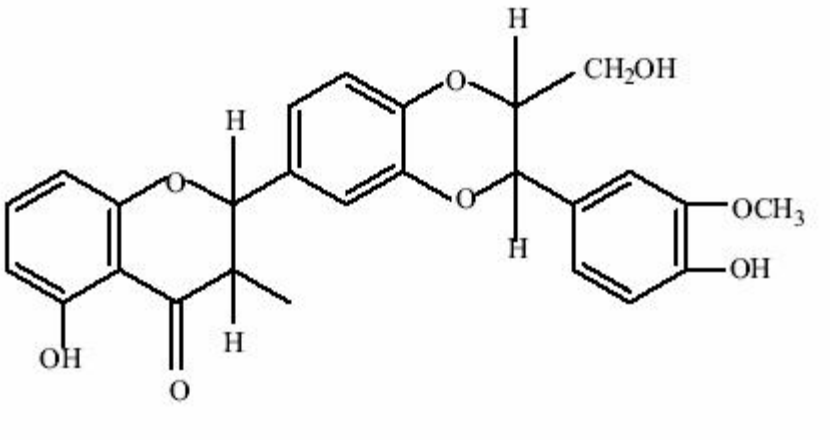


Fig 3: Structure of Silymarin

Mechanism of action:

The mechanism of action of Picrorhiza kurroa is not established.

The therapeutic activity of the drug may be based on two mechanisms:

1. Kutkins alter the structure of the outer membrane of the hepatocytes in such a way as to prevent penetration of the liver toxin into the interior of the cell.
2. Kutkins stimulate the action of nucleolar polymerase A, resulting in ribosomal protein synthesis and, thus stimulates the regenerative ability of the liver and formation of new hepatocytes.
3. Apocynin, is one of its constituents, has been found to exhibit powerful anti-inflammatory effects on a variety of inflammatory models.

Conclusion:

Mono- and polyherbal preparations with potent hepatoprotecive activity have been used in various liver disorders, More than 700 mono- and polyherbal preparations in the form of decoction, tincture, tablets and capsules from more than 100 plants are in clinical use. Silymarin has emerged as potential candidate with hepatoprotective agent. Kutkins have significant, even better hepatoprotective activity than silymarin and the drug should be screed for large-scale clinical trials.

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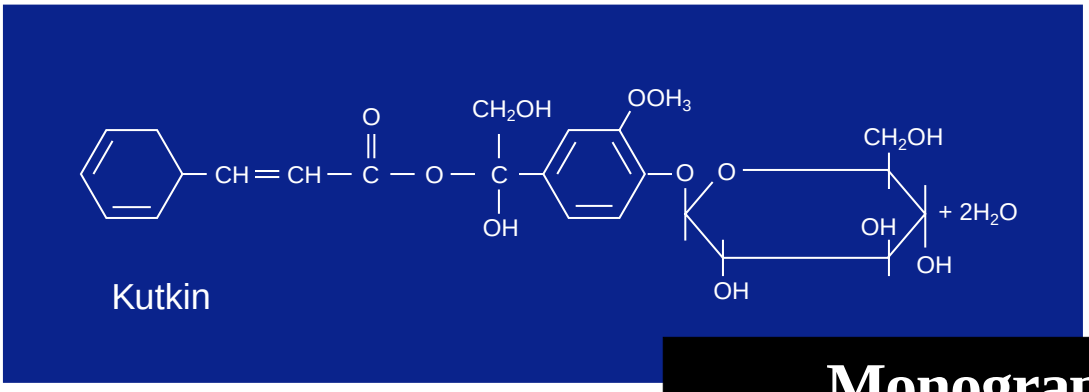

Monograph

Picrorhiza kurroa

Introduction

Picrorhiza kurroa is a well-known herb in the Ayurvedic system of medicine and has traditionally been used to treat disorders of the liver and upper respiratory tract, reduce fevers, and to treat dyspepsia, chronic diarrhea, and scorpion sting. It is a small perennial herb from the Scrophulariaceae family, found in the Himalayan region growing at elevations of 3,000-5,000 meters. *Picrorhiza kurroa* has a long, creeping rootstock that is bitter in taste, and grows in rock crevices and moist, sandy soil. The leaves of the plant are flat, oval, and sharply serrated. The flowers, which appear June through August, are white or pale purple and borne on a tall spike; manual harvesting of the plant takes place October through December. The active constituents are obtained from the root and rhizomes. The plant is self-regenerating but unregulated over-harvesting has caused it to be threatened to near extinction. Current research on *Picrorhiza kurroa* has focused on its hepatoprotective, anticholestatic, antioxidant, and immune-modulating activity.[1,2]

Active Constituents

Kutkin is the active principal of *Picrorhiza kurroa* and is comprised of kutkoside and the iridoid glycoside picrosides I, II, and III. Other identified active constituents are apocynin, drosin, and nine cucurbitacin glycosides.[3,4] Apocynin is a catechol that has been shown to inhibit neutrophil oxidative burst in addition to being a powerful anti-inflammatory agent,[5] while the curcubitacins have been shown to be highly cytotoxic and possess antitumor effects.[6]

Mechanisms of Action

The hepatoprotective action of *Picrorhiza kurroa* is not fully understood but may be attributed to Picrorhiza's ability to inhibit the generation of oxygen anions and to scavenge free radicals.[7] Picrorhiza's antioxidant effect has been shown to be similar to that of superoxide dismutase, metal-ion chelators, and xanthine oxidase inhibitors.[8] In rats infected with malaria, Picrorhiza restored depleted glutathione levels, thereby enhancing detoxification and antioxidation, and helping maintain a normal oxidation-reduction balance.[9] In this same animal model, Picrorhiza also demonstrated an anti-lipid peroxidative effect.[10] Like silymarin, Picrorhiza has been shown to stimulate liver regeneration in rats, possibly via stimulation of nucleic acid and protein synthesis.[11] Picrorhiza's anti-inflammatory action is attributed to the apocynin constituent, which has been shown to have potent anti-inflammatory properties in addition to inhibiting oxidative burst in neutrophils.[5] Although the mechanism is unclear,

animal studies indicate Picrorhiza's constituents exhibit a strong anticholestatic activity against a variety of liver-toxic substances, appearing to be even more potent than silymarin. Picrorhiza also exhibits a dose-dependent choleretic activity, evidenced by an increase in bile salts and acids, and bile flow.[12]

Clinical Indications

Hepatic Insult and Damage

Numerous animal studies, primarily in rats, have demonstrated that the active constituents of *Picrorhiza kurroa* are effective at preventing liver toxicity and the subsequent biochemical changes caused by numerous toxic agents. Hepatocytes damaged by exposure to galactosamine, thiocetamide, and carbon tetrachloride were incubated with Picrorhiza constituents. A concentration-dependent restorative effect was observed in regard to normal hepatocyte function.[13] A similar effect was seen when 25 mg/kg/day oral Picrorhiza extract was administered to rats poisoned by aflatoxin B1 exposure. *Picrorhiza kurroa* significantly prevented the biochemical changes induced by aflatoxin B1.[14] Picrorhiza extract, when given at a dose of 3-12 mg/kg orally for 45 days, was also shown to be effective in reversing ethanol-induced liver damage in rats. [15] In an animal model of hepatic ischemia, rats given Picrorhiza orally at 12 mg/kg daily for 7 days, prior to induced ischemia, demonstrated improved hepatocyte glycogen preservation and reduced apoptosis, compared to control animals.[16] Picrorhiza principals have also shown to be effective in treating Amanita mushroom poisoning in an *in vivo* animal model.[17] An *in vitro* study demonstrated Picrorhiza's antioxidant activity by subjecting human Glioma and Hep 3B cells to a hypoxic state. Picrorhiza treatment reduced the cellular damage cause by hypoxia, indicating Picrorhiza constituents may protect against hypoxia/reoxygenation-induced injuries.[18]

Viral Hepatitis

Studies indicate Picrorhiza extracts may be of therapeutic value in treating viral hepatitis. An *in vitro* study investigated anti-hepatitis B-like activity of Picrorhiza and found it to have promising anti-hepatitis B surface antigen activity.[19] In a randomized, double-blind, placebo-controlled trial of 33 patients diagnosed with acute viral hepatitis, 375 mg Picrorhiza root powder was given three times daily for two weeks. The treatment group was comprised of 15 patients; the remaining 18 subjects acted as controls and received placebo. Bilirubin, SGOT, and SGPT values were significantly lower in the treatment group, and the time required for bilirubin values to drop to 2.5 mg% was 27.4 days in the treatment group versus 75.9 days for the placebo group.[20]

Asthma/Allergy

In vivo studies of bronchial obstruction indicate that the drosin constituent of *Picrorhiza kurroa* prevented allergen- and platelet activating factor-induced bronchial obstruction when given to guinea pigs via inhalant and oral routes. *In vitro* histamine release was also inhibited by the plant extract.[21] Picrorhiza extract given orally at 25 mg/kg to mice and rats resulted in a concentration-dependent decrease in mast cell degranulation. However, induced bronchospasm was not prevented, indicating a lack of direct post-synaptic histamine receptor blocking activity.[22]

Dosage and Toxicity

Picrorhiza is not readily water-soluble and is therefore not usually taken as a tea. While it is ethanol soluble, the bitter taste makes tinctures unpalatable, so it is therefore usually administered as a standardized (4% kutkin) encapsulated powder extract. Typical adult dosage is 400 to 1500 mg/day, with dosages up to 3.5 g/day sometimes being recommended for fevers. Picrorhiza root extracts are

widely used in India with no adverse effects having been reported. The LD_{50} of kutkin is greater than 2600 mg/kg in rats with no data available for humans.[23]

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CONFERENCE PROCEEDINGS

Herbal medicines for liver diseases in India

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Abstract 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 antiviral 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.

