tribute in the proposed method.

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Protective Effect by Aqueous Extract of *Phyllanthus amarus* Linn., Phyllanthin and Nirocil against Carbontetrachloride-induced Liver and Brain Toxicity

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Effect of carbontetrachloride treatment on hepatic and brain antioxidant status in rats pretreated with aqueous extract of *Phyllanthus amarus* Linn. (Euphorbiaceae), nirocil (a tablet made up of aqueous extract of *P. amarus*), phyllanthin (a bioactive lignan from *P. amarus*), and silymarin were studied. Plasma aspartate aminotransferase and alanine aminotransferase were estimated to monitor the extent of hepatocellur damage. Tissue lipid peroxide, ascorbic acid and total protein levels were used as the markers for functional and antioxidant efficiency of liver and brain cells. Phyllanthin reversed the elevated plasma aminotransferase levels but did not affect hepatic antioxidant status. In all the paradigms tested for hepatoprotection, nirocil, silymarin and aqueous extract (90 mg/kg) showed significant protection. There was a drastic impairment in the functional and antioxidant status of brain on treatment with carbontetrachloride. None of the drugs except silymarin showed good protection against carbontetrachloride-induced lipid peroxidation in the brain, but all these produced a significant increase in the protein levels. All the drugs administered, augmented the ascorbate levels in liver and brain, with the aqueous extract of *P. amarus* clearly outdoing the others.

Phyllanthus amarus has been traditionally used as a folk remedy for the treatment of jaundice in India and various other parts of the world. Its protective activity has been demonstrated in chemically-induced liver toxicity model[1,2]. In this study, the antioxidant-cytoprotective property of *P. amarus* and its formulations was evaluated and compared with silymarin, a standard antioxidant-cytoprotective agent. Further, a recent report indicates that in acute liver failure, increased accumulation of glutamine, glutamate and inflam-

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matory cytokines from necrotic liver leads to brain edema and intracranial hypertension[3]. Glutamate, a neurotransmitter, leads to a cascade of events like increase in Ca^{++} influx, formation of arachidonic acid and eicosanoids probably resulting in the formation of oxygen free radicals that may cause lipid peroxidation[4]. Hence, the functional status of brain was also determined in the drug-treated animals.

Whole plants of *Phyllanthus amarus* were collected within the campus of Banaras Hindu University in August and were identified by Department of Pharmacognosy, Institute of Pharmaceutics, Banaras Hindu University, Varanasi. Nirocil tablets were obtained from Solumiks division, NPIL, Mumbai. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) kits were of Enzopak kits, Reckon Diagnostics, Vadodara.

Fresh green leaves of *P. amarus* were used, and the isolation of phyllanthin was carried out as per reported procedure[5]. The aqueous extract was prepared by boiling 10 g of shade dried powder of *P. amarus* (whole plant) in 250 ml double distilled water under reduced pressure for 8 h. The hot extract was filtered under suction, concentrated *in vacuo* and evaporated at room temperature to a semisolid consistency (yield-13.37%). The dose for administration was calculated to the equivalent present in nirocil tablets (each tablet contains 1 g of aqueous extract of *P. amarus*).

Wistar rats (150-180 g) were used for hepatoprotective and antioxidant studies. The animals were maintained in controlled temperature and humidity conditions with a 12 h light/dark cycle and were fed with standard laboratory diet and water *ad libitum*. All the experimental protocols were approved by the Institutional Animal Ethics Committee. The animals were divided into seven groups of 6 animals each. All formulations were administered by oral route. Group I and II were administered the vehicle. Groups III, IV, V, VI and VII were given silymarin 50 mg/kg, phyllanthin 10 mg/kg, nirocil 90 mg/kg and aqueous extract of *P. amarus* 40 and 90 mg/kg, respectively for six consecutive days. All treatments were administered isovolumetrically in 1% sodium carboxymethyl cellulose suspension[2]. On day 5, animals in all groups except the group I were administered carbon tetrachloride (CCl_4, 2 ml/kg, p.o., in 50% dilution with olive oil), while animals in group I were administered equivalent of olive oil only. On day 7 (i.e. 48 h after CCl_4 administration) blood was collected by cardiac puncture and centrifuged for 10 min at 3000 rpm to collect plasma. Plasma was analyzed for ALT and AST levels using Enzopak kits[6]. The liver and brain were dissected out, washed, weighed and a 10% tissue homogenate was prepared in ice cold 1.15% w/v KCl solution using a Teflon homogeniser. The homogenate was centrifuged at 4000 rpm for 10 min to remove nuclear fraction[7]. The supernatant was used for the estimation of lipid peroxide[7], ascorbic acid[8] and total protein levels[9]. Each value was expressed as mean±S.E. Statistical evaluation of the data was done using one way ANOVA (GraphPad Instar Software). A value of $p<0.05$ was considered to be significant.

