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An overview of the hepatoprotective potentials of *Phyllanthus amarus*

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Abstract
The liver played a crucial role in the metabolism of toxins from the body thereby predisposing it to challenges that impedes its optimum performance. *P. amarus* Schum & Thonn (Euphorbiaceae) is a small erect, annual herb that contain various phytochemicals that are attributed to its leaves, stem, and roots. It is a hepatoprotective herb that has been used in folk medicine for various liver disorders, and this property has been attributed to its active compounds mainly phyllanthin. The present study is a review of the hepatoprotective activity of the plant and the mechanisms that might play significant role. Several studies have indicated that the stone-breaker plant possess remarkable hepatoprotective and antihepatotoxic potentials against liver toxicity models such as acetaminophen, alcohol, and carbon tetrachloride. The suggested mechanism relates principally to the phytochemical lignan - phyllanthin which exhibit antioxidant effect against the oxidative stress generated by the hepatotoxins.

Keywords: *Phyllanthus amarus*, phyllanthin, hepatoprotective, antioxidant

Introduction
The liver is the second largest organ in the body and played a very crucial role in the metabolism of poisonous substances from the body [1]. An impediment to liver functions will greatly affect several homeostatic mechanisms and can thus lead to potentially severe implications [2]. Liver disease is a global health concern. Regrettably, conventional or synthetic drugs used in the treatment of this condition still remain inadequate and sometimes present serious side effects [3]. Without a reliable liver protective drug in contemporary medicine, herbal preparations in Ayurveda have been recommended for the treatment of liver disorders as they are apparently harmless owing to their natural source [4].
Liver disease may be described categorically as acute or chronic hepatitis, hepatosis (non-inflammatory diseases) and cirrhosis (degenerative disorder resulting in fibrosis of the liver). Its etiology mainly include certain drugs and chemicals (certain antibiotics, chemotherapy, aflatoxin, peroxidized oil, carbon tetrachloride, chlorinated hydrocarbons etc.), excess alcohol intake, infections and autoimmune disorders [5]. Hepatoprotectives are therapeutic agents that confer protection to the liver or promote regeneration of hepatic cells. Certain plants possess medicinal potentials for preventing or treating hepatic disturbances. Medicinal herbs are a significant source of hepatoprotectives. About 170 phytoconstituents isolated from 110 plants belonging to 55 families have been reported to possess hepatoprotective activity [6]. Liver damage usually present with cellular necrosis, increase in tissue lipid peroxidation and depletion of the tissue glutathione (GSH) levels. Furthermore, serum levels of many biochemical markers such as serum glutamate oxalo-transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), triglycerides, cholesterol, bilirubin, alkaline phosphatase (ALP) are elevated [7]. Hepatotoxicity of CCl_4 produced trichloromethyl and trichloromethyl peroxy radicals, which initiate lipid peroxidation and thereby result in fibrosis and cell necrosis [8].

Mechanism of hepatotoxicity
Direct hepatotoxicity and adverse immune reactions are the two pathways that ultimately result to liver toxicity, although the precise mechanism still remain obscure [9]. Most often, hepatic injury is initiated by the bioactivation of drugs to chemically reactive metabolites which interact with cellular macromolecules such as proteins, lipids, and nucleic acids, leading to protein dysfunction, lipid peroxidation, DNA damage and oxidative stress [10]. Additionally, these reactive metabolites may stimulate the disruption of ionic gradients and intracellular calcium stores, which result in mitochondrial dysfunction and loss of energy production. This dysfunction produces an overload of oxidants and eventually injures hepatic cells. Activation of some cytochrome P450 enzymes e.g CYP2E1 also cause oxidative stress. The accumulation of bile acid in the liver due to injury to the liver and bile duct cells, can promote further liver

damage. This abnormality in cellular function can climax in cell shutdown and ultimately liver failure [11].

Immunological reactions can arise consequent to hepatocyte dysfunction. Stress and hepatic injury triggered the release of signals that stimulate activation of other cells, particularly those of the innate immune portfolio, including kupffer cells, natural killer cells, and natural killer T-cells. These cells aid the progression of liver injury by producing proinflammatory mediators and secreting chemokines to further recruit inflammatory cells to the liver. It has been shown that inflammatory cytokines, such as tumour necrosis factor (TNF)-α, interferon (IFN)-γ, and interleukin (IL)-1β, produced during hepatic injury, participatorily promote tissue damage [12]. Conversely, innate immune cells are also the principal source of IL-10, IL-6, and certain prostaglandins, all of which displayed hepatoprotective role [13]. Consequently, it is the delicate balance of inflammatory and hepatoprotective mediators generated after activation of the innate system that determines an individual's susceptibility and adaptation to hepatic injury [9].