Key words: herbal medicine for liver diseases, Indian systems of medicine, *Phyllanthus amarus*, *Picrorhiza kurroa*.

INTRODUCTION

The use of herbal medicine can be traced back to 2100 BC in ancient China at the time of Xia dynasty, and in India during the Vedic period. The first written reports are timed to 600 BC with Charaka samhita of India, and in China the same became systematic by 400 BC.[1] The basic concept in these medicinal systems is that the disease is a manifestation of a general imbalance of the dichotomous energies that govern life as a whole and human life in particular, and they focus on medicine that can balance these energies and maintain good health. In Ayurvedha of India, the forces are said to be *agni* (strength, health and innovation) and *ama* (weakness, disease and intoxication). In India there are also other systems of traditional medicine besides Ayurvedha and these are called Siddha, which originated almost at the same time as Ayurvedha from southern India, and Unani, which entered India during the Mogul dynasty periods. Like Ayurvedha, practitioners of Siddha medicine believe in a perfect balance of three *doshas* known as *vatha* (space and air elements), *pitta* (fire and water elements) and *kapha* (water and earth elements). All these Indian systems of medicine have primarily claimed a curative potential for their medicinal preparations for all kinds of liver diseases.

In spite of the significant popularity of these medicinal systems, they are still to be recognized as being universally acceptable treatment modalities for chronic liver disease.

The limiting factors that contribute to such an eventuality are (i) lack of standardization of the herbal drugs; (ii) lack of randomized placebo controlled clinical trials; and (iii) lack of traditional toxicologic evaluations.

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Table 1 Siddha medicine preparations approved by the Indian Medicinal Practitioner's Co-operative Pharmacy and Stores for the treatment of jaundice and chronic liver diseases[2]

SL. no.	Preparation
1.	Arumuga chendooram.
2.	Annabedhi chendooram no. 1 and 2.
3.	Ayakantha chendooram.
4.	Mandooradi kudineer.
5.	Ayabringaraja karpam.
6.	Karisalai lehyam.
7.	Kantha chendooram and
8.	Loha mandooram.

Table 2 Ayurvedic preparations approved by the Indian Medicinal Practitioner's Co-operative Pharmacy and Stores for the treatment of jaundice and chronic liver diseases[2]

SL. no.	Preparation
1.	Bhringarajasava.
2.	Chandraprabhavati.
3.	Drakahadi rasayam.
4.	Guduchi satwam.
5.	Jambeeradi panakam.
6.	Panchatiktakwatha churnam.
7.	Dhathri loham.
8.	Tapyadi loham.
9.	Pipilyadi loham.
10.	Saptamiruda loham.

HERBAL PREPARATIONS USED IN INDIAN SYSTEMS OF MEDICINE

There are more than 300 preparations in the Indian systems of medicine for the treatment of jaundice and chronic liver diseases (Tables 1–3).

In India more than 87 medicinal plants are used in different combinations as herbal drugs for liver diseases.[3–6] However, not all the plants have been evaluated for their pharmacological and antiviral efficacy although several plants were reported as being hepatoprotective (Table 4).[7–36]

SCIENTIFIC VALIDATION IN MEDICINAL PLANT RESEARCH IN INDIA

There are data available on the following plants: (i) *Picrorhiza kurroa*; (ii) *Phyllanthus amarus* (*niruri*); (iii) *Silybum marianum*; and (iv) *Glycyrrhiza glabra*.

PICRORHIZA KURROA

Picroside 1 and 2, catapol, kutkoside I and kutkoside have been identified as major bioactive components of *P. kurroa*.[37,38] *Picrorhiza kurroa*, a known hepatoprotective plant, was studied in experimental and clinical situations. Basic research has been carried out on the whole plant, picroside 1 alone, and on picroliv, which contain picroside 1, catapol, kutkoside I and kutkoside.

Table 3 Unani preparations approved by the Indian Medicinal Practitioner's Co-operative Pharmacy and Stores for the treatment of jaundice and chronic liver diseases[2]

SL. no.	Preparation
1.	Jawarish-e-Amilasada.
2.	Jawarish-e-Amila luluvi.
3.	Jawarish-e-Tabashir.
4.	Kurs-e-gul.
5.	Rue-e-amila.
6.	Sherbeth-e-anarshreen.
7.	Sherbeth-e-deenar.
8.	Muffarah-e-Ahmedi.
9.	Gul-e-Nilofer.
10.	Bhoi-Amla.

Table 4 List of the Indian medicinal plants having liver protection properties against chemical-induced liver damage in experimental animals[7–36]

SL. no.	Preparation
1.	Acacia catechu.
2.	Achillea millfolium.
3.	Azadirachta indica.
4.	Andrographis paniculata.
5.	Boerhaavia diffusa.
6.	Capparis spinosa.
7.	Chelidonium majus.
8.	Cichorium intybus.
9.	Daucus carota.
10.	Eclipta alba.
11.	Geophila renifbrmis.
12.	Glycosmis pentaphylla.
13.	Mikania cordata.
14.	Moringa oleffera.
15.	Ocimum sanctum.
16.	Phyllanthus embilica.
17.	Phyllanthus debilis.
18.	Phyllanthus kozhikodianus.
19.	Phyllanthus maderaspatensis.
20.	Phyllanthus niruri/amarus.
21.	Picrorhiza kurroa.
22.	Ricinus communis.
23.	Sida cordifolia.
24.	Sida rhombifolia.
25.	Swertia chirata.
26.	Tephrosia purpuria.
27.	Trichopus zeylanicus.
28.	Verbena officinalis.
29.	Wedelia calendulacea.
30.	Withania somnifera.

Dwivedi *et al.* have shown significant hepato-protective properties of picroliv using models such as monocrotaline- and CCl_4-induced liver injury in rats.[38–40] Shukla *et al.* compared picroliv with silymarin in rat and guinea pig models and found potent choleretic and anticholestatic functions.[41] Chander *et al.* also found hepatoprotective properties in picroliv.[42] Using *in vitro* HBsAg binding assay, Mehrotra *et al.* have shown HBsAg inhibition in picroliv and its major component, catapol.[37] Pandey and Das, and Atal Mehrotra *et al.* have demonstrated anti-inflammatory and immunomodulating potential in *P. kurroa*, stimulating both cell-mediated and humoral immunity.[43–45]

Picrorhiza kurroa has also been found to be helpful in clinical and biochemical recovery in acute hepatitis.[46]

Jayaram found *P. kurroa* to be effective in HBeAg seroconversion in 28.5% of patients compared to 16% seroconversion with placebo.[47]

The existing literature on *P. kurroa* suggests that it is a powerful immuno-modulator rather than an antiviral drug in liver diseases.

PHYLLANTHUS AMARUS

Historical use of *Phyllanthus niruri* in jaundice

Even though clinical uses of *P. niruri* and other species, namely *P. amarus*, have been cited for over a century in the Ayurvedha and Siddha literature, scientific studies were carried out only during the last 50 years.

Studies on *Phyllanthus nirurilamarus* against hepatitis B virus

Thyagarajan *et al.* have shown that there exist antiviral properties against HBV for the whole plant extract of *P. niruri*.[48,49] This plant from Tamilnadu, India, has been identified taxonomically as *P. amarus*. The aqueous extracts of the plant inhibits the viral DNA polymerase (DNAp) of HBV and woodchuck hepatitis virus (WHV) *in vitro*.[50–52]

Yanagi *et al.* have also reported that aqueous extracts of high dilutions of *P. amarus* collected from South India inhibited HBV DNAp, DNApI, T4-DNAp, the Klenow fragment and reverse transcriptase of avian myeloblastosis virus.[52] Shead *et al.* have shown the aqueous extracts to inhibit the endogenous DNAp of Duck hepatitis virus (DHBV) at high dilutions.[53] Niu *et al.* with suitable controls found that after 10 weeks treatment, there was a transient reduction of duck hepatitis B viral DNA in the serum of congenitally infected ducks, but there was no effect on the level of virus DNA or surface antigen in the liver.[54]

Jayaram and Thyagarajan reported *in vitro* inhibition of HBsAg secretion by PLC/PRF/5 (Alexander) cell line for 48 h when the cell line was treated with 1 mg/mL concentration of *P. amarus* as a single dose.[55] Lee *et al.* found that *P. amarus* downregulated HBVm transcription and replication in transgenic mice and transgenic cell lines.[56] *Phyllanthus amarus* possibly interrupts the interaction between HBV enhancer I and cellular transcription factors.[57]

Phyllanthus amarus is reported to be safe and non-toxic to mice in a dose of 10 g/kg bodyweight.[58] A 20% aqueous extract of *P. niruri* is effective as an oral pretreatment at 0.2 mL/100 mg bodyweight against CCl_4-induced hepatotoxicity in rats.[59] The hexane-extracted compounds, phyllanthin and hypophyllanthin, were found to reduce CCl_4- or galactosamine-induced cytotoxicity to cultured rat hepatocytes.[60]

The aqueous extract of the dried whole plant did not produce any chronic toxicity in mice at 0.2 mg daily dose per animal for 90 days, as determined by physiological, biochemical and histopathological parameters.[61] Venkateswaran *et al.* demonstrated its *in vivo* safety using woodchucks as animal models,[50] while Niu *et al.* have shown *P. amarus* to be non-toxic in Pekin ducks chronically infected with duck hepatitis B virus.[54] Jayaram *et al.*, studying the effect of *P. amarus* on β-galactosamine-induced hepatotoxicity on isolated rat hepatocytes, found that *P. amarus* by itself was not hepatotoxic and at 1 mg/mL concentration it was found to be hepatoprotective.[62]

In the traditional medicine systems, several formulatory medicines are used for the treatment of jaundice without taking into consideration the etiology. *Phyllanthus niruri* is one of the constituents of such multiherbal preparations, which contain anywhere up to 12 medicinal herbs. Most of the treatment evaluations were based only on improvement in the clinical condition of the patient.

