

Antihepatotoxic Activity of *Phyllanthus atropurpureus* Cultivated in Egypt

Taha Sarg, Afaf Abdel Ghani, Rawia Zayed*, and May El-Sayed

Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, 44519 Zagazig, Egypt. E-mail: rawiazayed@hotmail.com

* Author for correspondence and reprint requests

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The genus *Phyllanthus* (family Euphorbiaceae) is considered one of the important medicinal and ornamental plants. A phytochemical analysis of the extracts was performed to search for the active ingredient. Results of the investigation of the hepatoprotective activity of *Phyllanthus atropurpureus* Boj. Hort. Maurit. revealed that the activities of alcoholic extracts of its aerial parts and roots were quite similar to those of silymarin. Both of them improve the parameters of CCl₄-induced liver injury including serum aspartate aminotransferase and alanine aminotransferase. Among the extracts tested, the root extract showed maximum activity compared to the aerial parts extract and to silymarin.

Key words: *Phyllanthus*, Euphorbiaceae, Hepatoprotective Activity

Introduction

The liver is one of the largest glands and most complex organs in the body. It performs multiple functions, including the production of proteins and enzymes, detoxification, metabolic functions, the regulation of cholesterol and blood clotting (Marsano *et al.*, 2003). It contains the highest concentration of enzymes involved in phase I oxidation-reduction reactions (Guegenrich, 1994). It is the primary site of biotransformation and detoxification of exogenous toxic xenobiotics (Lee, 1995)

Unfortunately, the liver is often the most abused organ in the body. It is exposed to alcohol, drugs, and a multitude of environmental toxins. An overstressed liver can impair detoxification and manifest in what may appear to be unrelated symptoms. Eventually, a dysfunctional liver can not perform its tasks properly and, consequently, the body becomes subject to toxicity and an overall decline in metabolic function (Treadway, 1998).

Problems associated with liver dysfunction can ultimately lead to serious illness such as hepatitis, cirrhosis, fatty liver, alcoholic liver disease, and biliary cirrhosis (Scott, 1998). Cirrhosis is a complex disease in which several biological and biochemical alterations are combined, and no proven effective treatment capable of reversing it has been developed (Matsuda *et al.*, 1995). Many plants demonstrate hepatoprotective activity. Some *Phyllanthus* plants were used as remedies

against hepatic disorders (Hawkins, 2001; Harish and Shivanandappa, 2006; Pramyothin *et al.*, 2007). Karuna *et al.* (2009) suggested that consumption of the non-toxic *Phyllanthus amarus* aqueous extract can be linked to an improved antioxidant status and reduction in the risk of oxidative stress. Ahmed *et al.* (2009) stated that the whole plant of *Phyllanthus debilis* afforded a new oxirano-furanocoumarin (debelalactone) which showed antihepatotoxic activity. Nworu *et al.* (2010) confirmed that the decoctions of *Phyllanthus niruri* are promoted in traditional medicine of Africa, Asia, and South America as beneficial supplement for different infectious diseases, especially for viral hepatitis and tumour, and for immune compromised patients.

Generally many *Phyllanthus* species contain many tannins, lignans, and flavonoids which possess antioxidant and hepatoprotective activity (Anila and Vijayalakshmi, 2003; Pramyothin *et al.*, 2007; Singh *et al.*, 2009). This stimulated the interest to study the ability of an ethanolic extract of *Phyllanthus atropurpureus* to repair rat liver damage induced by CCl₄ and also the possible mechanisms of the hepatoprotection.

Material and Methods

Experimental animals

Fifty adult male albino rats weighing about 200–250 g were used in the present investigation.

Table I. Groups of animals and treatments.

Group	Dose	Treatment
(1) $n = 10$, control group	30 μ l/100 g body weight (IP)	Received liquid paraffin for 45 days
(2) $n = 40$, subacute cirrhotic group	25 μ l/100 g body weight (IP)	Received CCl ₄ diluted (1:6) with liquid paraffin, three times a week for 45 days

n , Number of animals; IP, intraperitoneal.

The animals were housed in cages with wood shaving bedding, and allowed to become acclimatized to laboratory conditions for one week before the experiment. The animals were randomly divided into two groups [(1) and (2); Table I]. Group (2) was subdivided into 4 subgroups [(A)–(D)] which are listed in Table II.