Phyllanthus amarus

P. amarus Schum & Thonn is a cosmopolitan herb that belongs to the family Euphorbiaceae. Its common names include the stone breaker, hurricane weed, shatterstone, dukun anak (Malaysian), chanca-piedra (Spanish), quebra-pedra (Brazil), and carry-me- seed [14, 15]. *P. amarus* is synonymous to *Phyllanthus niruri* L. and sometimes the two are misidentified for each other. However, reorganization of the *Phyllanthus* genus has classified *P. amarus* as a type of *P. niruri* [16]. *P. amarus* has shown a wide spectrum of pharmacological effects which includes hepatoprotective, antiviral, antibacterial, antioxidant, anti-inflammatory, anticancer, hypolipidemic, antidiabetic, antispasmodial, nephroprotective, and diuretic properties [17]. The use of *P. amarus* has gained considerable momentum because of its novel antiviral activity against hepatitis B virus and for its several other biological activities [18].

Phytochemical studies of *P. amarus* have indicated the presence of many valuable phytochemicals including lignans, flavonoids, hydrolysable tannins (ellagitannins), triterpenes, alkaloids, sterols, and volatile oil [17]. An elaborate list of the active metabolites is available in the review by Sarin *et al* [19]. Of all the *Phyllanthus* species, the phytochemistry of *P. amarus* is well studied and phyllanthin is the predominant compound responsible for many biological actions. The present review highlights the hepatoprotective activities of the plant.

Hepatoprotective Studies of *P. amarus*

Several studies have indicated the extract of *P. amarus* significantly restores the normal function of the liver cells, enzymes and other markers thereby affirming the hepatoprotective potential of the herb. Krithika and Verma [4] determined the hepatoprotective activity of *P. amarus* extract against carbon tetrachloride (CCl_4)-induced liver damage in female mice where administration of the hepatotoxin caused a significant increase in liver and serum alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and acid phosphatase (ACP), while total protein content significantly decreased. Oral administration of the extract (LD_{50} = 8260 mg/kg) along with CCl_4 for 30 days significantly mitigated the toxic effects of CCl_4 in the liver and serum parameters in a dose-dependent manner. They attributed this activity to the antioxidative property of the active constituents such as lignans, flavonoids, ellagitannins and hydrolysable tannins [20] present in the plant. In a similar study by Tripathi *et al* [21], albino rats were treated with ethanol orally for 30 days, after which the *P. amarus* whole plant powder was administered at a dosage of 200 mg/rat per day for 45 days. The increased deposition of triglyceride, cholesterol and phospholipids found in liver, brain, kidney and heart due to ethanol administration were restored to normal after administration of *P. amarus* powder, thereby indicated the antihepatotoxic effect of the plant. Similarly, administration of whole plant powder was found to be effective in the treatment of liver damage induced by CCl_4 in rats by restoring the levels of biochemical parameters (SGOT, SGPT, ALP and cholesterol levels) to considerable normalcy within 48 hours [22].

The hepatoprotective, nephroprotective and cardioprotective property of *P. amarus* was further demonstrated by Obianime and Uche [23] where the methanol extract of its leaves (50-800 mg/kg) caused a statistically significant decrease in the levels of total cholesterol, AST, ALT, urea, uric acid, total protein, prostatic, alkaline, and acid phosphatases. The highest reduction effect was obtained with uric acid at 400 mg/kg of *P. amarus* extract while the least effect was observed in total cholesterol. All effects were dose- and time-dependent. Syed Assad *et al* [24] showed that *P. amarus* seed extracts produced percentage increases in scavenging activities of superoxide, hydroxyl and nitric oxide radicals due to its antioxidant activities. Conversely, *in vivo* studies showed the methanolic and aqueous extracts of the seeds of *P. amarus* at 250 mg/kg displayed hepatoprotective properties in rats with CCl_4-induced liver damage as adjudged from serum biochemical enzyme marker activities and histopathological studies with a statistically significant decrease in the biomarkers nearly comparable to the effect seen in Liv-52 standard drug.