In a clinical trial in acute viral hepatitis (AVH) patients, Jayanthi *et al.* used *P. niruri* and compared it with other herbal medicines.[63] A significantly greater decrease in transaminase levels after 2 weeks' treatment with *P. niruri* in both HBsAg-positive and -negative groups was observed. In another study, *P. amarus* treatment improved liver functions significantly faster in both acute hepatitis A and B, with a higher rate of HBsAg clearance.[64]

In a clinical trial on chronic HBV carriers, HBsAg clearance in the *P. amarus*-treated group was 59%, *versus* 4% in the placebo group.[65] The second open trial in 1990 showed 20% HBsAg clearance and 63.6% loss of infectivity by HBeAg sero-conversion.[66] Additional clinical trials were undertaken in subsequent years (Table 5).[69] However, several investigators from other countries including Leelarasamee *et al.* from Thailand and Wang *et al.* from China could not reproduce the treatment efficacy.[67,68] This could partly be due to the use of the local variety of *P. amarus* grown in their respective countries.

STANDARDIZATION OF *PHYLLANTHUS AMARUS*

In order to determine the reasons for variation in the *in vitro* efficacy of different collections of *P. amarus* made from different parts of India, *in vitro* analysis for biological properties was done. It revealed that the quantita-

Table 5 Clinical trials of *Phylllanthus amarus* on patients with chronic HBV

Clinical trial no.	Study/year	Dosage mg/tds	Duration	No. treated		HBsAg		HBeAg seroconversion %	
				Test	Placebo	Test	Placebo	Test	Placebo
1	Thyagarajan *et al.* (1988)[65], Madras	200	1 month	40	38	59	4	ND	ND
2	Thyagarajan *et al.* (1990)[66], Madras	250	3 months	20	Nil	20	–	63.6	–
3	Samuel *et al.* (1991)[69], Vellore	250	2 months	10	12	20	8.3	37.5	0
4	Thyagarajan etal (1992)[69], Madras	250	6 months	72	Nil	25	–	54.0	–
5	Thyagarajan etal (1993)[69], Madras	500	3 months	8	8	25	0	71.4	16.0
6	Walker *et al.* (1993–1995)[69], Glasgow	500	4–6 months	26	Nil	11.6	–	45.4	–
7	Thyagarajan *et al.* (1996–1997)[69], Madras	500	6 months	37	Nil	18.9	–	60.0	–

ND, not done. In summary, these trials enumerated in Table 5 have shown a mean HBsAg clearance rate of 25.6% and mean HBeAg seroconversion rate of 55.3% with a recommended dose of 500 mg dosage of *P. amarus* preparation in capsules given orally three times daily for 6 months. *P. amarus* grown in Tamilnadu; for convenience it is termed as 'university preparation'.

tive HBsAg binding ability and HBV DNA polymerase inhibition ability varied significantly in different plant collections, and some of them did not possess demonstrable anti-hepatitis B properties. When the extracts of these collections were analyzed by HPLC profiles, there were similar variations of diminished elution peaks or even absence of such peaks that could be correlated with the absence of biological properties. These observations were considered along with the report of Mitra and Jain on the botanical survey of India, which stated that the *P. niruri* is a mixture of three distinct species, namely *P. amarus* Schum and Thonn, *P. fraternus* Webster and *P. debilis* Kleinx Wild.[70] From the reports it is now understood that the variety that is predominant in South India is *P. amarus* only.[71]

Based on these observations it was necessary to define a multistep standardization procedure for assuring the reproducible, maximized bio-efficacy of *P. amarus* when used in the treatment of chronic liver disease with special reference to chronic HBV infection. These steps are: (i) taxonomic identification of the specific variety of *P. amarus* as the source material; (ii) preparation of the soil for the optimum growth of the selected *P. amarus* variety; (iii) a formulation of the combination of specific extracts that will possess the following six demonstrable *in vitro* activities: (a) HBsAg binding property, which will facilitate the inactivation of the virus in circulation, ultimately leading to viral clearance; (b) HBV-DNA polymerase enzyme-inhibiting potential, thus acting as an antiviral and preventing the multiplication of HBV; (c) reverse transcriptase enzyme inhibition, also required for the prevention of initiation of HBV replication; (d) inhibition of HBsAg secretion from HBV-transinfected liver cells, thus possessing activity against virus-infected chronic liver disease conditions; (e) hepatoprotective and antihepatotoxic properties against the liver cell toxicity brought about by all hepatitis viruses (A,B,C,D,E) and other hepatotoxic agents; and (f) immunomodulating property to potentiate the immune system of HBV-infected patients towards virus clearance and protective antibody (anti-HBs) responses; and (iv) a 'chemobiological finger printing methodology' using the aforementioned six biological tests along with a matching HPLC profile to assess batch-to-batch *in vitro* reproducibility.

The evaluation of herbal products face several major problems. The first is the use of mixed extracts (concoctions) and variations in methods of harvesting, preparing and extracting the herb, which can result in dramatically different levels of certain alkaloids. The biologically active substances have been structurally defined and standardized for only a few of the herbs. Even then it may not be known whether this is the sole active principal or if efficacy depends on the mixture of compounds.

Second, there is a lack of randomized placebo controlled clinical trials for many of these preparations using end-points of treatment efficacy such as viral clearance, and histological improvement.

Numerous reports on toxic effects contradict the popular view that herbal drugs are natural and are harmless.[72–76] A survey by the National Poison Information Service for the years 1991–1995 documented 785 cases of possible or confirmed adverse reactions to herbal drugs, among which hepatotoxicity was the most frequent.[73] Hence, safety studies are needed to generate scientifically sufficient data that may serve as a basis for future herbal drug development.

In addition to the well-accepted laboratory parameters as described here using *P. amarus* as an example, if the aforementioned three aspects viz., herbal drug standardization, randomized controlled clinical trials and well-designed safety studies are incorporated as integral components, then herbal drug development, potent, safe and acceptable herbal drugs can be launched for the treatment of acute and chronic liver diseases. The drug development of *P. amarus* in India may be an example of such an effort in an international setting.

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Review Article

YARROW (*ACHILLEA MILLEFOLIUM LINN.)* A HERBAL MEDICINAL PLANT WITH BROAD THERAPEUTIC USE – A REVIEW.

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ABSTRACT

This review gives an account of the current knowledge on the morphology, phytochemistry, and pharmacological aspects of yarrow (*Achillea millefolium).* Yarrow *(Achillea millefolium)* is a flowering plant in the family Asteraceae, it is called *plumajillo*, or "little feather", for the shape of the leaves. A wide range of chemical compounds have been isolated, mainlyisovaleric acid, salicylic acid, asparagin, sterols, flavonoids, bitters, tannins, and coumarins. Different parts of the plant have been used in traditional medicine as diaphoretic, astringent, tonic, stimulant and mild aromatic, cold and influenza, Amenorrhea, antiphlogistic. The aerial parts of the plant are used for phlegm conditions, as a bitter digestive tonic to encourage bile flow, and as a diuretic. The alkaloids extracted from the leaves of yarrow *(Achillea millefolium*) are reported to have anti-inflammatory and analgesic activity. yarrow *(Achillea millefolium)* shows several characteristic pharmacological effects like gastroprotective, Antibacterial, Antioxidant, Antiseptic, Expectorant, carminative activity which are consistent with the reported uses of the plant extracts in the indigenous system of medicine. Hence the present article includes the detailed exploration of morphology, phytochemistry, and pharmacological aspects of yarrow *(Achillea millefolium)* in an attempt to provide a direction for further research.

Keywords: Alkaloids, antibacterial, Antioxidant, *Achillea millefolium,* flavonoids.

INTRODUCTION

Plants have been used by men from prehistoric times to get rid of suffering &curing ailments. The folk medicines of almost around the world rely chiefly on herbal medicine even today[1]. Plants are of the important sources of medicine & alarge numbers of drugs in use are derived from plants. The therapeutic uses of plant are safe & economical & effective as their ease of availability[2]. In India the indigenous system of medicine namely Ayurvedic, Siddha & Unani have been in existence for several centuries. Yarrow, a member of the aster family, is closely related to chrysanthemums and chamomile.yarrow (*Achillea millefolium*) was named after Achilles, the Greek mythical figure who used it to stop the bleeding wounds of his soldiers. Decoctions have been used to treat inflammations, such as hemorrhoids, and headaches. The most medicinally active part of the plant is the flowering tops. They also have a mild stimulant effect, and have been used as a snuff. Popular in European folk medicine, yarrow contains flavonoids (plant-based chemicals) that increase saliva and stomach acid, helping to improve digestion. Yarrow may also relax smooth muscle in the intestine and uterus, which can relieve stomach and menstrual cramps. The flowers are used to treat various allergic mucus problems, including hay fever The dark blue essential oil, extracted by steam distillation of the flowers, is generally used as an anti-inflammatory[3] or in chest rubs for colds and influenza. The leaves encourage clotting, so it can be used fresh for nosebleeds.[4] The aerial parts of the plant are used for phlegm conditions, as a bitter digestive tonic to encourage bile flow, and as a diuretic.[5] The aerial parts act as a tonic for the blood, stimulate the circulation, and can be used for high blood pressure. It is also useful in menstrual disorders, and as an effective sweating remedy to bring down fevers. Yarrow intensifies the medicinal action of other herbs taken with it,[6] and helps eliminate toxins from the body. It has Analgesic[7,8] Amenorrhea, antiphlogistic,[9,10] anti-inflammatory agent, used to control bleeding, blood clots, blood pressure (lowers), blood purifier, blood vessels (tones), colds, chicken pox, circulation, cystitis, diabetes treatment, digestion (stimulates)gastro-intestinal disorders[9], choleretic[11] dyspepsia, eczema, fevers, flu's, gastritis, glandular system, gum ailments, heartbeat (slow), influenza, insect repellant, inflammation[12], emmenagogue,[13] internal bleeding, liver (stimulates and regulates), lungs (hemorrhage), measles, menses (suppressed), menorrhagia, menstruation (regulates, relieves pain), nipples (soreness), nosebleeds, piles (bleeding), smallpox, stomach sickness, toothache, thrombosis, ulcers, urinary antiseptic, uterus (tighten and contract), gastroprotective[14] varicose veins, vision, may reduce autoimmune responses[15]. A decoction of the whole plant is employed for bleeding piles, and is good for kidney disorders. It has the reputation also of being a preventative of baldness, if the head be washed with it. Internally it is used for loss of appetite and dyspepsia. Externally it is used as a sitz bath for female disorders.[16]