TABLE 1: EFFECT OF VARIOUS DRUG TREATMENTS ON CCl_4-INDUCED ELEVATION IN PLASMA TRANSAMINASES[a].

Treatment	Hepatocellular Injury	
	AST (IU/L)	ALT (IU/L)
Vehicle	4.40±2.23	37.9±1.23
CCl_4	85.7±2.80*	170±3.49*
Silymarin + CCl_4	54.9±4.83**	47.3±4.05**
Phyllanthin + CCl_4	68.3±0.98**	45.4±3.66**
Nirocil + CCl_4	58.5±3.31**	42.8±3.31**
Aqueous extract of *P. amarus* (40 mg/kg)	76.4±8.12	84.1±5.98**
Aqueous extract of *P. amarus* (90 mg/kg)	61.2±2.52**	49.3±3.65**

[a]All values are mean±S.E. (n=6). *represents $P<0.05$ compared to vehicle control alone; **represents $P<0.05$ compared to CCl_4-treated group.

As shown in Table 1 and 2, there was a drastic impairment in the hepatic functions after 48 h of CCl_4 administration, which was indicated by elevation in plasma aminotransferases (AST/ALT) and hepatic lipid peroxide levels. Nirocil and aqueous extract of *P. amarus* (90 mg/kg) significantly reversed these changes, which were comparable to that of silymarin. Phyllanthin reversed the transferase and protein levels but did not affect the hepatic antioxidant status (peroxide and ascorbic acid levels).

On treatment with CCl_4 there was a severe effect on brain lipid peroxidation, but contrary to hepatic ascorbic acid level, the brain ascorbic acid level increased reflecting the involvement of a secondary mechanism in CCl_4 metabolism, which may be affecting distant loci. In acute liver injury glutamate-free radicals mediated brain edema is also re-

TABLE 2: EFFECT OF VARIOUS DRUG TREATMENTS ON CHANGES IN CCL_4-INDUCED ALTERATIONS IN RAT LIVER[a].

Treatment	Functional evaluation		Antioxidant Status
	Total Proteins (mg/g of tissue)	Lipid peroxides (nmol MDA/100g protein)	Ascorbic acid (mg/g of tissue)
Vehicle	130±5.19	409±14.07	0.73±0.46
CCl_4	84.8±1.69*	808±25.18*	0.56±0.02
Silymarin + CCl_4	114±3.96**	557±26.17**	0.66±0.04
Phyllanthin + CCl_4	104±0.65**	766±29.48	0.86±0.08
Nirocil + CCl_4	102±3.04**	458±22.84**	0.89±0.09
Aqueous extract of *P. amarus* + CCl_4			
40 mg/ kg	95.3±3.61	682±41.95	1.12±0.11
90 mg/ kg	101±2.17**	562±34.39**	3.05±0.31**

[a]All values are mean±S.E. (n=6). *represents P<0.05 compared to vehicle control alone; **represents P<0.05 compared to CCl_4-treated group.

ported[3]. The decrease in hepatic ascorbic acid level may be due to the impairment of enzyme, L-gulonolactone oxidase that is essential for its synthesis; or may be due to oxidation of ascorbic acid to dehydroascorbic acid, as a natural response to thwart the oxidative stress. The increase in brain ascorbic acid level may be due to its release from adrenal medulla as a response to oxidative stress concomitantly with catecolamines. Such release of ascorbic acid along with adrenal catecholamines under various conditions has been reported[10]. Even physical trauma to the brain can cause massive release of ascorbic acid into the extracellular compartment[11].