P. amarus aqueous extract (1–4 mg/mL) increased percentage of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and decreased the release of AST and ALT in rat primary cultured hepatocytes being treated with ethanol. The acute toxicity study indicated a single dose of *P. amarus* (25, 50 and 75 mg/kg, p.o.) or SL (Silymarin, 5 mg/kg), 24 h before ethanol (5 g/kg, p.o) in male Wistar rats lowered the ethanol-induced levels of AST and/or ALT. The 75 mg/kg *P. amarus* dose gave the best result similar to SL. Histopathological observations confirmed the beneficial roles of *P. amarus* and SL against ethanol-induced liver injury in rats [25]. The assessment for hepatic effect of the whole plant of *P. amarus* showed a significant reduction in the plasma activities of the transaminases and total bilirubin concentration with a concomitant increase in protein concentration which suggest that the plant has hepatic cell protective function and enhancement potential [26]. The β- glucuronidase inhibitory action of extracts and isolated active constituents such as corilagin, brevifolin carboxylic acid, phyllanthin, and hypophyllanthin from *P. amarus* was demonstrated by Joshi and Priya [27]. They showed that lignans isolated from the methanol extracts of *P. amarus* (phyllanthin and hypophyllanthin) exhibit their hepatoprotective effect through a mechanism independent of β-glucuronidase inhibition.

Naaz *et al* [28] validated the hepatoprotective effect of *P. amarus* using aflatoxin-induced liver damage in mice. The plant extract displayed hepatoprotective effect by lowering the content of thiobarbituric acid reactive substances (TBARS) and enhancing the reduced glutathione level and the activities of antioxidant enzymes, glutathione peroxidase (GPx), glutathione-S-transferase (GST), superoxide dismutase (SOD)

and catalase (CAT). This study is in line with findings by Faremi *et al* [29] in which administration of *P. amarus* (250 and 500 mg/kg/day) significantly increased the activities of hepatic ALT, AST and ALP, with a simultaneous marked reduction in the plasma activity of the transaminases in the ethanol-challenged rats. Lipid peroxidation level was significantly reduced in the liver by *P. amarus* co-treatment. *P. amarus* leaves extract could protect the liver against ethanol-induced oxidative damage by possibly reducing the rate of lipid peroxidation and increasing the antioxidant defence mechanism in rats.

The preventive role of a standardized extract of *P. amarus* against CCl_4-induced hepatotoxicity *in vivo* and *in vitro* using mice model and human hepatoma HepG2 cell line, respectively was investigated [30]. Oral administration of CCl_4 increased oxidative stress, decreased antioxidative defense, and liver injury. Treatment with *P. amarus* significantly mitigated the increase in activities of liver marker enzymes, lipid peroxidation, and bilirubin content. It also increased the antioxidant enzymatic and non-enzymatic defense parameter levels. The results of the *in vitro* study conducted in HepG2 cells indicated that the hepatotoxin lowered MTT metabolism and increased the release of transaminases which were corrected with co-incubation with *P. amarus*. The study established a significant liver-protecting role of standardized *P. amarus* extract due to the presence of active constituent, phyllanthin. Chirdchupunseree & Pramyothin [31] also investigated the effect of phyllanthin on ethanol-induced rat liver cell injury using a 24-hour primary culture of rat hepatocytes. Results showed ethanol decreased percentage MTT, increased the release of transaminases with the increase in the production of intracellular reactive oxygen species (ROS) and lipid peroxidation. Phyllanthin demonstrated its role in protection by antagonizing the above effect induced by ethanol. It also restored the antioxidant capability of rat hepatocytes including the level of total glutathione, and activities of superoxide dismutase (SOD) and glutathione reductase (GR) which were reduced by ethanol.