Table 1: Scientific classification of Yarrow

Kingdom	Plantae
Family	Asteraceae
Genus	*Achillea*
Species	*A. millefolium*
Binomial Name	*Achilleamillefolium*L.
References Species	Plantarum 2: 899 L

Fig 1: *Achillea millefolium*

Fig 2: Budding

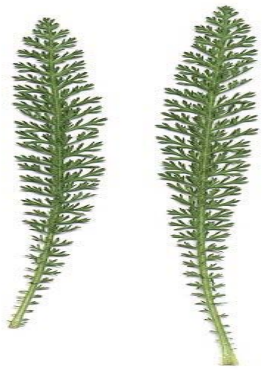

Fig 3

Leaves of Yarrow

Fig 4

Flowers of Yarrow

Table 2: Common Names of Yarrow

Green arrow	Milfoil
Noble yarrow	Nosebleed plant
Sanguinary	Solder's woundwort
Thousandleaf	Yarrow

Binomial Distribution[17]

Yarrow (*Achillea millefolium*) is an erect herbaceousperennial plant that produces one to several stems (0.2 to 1m tall) and has a rhizomatous growth form. Leaves are evenly distributed along the stem, with the leaves near the middle and bottom of the stem being the largest. The leaves have varying degrees of hairiness (pubescence). The leaves are 5–20 cm long, bipinnate or tripinnate, almost feathery, and arranged spirally on the stems. Yarrow grows up to 3500m above sea level. The plant commonly flowers from May through June, and is a frequent component in butterfly gardens. Common yarrow is frequently found in the mildly disturbed soil of grasslands and open forests. Active growth occurs in the spring. In North America, there are both native and introduced genotypes, and both diploid and polyploid plants.

Habitat[18]

Yarrow (*Achillea millefolium*) flourishes in a sunny and warm habitat, and is frequently found in meadows and along roadsides, as well as on dry, sunny slopes. It grows as a simple, upright, and hairy stem, usually under 3 feet. Yarrow blooms between June and September. The flowers are typically white, but either pink or pale purple flowers are common in mountain areas. The petals are densely arranged in flattened clusters, and the leaves look like feathers. The plant spreads rapidly. Yarrows can be planted to combat soil erosion due to the plant's resistance to drought. It is Found in, Europe, North America northern Asia, and southern Australia.

Establishment[19]

Common Yarrow (*Achillea millefolium*) is a drought tolerant species of which there are several different ornamental cultivars. Seeds require light for germination, so optimal germination occurs when planted no deeper than ¼ inch. Seeds also require a germination temperature of 18–24°C (64–75°F). Common yarrow responds best to soil that is poorly developed and well drained. The plant has a relatively short life, but may be prolonged by dividing the plant every other year, and planting 12 to 18 inches apart. Common yarrow is a weedy species and can become invasive. It may suffer from mildew if not planted in well-drained soil.

Parts Used

The flowers, leaves, and stems of the Yarrow (*Achillea millefolium*) plant are used for medicinal purposes. It is collected while in bloom.

Phytochemical constituents of Yarrow (*Achillea millefolium*) [20]

- Alkaloids (betonicine, stachydrine, trigonelline)
- Coumarins
- Flavonoids (apigenin, luteolin, quercitin)
- Salicylic acid
- Sesquiterpene lactones (achillin, achillicin)
- Polyacetylenes
- Volatile oil with variable content (linalool, camphor, sabinene, chamazulene)
- Triterpenes
- Tannins.
- Sterols and plant acids.

Ethanobotanical uses[21]

Various parts of the globe, use of plants has been mentioned by all the cultures. The physical evidence for the use of herbal remedies dates back to 60,000 years of a Neanderthal man uncovered in 1960. As per history of phytotherapy, Women of those days prepared herbal medicines every day and took care of the ills of the members of the family; while the men complied the remedies used by the women and wrote it down. the ancient civilization of India, China, Greece and other countries of the world developed their own system of medicines India plants have been traditionally

independent of each other but all of them were predominantly plant based.

In India plants have been traditionally used for human and veterinary health care and also in the food and textile industry. 90% percent of the local food resources known to indigenous people were undocumented to nutritional literature, trade, cosmetics and perfumes: but India has a special position in area of herbal medicines *A. millefolium* Linn., Family composite used in various purposes such as leaves, flowers, Aerial parts, root, essential oil etc. are known to have potential pharmacological activity such as expectorant, analgesic, anticancer, hypotensive, and antiinflammatory agent. It also plays a significant role in treatment of fevers, arthritis, asthma, bronchitis, Cardio vascular complaints etc. in traditional medical practices.

Uses of whole plant

Yarrow (*Achillea millefolium*) plant has flexible medicinal use and it is very resourceful as a herbal medication is established from the fact that various parts of the plant can be used for healing different disorders. The flowers, essential oil, leaves as well as aerial parts are useful in some way or the other.

Flowers

Infusion: An infusion of yarrow flowers can be prepared by steeping the flowers into boiling water for some time. If taken internally, the infusion is useful for upper respiratory phlegm. It may also be useful to heal eczema when applied externally as a wash.

Inhalation: Fresh yarrow flowers may be added to boiling water and the aroma inhaled to cure hay fever and mild asthma.

Essential oil

Massage oil: With a view to get relief from swollen joints, dilute 5 to 10 drops of yarrow oil in 25 ml of permeated St. John's wort oil and massage the amalgamation on the affected areas.

Chest rub: To alleviate chesty colds and drive out influenza, dilute 20 drops of essential yarrow (Achillea millefolium) oil in 25 ml of almond or sunflower oil and blend it with eucalyptus, peppermint, hyssop or thyme oil and rub the mixture on the chest.

Leaves

Fresh: Inserting yarrow (*Achillea millefolium*) leaf into the nostril helps in curbing nose bleed. Poultice: Cuts and scratches on the body can be healed by wrapping cleansed fresh yarrow leaves on the affected regions.

Aerial parts

Infusion: An infusion prepared with the aerial parts of the yarrow plant is useful in reducing fevers. The infusion is also useful as a digestive stimulant.

Tincture: Yarrow (*Achillea millefolium*) tincture is useful for healing urinary disorders and menstrual problems. It is also recommended for cardiovascular complaints.

Compress: soak a pad in the yarrow infusion or dilute the yarrow tincture to get relief from varicose veins

PHARMACOLOGICAL ACTIONS

The volatile oils work as antibacterial, anti-inflammatory and diuretic agents. The tannins are aggressive astringents. The alkaloids are both hypotensive and hypoglycemic. Yarrow (*Achillea millefolium*) even has coumarin in its cells which works as an anti-thrombotic to reduce high blood pressure. The bitter compounds that the tongue detects are due to flavonoids such as saponins and unpleasant tasting but powerful alkaloids like achilleine, trigonelline and betonicine. These are the proven facts for yarrow's actions in the digestive system, tissues and the blood stream.

Biological activity

Anti Microbial activity[22-30]

Yarrow (*A. millefolium*) is recognized as a powerful medicinal plant is widely distributed and has been used medicinally for thousands of years. In a study, *A. millefolium* showed antibacterial activity against *S. typhimurium* and *S. aureus* with predicted MICs on the order of 10 s of µg/mL or 10 s of mg/mL, respectively. A number of studies have investigated the antibacterial properties of this species and found similar results to those presented here. One difference, however, is that two studies found that ether-hexane-methanol extracts of Yarrow caused inhibition zones against *E. coli* in disc diffusion assays, whereas the study with aqueous extracts of flower, leaves, roots, and shoots and a separate study of essential oil and methanolic extracts did not. These differing results could be due to the different extraction methods used or regional variation in the chemical constituents of the plants. It is well known that Yarrow represents a diverse, polyploid complex that is probably composed of dozens of species with varying biochemical compositions. The biochemical diversity of this complex has been fairly well described, and it has been hypothesized that phenolic compounds such as flavonoids and phenolcarbonic acids may underlie the observed antimicrobial activity.

Cholagogue Activity

The cholagogue activity of yarrow (*Achillea millefolium*) may be due to the presence of unsaturated fatty acids. At the least, it has been observed unsaturated fatty acids have good cholagogue effects, and they are present in yarrow.

Anti Hypertensive activity[31]

The control of Hypertension is an important element in the management of cardiovascular diseases. Study showed that yarrow (*Achillea millefolium*) exhibits a high

angiotensin converting enzyme inhibition, hence used in the treatment of hypertension.

Anti Inflammatory activity[32]

Yarrow oil (*Achillea millefolium*) possesses good Anti inflammatory activity and used to treat rheumatism, muscle aches and heal inflamed cuts or wounds. Its phytochemical constituent, Flavonoids, along with having an effect on prostaglandin production, possess anti-inflammatory. Another component of yarrow that is a powerful anti-inflammatory is azuline, which comprises almost half of yarrow's chemical composition.