Induction of liver cirrhosis

Liver cirrhosis was induced in rats by intraperitoneal (IP) injection (Hernandez-Munoz *et al.*, 1997) of CCl₄ 3 times a week for 45 d in a dose of 25 μ l/100 g body weight. CCl₄ was freshly diluted (1:6) in liquid paraffin directly before the injection.

Blood sampling and serum preparation

Blood samples were collected in clean dry test tubes from the orbital sinus of fasted rats using heparinized microcapillary tubes according to Riley (1960). Blood samples were centrifuged directly at 2000 $\times$ g for 15 min using a Labofuge 200 Heraeus Sepatech centrifuge. Liver enzymes (ALT, AST), proteins (total protein, albumin), and antioxidant parameters (malondialdehyde and glutathione) were determined in the collected plasma and serum.

Determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT)

The serum AST and ALT levels were determined by colorimetric methods (Rietman and Frankel, 1957) using diagnostic kits supplied by Plasmatek (Weil, Germany).

Determination of total protein (Biuret method)

Total protein was determined colorimetrically with biuret (Chromy and Fischer, 1977) using a diagnostic kit supplied by Biocon (Mönchberg, Germany).

Determination of serum albumin

Serum albumin was determined colorimetrically by the bromocresol green method (Doumas *et al.*, 1971; Webster *et al.*, 1992) using a diagnostic kit supplied by Biocon.

Determination of malondialdehyde (MDA)

MDA was identified as the product of lipid peroxidation that reacts with thiobarbituric acid in acidic medium at 95°C for 30 min to form a pink coloured compound absorbing at 534 nm (Ohkawa *et al.*, 1979).

Determination of reduced glutathione (GSH)

GSH reduces 5,5'-dithio-bis-(2-nitrobenzoic acid) to produce a yellow compound which has an absorption maximum at 405 nm (Beutler *et al.*, 1963).

Plant materials

Phyllanthus atropurpureus Boj. Hort. Maurit., family Euphorbiaceae (spurge), was collected in the flowering stage from plants cultivated in the medicinal plants garden of the Faculty of Science,

Table II. Drugs and chemicals used in the antihepatotoxic experiment. The subgroups received the drugs for 30 days (all drug solutions were freshly prepared just before use).

Subgroup	Drug name	Dose	Note
(A) $n = 10$			Cirrhotic rats without treatment
(B) $n = 10$	Ethanolic aerial parts extract	200 mg/kg (orally)	The drugs were suspended or emulsified in 10% gum
(C) $n = 10$	Ethanolic roots extract	200 mg/kg (orally)	The drugs were suspended or emulsified in 10% gum
(D) $n = 10$	Silymarin	100 mg/kg (orally)	The drug was dissolved in normal saline

n , Number of animals.

Table III. Phytochemical screening of powdered *P. atropurpureus*.

Chemical test	Petroleum ether		Diethyl ether		Chloroform		Ethanol	
	AP	R	AP	R	AP	R	AP	R
For sterols and/or triterpenes	+	+	+	+	-	-	-	-
For alkaloids	-	-	-	-	-	-	-	-
For flavonoids	-	-	-	-	+	+	+	+
For glycosides	-	-	-	-	-	-	+	+
For tannins	-	-	-	-	-	-	+	+

AP, aerial parts; R, roots; +, detected; -, not detected.

Ain Shams University, Cairo, Egypt. The identification of the plant was kindly verified by Dr. Hesham Abd El-Aal Elshamy, Professor of Medicinal, Aromatic and Ornamental Plants, Horticulture Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. A voucher specimen is deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt. The plant material was shade-dried and ground by an electric mill to a moderately fine powder.

The air-dried and separately powdered aerial parts and roots of *Phyllanthus atropurpureus* were successively extracted till exhaustion in a Soxhlet apparatus with the following solvents: petroleum ether, diethyl ether, chloroform, and ethanol (95%), applying one after the other. The extracts were collected separately, and the column of the Soxhlet apparatus was washed with 200 ml of water and 100 ml of a similar solvent as an eluent after each type of solvent extraction procedure. The eluted materials and each of the extracts were concentrated at 40 °C to 100 ml in a rotary evaporator. Then each of the extracts was filtered, solvents were evaporated, and the solid residues were weighed and then investigated.

Statistical analysis

All results are expressed as mean $\pm$ standard error of the mean (S.E.M). ANOVA and post ANOVA test at $p > 0.05$ were used to test the significance of the differences between control and treated groups.