Krithika *et al* [32] reported the protective effect of *P. amarus* extract and phyllanthin on CCl_4-induced toxicity in human hepatoma HepG2 cell line. The results indicated that CCl_4 treatment caused a significant decrease in cell viability. In addition, the toxin treatment initiated lipid peroxidation (LPO), caused leakage of enzymes like ALT and lactate dehydrogenase (LDH) with a significant decrease in GSH levels. It was observed that phyllanthin effectively alleviated the changes induced by CCl_4 in a concentration-dependent manner, with much smaller strengths as compared to *P. amarus* extract. Sangeeta *et al* [33] explored the hepatoprotective activity of *P. amarus* on anti-tuberculosis drug (ATD)-induced liver injury. ATD combination includes isoniazid, rifampicin, pyrazinamide and ethambutol. Oral administration of *P. amarus* extract showed significant recoupment in a dose dependent manner from the elevated transaminases and reversal of the reduced GSH level and increased lipid peroxidation caused by cellular oxidative stress. At 300 and 400 mg/kg, *P. amarus* displayed beneficial hepatoprotection in albino rats as all the major parameters showed percent protection level above 50%.

Krithika and Verma [34] reported that phyllanthin ameliorated the CCl_4-induced hepatic oxidative damage in mice. Oral administration of the hepatotoxin caused a significant increase in lipid peroxidation. The hepatic levels of both enzymatic and non-enzymatic antioxidants were significantly lowered in CCl_4-treated mice as compared to control. Treatment with phyllanthin significantly mitigated these changes. Treatment with phyllanthin rescinded the alterations induced by the toxin and the subcellular features of phyllanthin-treated mice were similar to those present in the normal mouse liver. Furthermore, the hepatoprotective effect of *P. amarus* Schum & Thonn was studied on paracetamol hepatotoxicity in rats by monitoring serum transaminases (SGOT and SGPT), ALP and bilirubin as well as histopathological examination of the liver. The extract at 1.6 and 3.2 g/kg decreased the paracetamol-induced hepatotoxicity as indicated by the decrease in SGOT and SGPT, bilirubin and histopathological score while the ALP did not change. It is evident that the hepatoprotective mechanism of the plant was neither related to inhibition on cytochrome P450, nor induction on sulfate and glucuronide conjugation pathways of paracetamol, but partly due to the antioxidant activity and the protective effect on the decrease of hepatic reduced glutathione [35]. These outcomes provide justification for the folkloric use of the plant for the treatment of toxic liver diseases.

P. amarus has been studied for its joint hepatoprotective activity with other herbals. Aqueous extracts of *P. amarus* and *Andrographis paniculata* were used individually and in combination to investigate their hepato-recovery potential using CCl_4-treated, liver injured albino wistar rats. The efficacy of the polyherbal combination was less compared to individual extracts in reducing bilirubin concentration. Reduction in SGPT and SGOT by the polyherbal was found to be similar to that of individual extracts. Co-administration of extracts from both plants exhibited maximum reduction in γ-glutamyltransferase concentration. However, the polyherbal combination produced no significant effect in reducing blood cholesterol when compared to individual extracts. Gallic acid an active constituent from *P. amarus*, in its pure form, played significant role in reducing most liver injury biomarkers when compared to phyllanthin. However, the latter was more effective in reducing blood cholesterol compared to gallic acid. The polyherbal combination of *A. paniculata* and *P. amarus* did not consistently produce a uniform, beneficial effect in cases of liver injury when monitored by several biomarkers [36].

The hepatoprotective effect produced by *P. amarus* can be compared to silymarin, a standard hepatoprotective agent used in conventional medicine. Silymarin (*Silybium marianum*, asteraceae) is a mixture of three flavolignans: silybin, silidianin and silychristine, with silybin being the most active. Silymarin has been used medicinally to treat liver disorders, including acute or chronic viral hepatitis, drug-induced hepatitis, cirrhosis and alcoholic liver diseases. Its mechanism of action includes inhibition of hepatotoxin binding to receptor sites on the hepatocyte membrane, reduction of glutathione oxidation to enhance the level of hepatocytes in the liver [9]. Administration of silymarin lowered the elevated serum level of bilirubin, AST and ALT [37]. In one study, the aqueous extract of *P. niruri* showed remarkable activity at dose of 6.25 mg/kg as compared to silymarin (100 mg/kg, positive control) in paracetamol-induced liver toxicity in chicks [38]. This demonstrated the possibility of the extract to be a better alternative hepatoremedial than silymarin in hepatotoxicity induced by paracetamol. A synergistic hepatoprotective effect was achieved when a standardized extract of *P. amarus* was co-administered with silymarin in CCl_4-induced hepatotoxicity in *Rattus norvegicus* [39]. Other standardized herbal products containing extracts of *P. amarus* or *P. niruri* have also been marketed as effective hepatoprotectives in Ayurvedic medicine. HPN-2, a multiherbal preparation containing *Glycyrrhiza glabra*, *Pichorhiza kurroa*, *Berberis aristata*,