Antioxidant Activity[33]

In a Study the in vitro antimicrobial and antioxidant activities of the essential oil and methanol extracts of *Achillea millefolium* subsp. *millefolium Afan.* (Asteraceae) were investigated. GC-MS analysis of the essential oil resulted in the identification of 36 compounds constituting 90.8% of the total oil. Eucalyptol, camphor, alpha-terpineol, beta-pinene, and borneol were the principal components comprising 60.7% of the oil. The oil strongly reduced the diphenylpicrylhydrazyl radical (IC(50)=1.56 micro g/ml) and exhibited hydroxyl radical scavenging effect in the Fe(3+)-EDTA-H(2)O(2) deoxyribose system (IC(50)=2.7 micro g/ml). It also inhibited the nonenzymatic lipid peroxidation of rat liver homogenate (IC(50)=13.5 micro g/ml). The polar phase of the extract showed antioxidant activity. The oil showed antimicrobial activity against Streptococcus pneumoniae, Clostridium perfringens, Candida albicans, Mycobacterium smegmatis, Acinetobacter woffii and Candida krusei while water-insoluble parts of the methanolic extracts exhibited slight or no activity. This study confirms that the essential oil of yarrow (*Achillea millefolium)* possesses antioxidant and antimicrobial properties in vitro.

Anti nociceptive activity[34]

In a study the hydroalcohol extracts of *Achillea millefolium L.* (AM) and *Artemisia vulgaris* L. (AV), both belonging to the Asteraceae family, were evaluated by the hot plate, writhing, formalin and intestinal transit tests in an attempt to confirm their folk use as analgesic, antiinflammatory and antispasmodic agents. AM 500 and 1000 mg/kg significantly inhibited abdominal contortions by 65% and 23%, respectively, whereas AV 500 and 1000 mg/kg inhibited them by 48% and 59%, respectively. None of the extracts produced differences in the intestinal transit in mice, nor in the response time in the hot plate or in the immediate or late responses in the formalin test. In HPLC/DAD analyses 'fingerprint', monitored at 360 and 270 nm, both hydroalcohol extracts showed the same flavonoid glycoside as a principal constituent, which was identified as rutin. A high content of caffeic acid derivatives were also found in both extracts. The main differences were observed at 240 nm: AM had a higher content of rutin, while in AV the hydroxybenzoic acid derivative was the major component.

Anti cancer activity[35,36]

Yarrow (*Achillea millefolium*) possesses good Anti cancer activity. The phytochemical constituent of yarrow that is the flavonoids and sesquiterpenoids have anti-proliferative effects against mouse P-388 leukemia cells and cervix epithelial adenocarcinoma (HeLa), breast epithelial adenocarcinoma (MCF-7) and skin epidermoid carcinoma (A431) cells[33].

Anti Diarrheal and Gastroprotective activity

The astringent feature of yarrow (*Achillea millefolium*) makes it a useful medication in stopping diarrhea and dysentery as well as impedes hemorrhage from the intestinal coatings. In addition, yarrow's sterile and anti-inflammatory qualities help in healing infections and swollen organs like in the case of gastritis and enteritis.

SAFETY FACTORS & TOXICITY[37]

Yarrow (*Achillea millefolium*) causes Contact dermatitis, photosensitization, and other allergic reactions may occur in sensitive individuals. This herb has approval status by the German Commission E. Yarrow (*Achillea Millefolium*) Extract is an extract of the yarrow plant, *Achillea millefolium*, supplied in polypropylene glycol, which is reported to function as a "biological additive" in cosmetic products. Sesquiterpene lactones, polyacetylenes, simple coumarins, and flavonoids have been identified among the many components of *A. millefolium*. Yarrow Extract was reportedly used in 65 cosmetic formulations. Historically, Yarrow (*Achillea Millefolium*) Extract was reported to be used at concentrations of < or =25%, but recent data indicate that this ingredient is supplied with actual Yarrow (*Achillea Millefolium*).

Extract content of 2% to 25% and used at concentrations of 0.5% to 10%. Only limited toxicity data were available. Guinea pigs were sensitized to crude extracts of the whole plant and the flowers of A. *millefolium*. *A. millefolium* tea was weakly genotoxic in a somatic mutation and recombination test using Drosophila melanogaster. In clinical testing, product formulations containing 0.1% to 0.5% of ingredient that actually contained 2% of Yarrow Extract were generally not irritating. In provocative testing, patients reacted to a Composite mix that contained yarrow, as well as to yarrow itself. Also in clinical testing, a formulation containing 0.1% Yarrow *(Achillea Millefolium*) Extract (2% Yarrow in propylene glycol and water) was not a sensitizer in a maximization test and alcoholic extracts of dried leaves and stalks of *A. millefolium* did not produce a phototoxic response.

These data were not considered sufficient to support the safety of this ingredient in cosmetics. The types of data (all testing is to be performed on cosmetic-grade ingredients) still required include (1) ultraviolet (UV) absorption data, if absorption occurs in the UVA or UVB

range, photosensitization data are needed; (2) gross pathology and histopathology in skin and other major organ systems associated with repeated exposures; (3) reproductive and developmental toxicity data; (4) two genotoxicity studies, one using a mammalian system, if positive, a 2-year dermal carcinogenicity assay performed using National Toxicology Program (NTP) methods may be needed; and (5) clinical sensitization testing at maximum concentration of use. In the absence of these data, it was concluded that the available data are insufficient to support the safety of Yarrow (*Achillea Millefolium)* Extract for use in cosmetic products.

Abortifacient activity[38]

Yarrow (*Achillea millefolium*) also has traditionally been used as an abortifacient, emmenagogue, contraceptive, and for stimulating uterine contractions. For this reason, it is contra-indicated for use in pregnancy. There has, however, been little scientific research carried out to either confirm or refute this recommendation Female rats were dosed, orally by gavage using 56 times the human dose of yarrow daily on either gestation days (GD) 1-8 or GD 8-15. Two groups of controls were included; the first received water and the second received an equivalent dose of ethanol to that found in the yarrow preparation over the two gestation periods. On GD 20, rats were sacrificed, placentae were weighed, and corpora lutea counted. The fetuses were weighed and examined for signs of external, internal or skeletal malformations.

In the study it was found that yarrow, when administered to rats at 56 times the human dose, was associated with reduced fetal weight and increased placental weight. In the absence of a no observable effect level for these variables it must be concluded that the consumption of yarrow is contraindicated during pregnancy until further investigations have been carried out.

CONCLUSION

Aromatic plants have a significant role to combat disease, from the dawn of civilization. The genus *Achillea* consists of about 140 perennial herbs native to the Northern hemisphere. The vast survey of literature showed that *A.millifolium* has an esteemed status in herbs with diverse pharmacological activity spectrum. Traditional indications of their use include digestive problems, liver and gall-bladder conditions, menstrual irregularities, cramps, fever, wound healing.

The Commission E approves its internal use for loss of appetite and dyspeptic ailments (gastric catarrh, spastic discomfort), externally it is used in form of sitz bath or as a compress against skin inflammation, slow healing wounds, bacterial or fungal infections. In the last decades, pharmacological studies became intensive, although human clinical investigations are still rare. Recent findings have confirmed several traditional uses.

The largest number of data accumulated for antioxidant and anti-inflammatory effects. There are positive results on the analgesic, anti-ulcer, choleretic, hepatoprotective and wound healing activities. First results on other interesting therapeutical areas - antihypertensive, antidiabetic, antitumor, antispermatogenic activities - need confirmation.

Yarrow can be used also as an insect repellent. Contact dermatitis as adverse effect may be connected to sesquiterpenes. The diversity and complexity of the effective compounds of yarrow species explains the broad spectrum of their activity. According to this literature the pharmacological effects are mainly due to the essential oil, proazulenes and other sesquiterpene lactones, dicaffeoylquinic acids and flavonoids. Achillea species have different chemical and therapeutical values. Despite of numerous data, correct evaluation of the results is difficult because of missing generally accepted taxonomical nomenclature. The used chemical-analytical methods and bio-assays are utmost diverse, making the comparison complicated. Further research on the activity is needed using exactly defined plant material, standardized methods and chemical analysis.

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Medical Sciences

Effects of an extract from *Phyllanthus niruri* on hepatitis B and woodchuck hepatitis viruses: *In vitro* and *in vivo* studies

(antiviral agent/*Marmota monax*/DNA polymerase/hepatitis B surface antigen/woodchuck hepatitis surface antigen)

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Contributed by B. S. Blumberg, September 18, 1986

ABSTRACT **An aqueous extract of the plant *Phyllanthus niruri* inhibits endogenous DNA polymerase of hepatitis B virus and binds to the surface antigen of hepatitis B virus *in vitro*. The extract also inhibits woodchuck hepatitis virus (WHV) DNA polymerase and binds to the surface antigen of WHV *in vitro*. The extract, nontoxic to mice, was tested for antiviral activity in woodchucks (*Marmota monax*). In a trial using six long-term WHV-carrier woodchucks, five treated animals showed a faster decrease in woodchuck hepatitis virus surface antigen titer compared to one untreated control. In animals recently infected with WHV, the extract was effective when administered i.p. in three out of four animals in reducing and within 3–6 weeks eliminating both the surface antigen titer and DNA polymerase activity in serum. The treatment was discontinued after 10 weeks, and the treated animals have remained free of detectable markers of WHV for more than 45 weeks. In contrast, three untreated controls remained positive for both markers for WHV. One of the controls died after 8 weeks; the other two controls have remained positive for WHV markers for more than 45 weeks. In a third trial with long-term carriers, test animals treated subcutaneously with the extract for 12 weeks did not respond; but on switching the mode of administration to i.p., two out of the five animals showed a significant decrease in woodchuck hepatitis virus surface antigen titer compared to controls.**

Chronic carriers of the hepatitis B virus (HBV) may remain asymptomatic for long periods, but many are at high risk of eventually developing post-hepatitic cirrhosis and primary hepatocellular carcinoma. Carriers are often infected within the first few years of life, but symptoms of chronic liver disease and primary hepatocellular carcinoma may not be perceived until the third, fourth, or later decades; pathogenesis, even though relentless, is slow (1, 2).