Results

Phytochemical investigation

From the preliminary chemical tests it could be suggested that the most bioactive compounds detected in *Phyllanthus atropurpureus* Boj. Hort.

Maurit. are flavonoids, isoflavonoids, tannins, and glycosides, as listed in Table III.

Hepatoprotective activity

As shown in Table IV, a reduction of the hepatic GSH level was observed in rats administered with CCl₄. However, treatment with *P. atropurpureus* root extracts at a dose of 200 mg/kg body weight exhibited a significant increase in the plasma levels of GSH. A significant increase in MDA levels was observed in CCl₄-treated rats. However, treatment with *P. atropurpureus* extracts (root and aerial parts) at a dose of 200 mg/kg body weight reduced significantly the MDA level elevated by CCl₄ treatment; also in silymarin-treated rats, MDA levels were significantly reduced. The antioxidant activity of extracts of *P. atropurpureus* was comparable to that of silymarin, the reference hepatoprotective drug.

The results presented in Table IV demonstrate that the activities of serum AST and ALT (marker enzymes for liver damage) were significantly elevated in CCl₄-treated animals compared to control rats indicating liver damage due to cytolysis resulting in higher levels of serum AST. The administration of silymarin at a dose of 100 mg/kg body weight caused a significant reduction in ALT and AST levels which was quite similar to the significant reduction caused by oral administration of *P. atropurpureus* extracts at a dose of 200 mg/kg body weight in CCl₄-intoxicated rats.

As listed in Table IV, only oral treatment of cirrhotic rats with the ethanolic extract of roots showed a significant elevation in the albumin level, while neither root extract nor aerial part extract produced any significant change in the total protein level.

These results indicate that the hepatoprotective activity of *P. atropurpureus* extracts is quite similar to that of silymarin. Both of them improve the

Table IV. Effect of total extracts of aerial parts and roots of *P. atropurpureus* (200 mg/kg body weight) taken orally for 30 days on liver enzymes, plasma protein, and antioxidant parameters in subacute male cirrhotic rats.

Parameter	Normal rats	Cirrhotic rats/ CCl ₄ -treated rats (before treatment with drugs and extracts)	Cirrhotic rats after treatment with drugs and extracts			
			Silymarin		Aerial part extract (200 mg/kg)	
			Mean $\pm$ S.E.M		Mean $\pm$ S.E.M	Effect relative to silymarin
ALT (IU/ml)	19.21 $\pm$ 1.05	50.49 $\pm$ 1.33	31.18 $\pm$ 1.16*	25.59 $\pm$ 0.66*	30.15 $\pm$ 1.13*	1.05
AST (IU/ml)	63.08 $\pm$ 5.37	108.55 $\pm$ 8.6	76.97 $\pm$ 6.7*	79.8 $\pm$ 5.37*	78.5 $\pm$ 2.66*	0.95
Total protein (g/l)	6.42 $\pm$ 0.47	5.48 $\pm$ 0.42	4.98 $\pm$ 0.43	4.96 $\pm$ 0.28	4.85 $\pm$ 0.28	1.26
Albumin (g/l)	4.11 $\pm$ 0.26	3.16 $\pm$ 0.37	3.57 $\pm$ 0.31	3.46 $\pm$ 0.05	4.68 $\pm$ 0.57*	3.71
MDA (nmol/ml)	30.91 $\pm$ 1.8	197.15 $\pm$ 25.3	43.36 $\pm$ 8.6*	42.66 $\pm$ 4.6*	36.7 $\pm$ 2.6*	1.04
GSH (nmol/ml)	1.97 $\pm$ 0.23	1.79 $\pm$ 0.32	2.36 $\pm$ 0.48	2.35 $\pm$ 0.122	2.91 $\pm$ 0.33*	1.97

Data are expressed as mean $\pm$ S.E.M.* Significant difference from cirrhotic rats without treatment (after 30 days) at $p > 0.05$.

parameters of CCl₄-induced liver injury including serum AST and ALT. Among the extracts tested, the root extract showed maximum activity, as shown in Table IV, compared with the aerial parts extract relative to silymarin.