Piper longum, P. niruri, Solanum dulcamara, Zingiber officinale, Curculigo orchioides, Elletaria cardamomum, Tinospora cordifolia, Desmodium trifoliumm and *Saccharum officinarum* when orally administered to male albino rats at 1 mL/100g body weight was effective against liver damage [40]. Another herbal product 'Catliv' containing extracts of *Swertia chirata, Eclipta alba, Fumaria vaillanti, Picorrhiza kurroa, A. paniculata* and *P. niruri* administered to CCl_4- induced hepatopathic animals at 25 mL orally twice daily for six days starting at 48 hours after administration of the hepatotoxin, effectively helped in the regeneration of hepatic cells in calves [41]. A possible combination of hepatoprotective herbal drugs will be ideal for achieving better therapeutic outcome in the management of chronic liver disease, since there is no single ideal herbal that can completely cure the numerous diseases of the liver.

P. amarus has been reported to be a potent antioxidant which was proved in most of the studies by the fact that elevation of the antioxidant enzymes in the intestine and decrease in lipid peroxidation levels were observed after its administration. Histopathological evaluation of the intestines of the laboratory animals revealed decreased damage to the intestinal walls which further demonstrated that *P. amarus* protected the intestine from oxidative damage. Also, antioxidant enzymes such as glutathione-s-transferase, glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase increased in level following *P. amarus* administration thus providing further evidence of the antioxidant potential of the plant [42]. The hepatoprotective property of *P. amarus* has been attributed to its major lignan constituent, phyllanthin [31, 24, 34] and/or hypophyllanthin [43-45]. However, other compounds which possess similar pharmacological activities to phyllanthin such as the flavonoids like rutin and quercetin-3-*O*-glucoside; tannins like amariin, corilagin, phyllanthusiin A, B, C, D [46] may play a part in that effect. Phyllanthin possess antioxidant effect [32], it scavenges the free radicals generated in hepatic cells thereby restoring the normal functioning of the liver. Another suggested mechanism is by reducing the rate of lipid peroxidation and increasing the antioxidant defence mechanism [29].

This review has gathered that the *P. amarus* or its active principles possess liver protecting property, thus confirming its application in traditional medicine as a hepatoprotective or hepatocurative agent as reported in the literature [47-50]. However, a few investigations have reported otherwise. In a study by Adedapo *et al* [51], the chromatographic fractions obtained from *P. amarus* were tested on the serum biochemistry of rats. Six fractions of *P. amarus* were orally administered to 8-week old albino rats in separate groups at doses 400, 800 and 1600 mg/kg. All the fractions caused an increase in ALT, ALP total bilirubin, serum creatinine, blood urea nitrogen, BUN (except one fraction), while three fractions caused a significant increase in AST; thereby suggesting that *P. amarus* may be hepatotoxic since the elevated BUN causes nephrotoxicity.

Conclusion

P. amarus Schum & Thonn have been used in herbal medicine over a long time for liver protecting potentials. Studies have reported the plant to be effective in suppressing human hepatitis B owing to its antiviral property. Extracts of this pant have been isolated and found to be valuable in reversing the hepatotoxicity and hepatic structures deformation occasioned by the use of hepatotoxins such as paracetamol, carbon tetrachloride, aflatoxins, alcohol, galactosamine and antitubercular drugs. The lignan, phyllanthin, have produced hepatoprotective effect far superior to that produced by the standard drug silymarin, even at a very low concentration which clearly indicates it is a highly promising drug candidate. The hepatoprotective capacity exhibited by this compound may be partly due to its antioxidant property as widely suggested by the literature. However, further research is required to unravel its detailed mechanism of action. Extracts of this plant can future be explored so as to produce an enhanced, standardized antihepatotoxic and hepatoprotective agent that can replace the less safe and efficacious synthetic drugs for hepatic disorders.

Conflict of interest

The authors have no conflict of interest to declare with regard to the publication of this paper.

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