Materials of animal, bacterial, and plant origin (3) have been described that appeared to interfere with the binding of the HBV surface antigen (HBsAg) to the HBsAg antibody (anti-HBs). Subsequently, about 1200 species of plant were tested, and about one-third were found to inhibit anti-HBs–HBsAg binding. To obtain more specificity and increase the probability of obtaining an effective therapeutic agent, in addition to the inhibition of HBsAg–anti-HBs binding, we examined plant extracts *in vitro* to determine if they inhibited the endogenous DNA polymerase (DNAp) of HBV, which is necessary for its replication. The first plant tested was *Phyllanthus niruri*, which has been and is used widely (4) in southern India and elsewhere for the treatment of jaundice. The treatment of HBV carriers has not been recognized in traditional indigenous medical systems. The inhibition of anti-HBs–HBsAg binding by *P. niruri in vitro* has been reported by Thyagarajan *et al.* (5).

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To assess the effects of *P. niruri* on the replication of HBV-like viruses *in vivo*, we used the woodchuck (*Marmota monax*) as an animal model. The carrier state in woodchucks and humans is similar. Liver diseases including primary hepatocellular carcinoma induced by woodchuck hepatitis virus (WHV) in woodchucks are very similar to those induced by HBV in humans. WHV is similar to HBV (6, 7) with substantial immunological cross-reactivity (8) and significant homology of DNA (9). The endogenous DNAp of both viruses exhibited optimal activities in the same range of pH, $MgCl_2$ concentration, and showed similar sensitivity to inhibitors like phosphonoformic acid and arabinofuranosyl nucleotides (10).

In this paper we report that *P. niruri* has profound effects *in vitro* on HBsAg, on woodchuck hepatitis virus surface antigen (WHsAg), and on the DNAp of both viruses and *in vivo* on the replication of WHV and on liver histopathology. In some controlled studies, it appeared to eliminate WHV from carriers.

MATERIALS AND METHODS

Preparation of the Aqueous Extract of *P. niruri*. Dried whole plant (40 g) was pulverized in a Waring blender and mixed with 200 ml of water. The mixture was shaken periodically (60°C) for 2 hr and filtered through nylon mesh. The filtrate was centrifuged at 8000 rpm for 1 hr in a Beckman JA10 rotor at 20°C. The supernatant was filtered through a 0.45-μm filter (Millipore) for *in vivo* studies.

Assay for HBsAg or WHsAg Binding Activity. Serial dilutions of the aqueous extract of *P. niruri* were mixed with an equal volume of sera positive for HBsAg or WHsAg, and the mixture was incubated for 1 hr at 20°C. The mixture was assayed directly for HBsAg or cross-reacting WHsAg using Ausria II ELISA kits (Abbott). Binding activity was expressed as the decrease (in percent) in the absorption of the test sample compared to that of the control composed of 1:1 (vol/vol) mixture of surface antigen positive serum and PBS. (PBS = 0.01 M sodium phosphate/0.85% NaCl, pH 7.2.)

Assay of WHsAg Titers in Serum. Serum titers of WHsAg were determined by assaying serial dilutions of serum with the Ausria II kit using the value obtained for sera of uninfected woodchucks as controls.

Inhibition of Endogenous Viral DNA Polymerase Activity. Suspensions of the virus were added to a reaction mixture containing the nucleotides required for DNA synthesis by DNAp. The formation of DNA was determined by gel electrophoresis. Serial dilutions of the extracts of the plant were added to determine their inhibitory ability.

Abbreviations: DNAp, DNA polymerase; HBV, hepatitis B virus; WHV, woodchuck hepatitis virus; WHsAg, woodchuck hepatitis surface antigen; HBsAg, hepatitis B surface antigen; anti-HBs, antibody to HBsAg.

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Table 1. Effect of *P. niruri* extract on the binding of HBs to HBsAg or WHsAg

P. niruri extract, mg/ml	% inhibition of anti-HBs binding	
	WHsAg	HBsAg
5	61	63
2.5	45	35
1.25	25	18
0.63	11	9
0.5	13	0
0.31	13	0
0.1	5	0

Serum (50 μl) containing HBV (or WHV) particles was layered over a 10–20% sucrose gradient and centrifuged at 45,000 rpm for 3 hr in a SW 55 Ti rotor in a Beckman ultracentrifuge. Pelleted virus was separated from the supernatant and was resuspended in 5 μl of 0.05 M Tris·HCl, pH 8.0. Equal volume of serial dilutions of the extract of *P. niruri* was mixed with the virus suspension. Then nucleotide triphosphates dATP, dGTP, dCTP, and [^{32}P]dTTP were added in the presence of 0.05 M Tris·HCl, pH 8.0, containing 10 μM $MgCl_2$, 0.15 M NaCl, 1 mM dithiothreitol, and 0.1% Nonidet P-40, and the mixture was incubated at 37°C for 2 hr. For control, the virus suspension was mixed with 0.05 M Tris·HCl buffer, pH 8.0, instead of the potential inhibitor. The reaction was stopped by the addition of 15 μl of Pronase (0.5 mg/ml) in 0.1% $NaDodSO_4$ containing 0.01 M EDTA in Tris·HCl, pH 7.4; and the mixture was electrophoresed on 1.5% agarose gel using bromophenol blue as the tracking dye. The DNA formed was detected by autoradiography.

A quantitative determination of the inhibition was obtained by substituting [^{3}H]dTTP and [^{3}H]dGTP for [^{32}P]dTTP as radiolabel according to the method reported by Hantz *et al.* (10) with modifications. In this experiment the reaction was terminated by the addition of 2.5 ml of 5% (wt/vol) trichloroacetic acid [containing 2% (wt/vol) pyrophosphate]. Then 40 μl of 2.5% bovine serum albumin and 100 μl of 5% (wt/vol) calf thymus DNA were added as carriers, and the mixture was filtered through a glass fiber filter (Whatman). The filter was washed thrice with 5% (wt/vol) trichloroacetic acid containing pyrophosphate, thrice with 95% (vol/vol) ethanol, dried under a heat lamp, and the radioactivity remaining on the filter was measured in a scintillation counter (Packard Instrument, Downers Grove, IL).

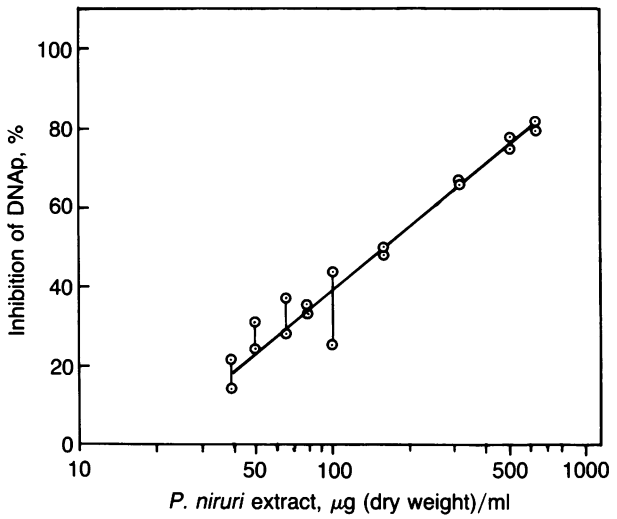

FIG. 1. Inhibition of DNAp of WHV by aqueous extracts of *P. niruri*. The data are presented as μg (dry weight) per ml of assay mixture.

To determine the WHV DNAp activity in sera of woodchucks used for experiments *in vivo*, a virus pellet, centrifuged from 50 μl of serum on sucrose density gradient, was used directly without addition of any diluent buffer as described above.

RESULTS

The Effect of *P. niruri* Extract on WHsAg and HBsAg *in Vitro*. *P. niruri* extract inhibits the reaction of HBsAg with anti-HBs and of WHsAg with anti-HBs (Table 1). The inhibition was concentration dependent for HBsAg and WHsAg.

Inhibition of WHV DNAp by the Extract *in Vitro*. Serial dilutions were assayed for their ability to inhibit WHV DNAp activity. Inhibition (Fig. 1) was directly proportional to the concentration of the extract up to 600 μg/ml at which the inhibition was 82%.