Discussion and Conclusion

Antioxidant activity

The extract of *P. atropurpureus* showed good antioxidant potential and prevented oxidation of proteins and lipids. By virtue of its ability to scavenge reactive oxygen species (ROS), it probably can modulate transcription factors and regulate the levels of antioxidant enzymes. Potent antioxidant activities in aerial parts of *Phyllanthus* may be due to the presence of phenolic and polyphenolic compounds, such as flavonoids (Agarwal and Tiwari, 1991), catechin (Deckar, 1995) and hydrolysable tannins, with geraniin being the most abundant (Foo, 1993, 1995; Foo and Wong, 1992), ellagic acid (Ishimaru *et al.*, 1991), and lignans (Singh *et al.*, 2009; Satynarayana *et al.*, 1988).

Polyphenolic compounds enhance the stability of low-density lipoprotein (LDL) to oxidation by scavenging the superoxide anion (Robak and Gryglewski, 1988), singlet oxygen (Husain *et al.*, 1987), and lipid peroxy radicals (Torrel *et al.*, 1986) and stabilizing free radicals involved in oxidative processes through hydrogenation or complex formation with oxidizing species (Lewis, 1993; Shahidi and Wanasusdara, 1992). La Casa *et al.* (2000) reported that rutin, a natural flavonol glycoside, induced a significant increase in the GSH activity. Flavonoids can reduce macrophage oxidative stress by inhibition of cellular oxygenases, such as NADPH oxidase, or by activating cellular antioxidants, such as GSH (Fuhrman and Aviram, 2001).

The potent antiperoxidative effect of *Phyllanthus* protects the liver by preventing trichloromethyl free radical (CCl₃•)-induced peroxidative disintegration of membranes (Dhuley and Naik, 1997). The enhancement in the hepatic GSH status was associated with corresponding decreases in MDA levels and ALT activities, indicating a significant reduction in the extent of oxidative hepatocellular damage. In conclusion, the extracts of *P. atropurpureus* act as antioxidant and the anti-lipid peroxidation activity of the root extract was found to be higher than that of the aerial part extract that may be attributed to the presence of

tannins in the root as reported previously (Bhattachary *et al.*, 1999).

Hepatoprotective activity

The increased cytolysis, as is evident from the higher levels of serum AST, suggests that the enhanced microsomal lipid peroxidation in the liver is associated with a damage of hepatic tissue, which is in agreement with earlier findings (Barber, 1963). Carbon tetrachloride (CCl_4) is a hepatotoxic agent causing centrilobular necrosis and is associated with fatty liver. CCl_4 is converted to the $\text{CCl}_3\cdot$ radicle by hepatic mixed function oxidases. $\text{CCl}_3\cdot$ can abstract hydrogen from polyunsaturated fatty acids to initiate lipid peroxidation; alternatively in the presence of oxygen, it forms the more reactive trichloro-methylperoxy free radical ($\text{CCl}_3\text{COO}\cdot$). $\text{CCl}_3\text{COO}\cdot$ can participate in lipid peroxidation or can decompose to phosgene (CCl_2O) (Brattin *et al.*, 1985).

Antioxidants and radical scavengers have been used to study the mechanism of CCl_4 toxicity as well as to protect liver cells from CCl_4 -induced damage by breaking the chain reaction of lipid peroxidation. Silymarin has been reported to protect liver cells from a wide variety of toxins (Muriel and Mourelle, 1990; Bosio *et al.*, 1992), including CCl_4 . The hepatoprotective mechanism of silymarin may be due to its antioxidant activ-

ity and/or inhibition of lipid peroxidation (Pietrangelo *et al.*, 1995; Basage *et al.*, 1997).

The pronounced hepatoprotective activity (compared to silymarin) of the ethanolic extract of *P. atropurpureus* found in this study is in agreement with that reported on the effect of other *Phyllanthus* species against various chemical liver toxins (Liu and Meintosh, 2001; Wang *et al.*, 2001; Khatoon *et al.*, 2006; Kumaran and Karunakaran, 2007; Narayan *et al.*, 2008; Syamasunder *et al.*, 1985).

It can be concluded that the antioxidant property of ethanolic extracts of root and aerial parts of *P. atropurpureus* could counteract CCl_4 toxicity. The hepatoprotective activity of *P. atropurpureus* may be due to the presence of polyphenolic compounds. Therefore, the hepatoprotective mechanism of *Phyllanthus* may involve its antioxidant activity against production of ROS. Thus, *P. atropurpureus* can be considered a new efficient hepatoprotective candidate, but clinical follow-up studies are needed to test the safe use in the whole organism.

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