Our results indicted that silymarin, a mixture of

TABLE 3: EFFECT OF VARIOUS DRUG TREATMENTS ON CHANGES IN CCL_4-INDUCED ALTERATIONS IN RAT BRAIN[a].

Treatment	Functional evaluation		Antioxidant Status
	Total Proteins (mg/g of tissue)	Lipid peroxides (nmol MDA/100g protein)	Ascorbic acid (mg/g of tissue)
Vehicle	53.0±1.99	234±12.11	0.64±0.02
CCl_4	24.8±1.38*	720±37.69*	1.05±0.07
Silymarin + CCl_4	31.6±2.24	529±24.22**	2.17±0.13**
Phyllanthin + CCl_4	36.8±2.37**	600±41.59	0.93±0.04
Nirocil + CCl_4	39.1±0.79**	651±30.77	1.68±0.14
Aqueous extract of *P. amarus* + CCl_4			
40 mg/ kg	37.4±0.88**	557±21.72	2.03±0.03**
90 mg/ kg	40.3±1.62**	620±26.55	3.20±0.32**

[a]All values are mean±S.E. (n=6). *represents P<0.05 compared to vehicle control alone; **represents P<0.05 compared to CCl_4-treated group.

flavonolignans, silybin, silydianin and silychristin, afforded good hepatoprotective activity by reversing the CCl_4-induced elevation of plasma ALT and AST levels, decreasing the lipid peroxides, increasing the protein and ascorbate levels in liver. Hepatoprotective and antioxidant activities of silimarin have been reported earlier[12,13], and these appear to be confirmed in the present investigation. Phyllanthin, a diarylbutane lignan, isolated from *P. amarus* showed a significant protection against CCl_4-induced elevation in aminotransferase levels as well as CCl_4-induced decrease in protein levels. However, phyllanthin did not reverse hepatic lipid peroxidation, which indicated a mechanism other than free radical scavenging activity. Reports indicated similar protection by phyllanthin in reversing the transferase levels[1]. Interestingly, phyllanthin marginally prevented CCl_4-induced ascorbic acid depletion.

Nirocil, which is devoid of phyllanthin (confirmed by HPTLC) but rich in flavonoids, is comparable to silymarin in protecting liver. Flavonoids are well known for their antioxidant and hepatoprotective activities[14,15]. Nirocil's ability to show significant protection against lipid peroxidation indicates a strong antioxidant property. Aqueous extract of *P. amarus* (40 mg/kg) showed moderate hepatoprotective action by significantly reversing plasma ALT. But hepatic lipid peroxides and protein levels were unaffected. However, at a dose of 90 mg/kg, activity of the aqueous extract was comparable to nirocil. More interestingly, none of the drugs tested except silymarin showed good protection against CCl_4-induced lipid peroxidation in the brain, but all the drugs produced a significant increase in the protein levels. All the drugs administered, augmented the ascorbate levels in liver and brain, and aqueous extract of P. *amarus* out doing the others can be explained by the presence of variable amounts of ascorbic acid in *P. amarus*. Reports exist regarding the uptake of exogenously administered ascorbic acid by different organs[11]. The reason for the elevation by the other drug treatments is not clear.

Balasubramanyam *et al.*[16] reported that surgical stress in the small intestine produced a free radical-mediated damage on distant organs. Olfsson *et al.*[3] reported a similar effect in the brain as a result of acute liver injury. Hence, the present investigation reinforces the need for hepatoprotective drugs that act against the free radical onslaught not only at the liver but also at distant loci such as the brain. Since brain is rich in polyunsaturated fatty acids with their double bonds easily accessible for free radicals, the free radical related pathology accounts for a variety of mental and cerebral disorders[17].

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