Toxicity Tests in Mice. Forty outbred albino female mice were used to determine the toxicity of the extract in accordance with a National Institutes of Health recommended assay to determine acute toxicity. The mice were divided into eight

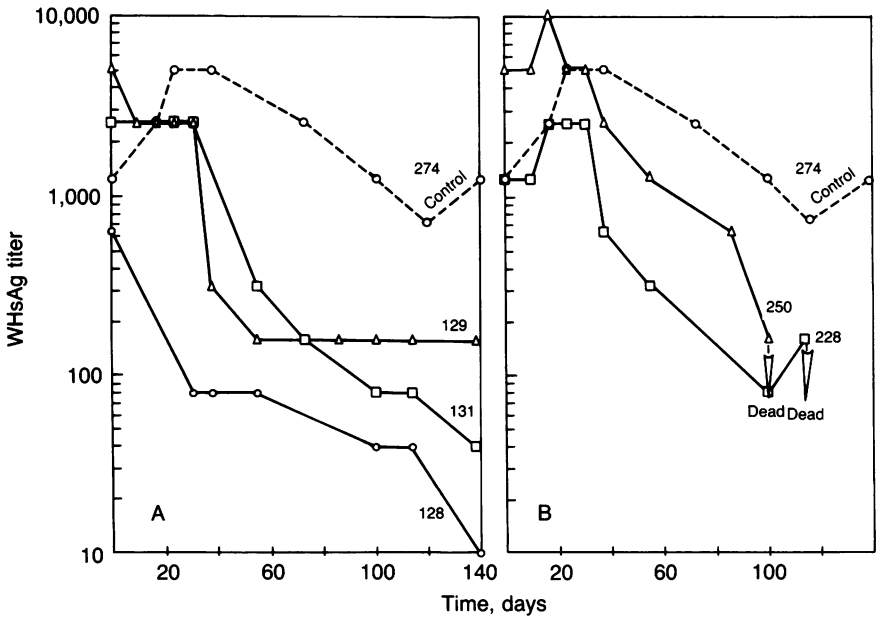

FIG. 2. Effect of aqueous extracts of *P. niruri* given i.p. on long-term chronic-carrier woodchucks (solid lines) compared to a single control carrier (dashed lines). For clarity, the results have been arbitrarily separated in two groups, *A* and *B*. The numbers of the animals are given next to the appropriate curve.

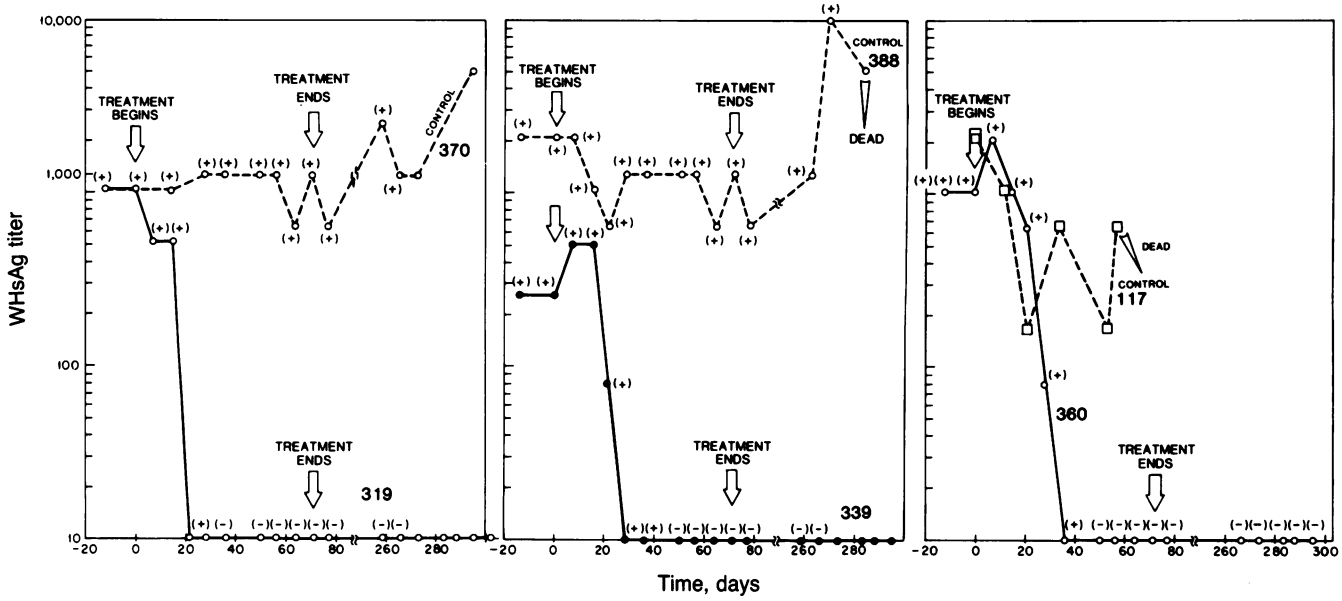

FIG. 3. Effect of aqueous extracts of *P. niruri* given i.p. on recently infected woodchucks. Solid lines indicate treated animals, and dashed lines indicate the controls. Arrows indicate the beginning and end of treatment. The DNAp activity [positive (+) or negative (−)] is indicated in parentheses.

sets of five mice and were weighed. Four sets designated "test" were given i.p. aqueous extract of *P. niruri* [0.1 ml, 1.8 mg (dry weight) per mouse], while the other four sets, designated "control," were given i.p. PBS, 0.1 ml per mouse. All sets were weighed after 3 and 7 days. There was no loss in weight among the treated or control mice after 3 days. There was a net gain in weight among all the mice after 7 days. One of the treated mice died on the 3rd day of internal injury unrelated to the test.

Long-Term Chronic-Carrier Woodchucks. An initial study was conducted on woodchuck carriers whose sera showed the presence of both the surface antigen and WHV DNAp activity at the time these animals were trapped. The length of time these animals were carriers of WHV was unknown, but it was probably more than several months. Of the six carrier woodchucks that were available, five animals, 128, 129, 131, 228, and 250, were treated; and one, 274, was used as control. Test animals were given 0.5 ml of the extract [9 mg (dry weight)] i.p. once a week, while the control animal was given the same volume of buffered saline i.p. once a week. The animals were bled periodically, and the titer of WHsAg was determined (Fig. 2). A comparison of the slope of the curves indicated that the extract appeared to reduce the titer of WHsAg in long-term chronic carriers.

Woodchucks Recently Infected with WHV. The second study was conducted on animals that were negative for WHV at the time of capture but became positive while in the enclosures in our woodchuck colony. By the time of the experiment, these animals had been infected for at least 1 month. The aqueous extract of *P. niruri* [0.5 ml, 9 mg (dry weight) per woodchuck] was administered i.p. twice a week to four woodchucks, 319, 339, 360, and 376A; the other three, 370, 388, and 117, received 0.5 ml of PBS twice a week. They were followed by weekly bleeding and assay for WHsAg and WHV DNAp. Treatment was terminated after 72 days, but the weekly bleedings were continued for over 300 days. Liver biopsy was performed on day 80, except for control woodchuck 117, which was autopsied when it died on day 57.

In woodchuck 319, WHsAg started dropping soon after the start of treatment, becoming undetectable about 21 days later (Fig. 3). WHV DNAp activity stayed positive for about 1 week after the surface antigen titer became undetectable, but subsequently it also became undetectable. Although the treatment with extract was terminated after 72 days, there were no detectable levels of the surface antigen or DNAp

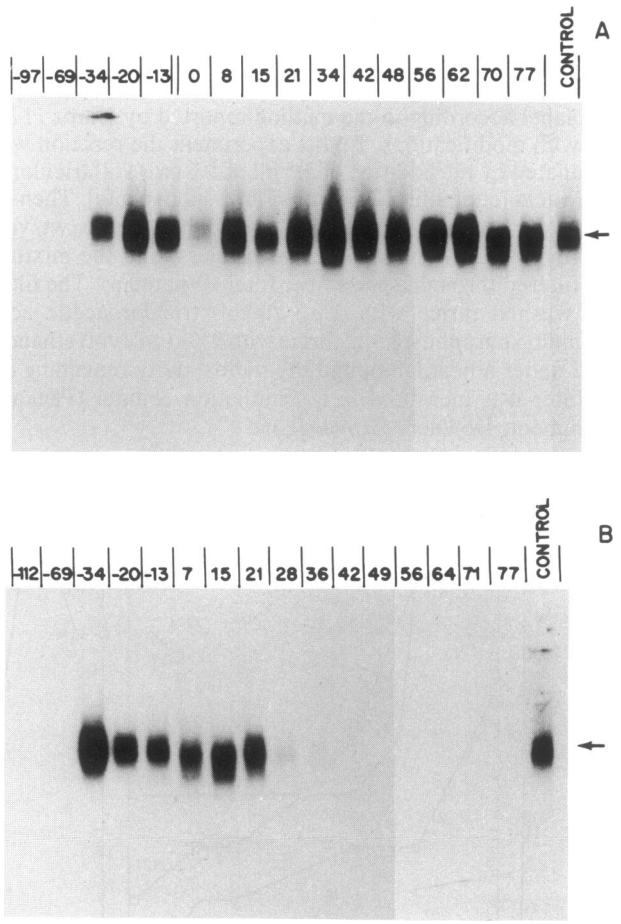

FIG. 4. Autoradiography of DNAp activity on the day indicated (−, before treatment) in the sera of a control animal, 370 (*A*) and a treated animal, 339 (*B*). These are typical of the other two control and treated animals (see Fig. 3). Arrow indicates the band of 3.3-kilobase, closed, circular DNA of WHV.

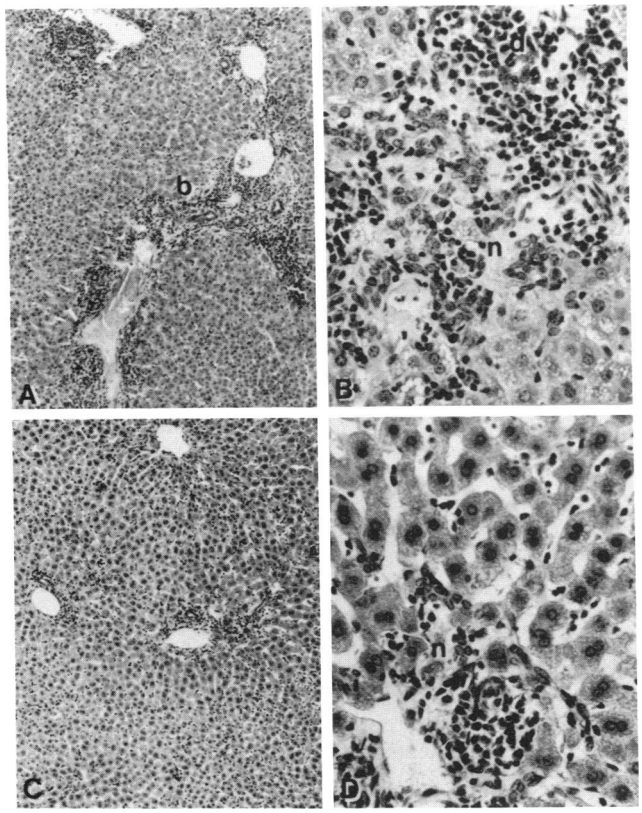

FIG. 5. Comparison of livers from the untreated woodchuck 370 (*A* and *B*) and the treated woodchuck 339 (*C* and *D*) that were infected with WHV and obtained at biopsy 8 days after the termination of treatment. All preparations were sectioned in paraffin at 5 μm and stained with hematoxylin and eosin. (*A*) Typical pattern of chronic progressive viral hepatitis with granulomatous inflammation centered chiefly around portal triads with frequent "bridging" intervening spaces between them (b). Note blurring of intralobular cords as compared with *C* (treated), owing to inflammatory swelling of individual hepatocytes. (×75.) (*B*) A periportal lesion, with an agglomeration of lymphocytes, plasmacytes, histiocytes, fibroblasts, and necrotic hepatocytes (n); proliferating biliary ductules (d) are conspicuous, which, along with fibrosis, indicate a progression to cirrhosis. (×300.) (*C*) Minimal inflammatory foci are barely visible in the portal triads of this treated animal. Hepatocytic swelling and loss of crispness of cords is seen to the right of center. (×75.) Overall, these effects are much less than in the untreated liver. (*D*) Small periportal granuloma; hepatocyte and cords are well preserved, apart from rare necrosis (n). (×300.)

activity in 319 up to 300 days after the start of treatment (i.e., 228 days after termination of treatment). The control animal 370, on the other hand, did not show a drop in either WHsAg titer or DNAp activity up to 300 days.

Treated animals 339 and 360 showed a similar drop in WHsAg between 21 and 35 days after the start of treatment, followed 7–14 days later by a drop in WHV DNAp; the markers stayed undetectable up to 300 days. Control animals 117 and 388, however, showed high levels of the WHsAg and DNAp during the same period. Autoradiography of the product of DNAp reaction in the serial bleedings of control woodchuck 370 and *P. niruri* extract-treated woodchuck 339 is given in Fig. 4. The presence of a band at 3.3 kilobases (arrow) indicated WHV DNAp activity up to 21 and 28 days. These became undetectable thereafter. DNAp of control animal 370 did not change.

Woodchuck 376A, one of the four treated animals, became bacteremic early in the experiment. Chloramphenicol was administered, but the animal succumbed to the infection. It did not respond to the extract.

The histopathology of the livers of control woodchuck 370 and treated animal 339 are shown in Fig. 5. Data on the liver biopsy performed 8 days after the termination of treatment are given in Table 2. (Liver biopsies before treatment were not available.) The three untreated controls, 370, 388, and 117, showed extensive portal infiltration and focal necrosis; all three were diagnosed as chronic active hepatitis. The livers of the treated animals 319, 339, and 360, on the other hand, showed marginal or negative portal infiltration and focal necrosis. The diagnosis of woodchuck 319 was early mild viral hepatitis, of 339 was early active hepatitis, and of 360 was minimal portal hepatitis.

Table 2. Data on liver biopsies performed 8 days after termination of treatment in experiment involving woodchucks recently infected with WHV

	Pathology		
	Portal infiltrate	Focal necrosis	Diagnosis
Control animal			
370	+++	++	Chronic, active hepatitis
388	++	++	Chronic, active hepatitis
117	+++	++	Chronic, active hepatitis
P. niruri extract-treated animal			
319	±	±	Mild viral hepatitis
339	+	+	Active hepatitis
360	±	0	Minimal, portal hepatitis

0, None. ±, Marginal. +, Minimal positive. ++, Positive. +++, Extensive.

Subcutaneous Administration of the Extract in Long-Term Carrier Woodchucks. Five of eight long-term WHV-carrier woodchucks (327, 437, 456, 488, and 492) were administered 0.5 ml [9 mg (dry weight)] of extract subcutaneously twice a week. The remaining three (318, 429, and 471) were given 0.5 ml of PBS subcutaneously twice a week. The animals were bled weekly, and the titer of WHsAg and WHV DNAp was monitored. During 3 months of treatment, there was no appreciable change in either marker in the treated or control animals.

We concluded that subcutaneous administration was ineffective. We hypothesized that either the active principle was not absorbed by this route, that antibodies were developed against the extract, or some other mechanism rendered it ineffective. After 90 days the mode of administration was changed to the intraperitoneal route that had apparently been successful in the previous studies. Two of the treated animals showed a drop in WHsAg about 60 days after switching to i.p. administration (Fig. 6). One control and two treated animals died due to bacteremic infections. None of the control animals showed any significant change in WHsAg.

DISCUSSION

London and Blumberg (1) have proposed a model to explain the observations on the relation between primary hepatocellular carcinoma and HBV. It postulates the existence of fully differentiated liver cells which, when infected, allow complete replication of HBV. (They are designated S cells; i.e., susceptible to replication.) Less-differentiated liver cells (common in the fetus and newborn but less so in the adult liver), when infected, do not allow replication, although penetration of the virus and integration of virus DNA may occur. (They are designated R cells; i.e., resistant to repli-

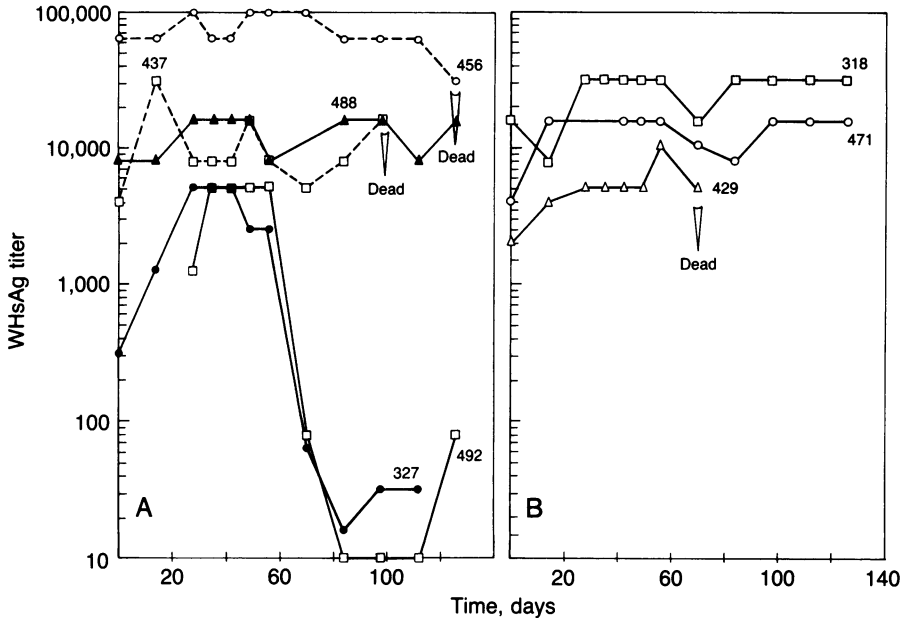

FIG. 6. Effects of *P. niruri* extracts given i.p. to animals that did not respond to subcutaneous administration of the same extract. (*A*) Treated animals; (*B*) controls.

cation.) The proliferating HBV in the differentiated cells leads to cell death, in part caused by the immune response of the host to its S cells altered by HBV. The less-differentiated R cells are not damaged by the virus, since the virus does not replicate. The R cells divide and multiply in response to the death of the S cells. With increased division of R cells, chromosomes are more liable to disruption, deletion, rearrangement, or mutation by the resident HBV (or, possibly, another agent). This could result in a favored clone that divides rapidly and is eventually perceived as a cancer.

The course of these events could be stopped if virus were eliminated from the carrier. If this is not possible, then decreasing the virus load or inhibiting its entry into liver cells could slow the death of liver cells so that perceptible disease would not be expected to occur until the carrier had lived out a life span. We have referred to this as "prevention by delay" (2).

In an attempt to achieve this goal, we looked for agents that would affect the virus or its entry into liver cells. The aqueous extract of *P. niruri* inhibits HBV DNAp and WHV DNAp and interferes with the binding of anti-HBs to HBsAg and WHsAg apparently because of its ability to bind the surface antigen.

The extract of *P. niruri* was tested in three independent *in vivo* experiments in woodchuck carriers. In the first, with long-term carriers, a significant drop in the titer of WHsAg was observed in the extract-treated animals compared to the control. In the second, using short-term carriers, the WHsAg and WHV DNAp in three of four treated animals became undetectable and stayed that way even after the treatment was terminated, while the levels of these markers stayed high in the controls. These results indicated a possible break in the carrier state directly attributable to the treatment with *P. niruri* extract. The conclusion from the third experiment was that the extract was not effective when administered subcutaneously. However, on switching the mode of administration to i.p., two out of three extract-treated animals showed a drop in WHsAg titer. The advantages of intraperitoneal chemotherapy for liver ailments have been reported (11) and may be applicable to WHV and HBV infections since replication of both these viruses takes place in the liver. Although this experiment was faulted because of its *post hoc* design, the results were in the direction predicted from the two earlier experiments.

Our preliminary results indicate that there are one or more active materials in *P. niruri* that inhibit the replication of WHV *in vivo* and decrease the pathological effects of WHV on woodchuck liver. By inference, the substance should affect HBV infection in humans similarly. Using a variety of techniques (including HPLC), we have identified fractions from *P. niruri* containing the DNAp inhibitory activity and the surface antigen binding activity.

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