**Enicostemma littorale** Blume — A potential hypolipidemic plant

R Gopalan, A Gnanamani, R Udayakumar and S Sadulla

1CHORD, Central Leather Research Institute, Adyar, Chennai-20, Tamil Nadu
2Department of Biochemistry, J. J. College of Arts and Science, Pudukkottai-622404, Tamil Nadu
*Correspondent author, E-mail: agmani_2000@yahoo.com

Abstract

Restoration of liver function by the application of *Enicostemma littorale* Blume (aerial part) powder in *p*-DAB (*p*-dimethylaminoazobenzene) induced animals was evaluated by analyzing the serum total cholesterol, triglycerides, HDL-cholesterol, serum γ-glutamyl transpeptidase and liver malondialdehyde levels. Administration of the chosen plant reduces the hyperlipidemia at significant level and also reduces the lipid peroxidation and significantly increased HDL-cholesterol level in serum. When comparing the results with the standard antilipidemia agent namely, vitamin E, the results obtained using the plant is highly comparable.

**Keywords:** *Enicostemma littorale*, Chhotachirayta, *p*-Dimethylaminoazobenzene (*p*-DAB), Hepatotoxicity, Lipid profile, Lipid peroxidation, γ-Glutamyl transpeptidase.

**IPC code; Int. cl.** A61K 35/78, A61P 1/16, A61P 3/06

Introduction

Liver is one of the most important organs for the metabolism of various chemicals and is responsible for the major detoxification. Liver damage/hepatocarcinoma, in general associated with the external agents comes under the classification as carcinogens (Raghuram et al., 2003). About 80-90% of cancer related diseases and the hepatic problems are mainly due to continuous exposure or contamination of chemical carcinogens in the food chain (WHO, 1987). Chemical carcinogens like *N*-nitrosodiethylamine (NDEA) and *p*-dimethylaminoazobenzene (*p*-DAB) are reported to induce damage in hepatic systems (WHO, 1975). *p*-DAB, a colouring agent commonly used in foods is reported to be a powerful liver carcinogen (Mukai & Goldstein, 1976; Weinberg, 1996; Fabiana et al., 2001). The electrophilic nature of the metabolites of *p*-DAB has the capacity to covalently bind with RNA, DNA and tissue protein (Labuc & Blunck, 1979; Ohnishi et al., 2001). In addition, reactive oxygen species such as superoxide, hydroxyl radicals and hydrogen peroxide (Halliwell & Gutteridge, 1989; Sies, 1991; Grisham, 1992; Moslen, 1994; Halliwell, 1996) are released due to the induction of *p*-DAB, which further stimulate the damage of liver by the metabolized products of lipid peroxidation, consequently leading to DNA aberrations (Biswas & Khuda-Bukhsh, 2002). Shamberger et al. (1974) reported that hydroxyl radicals are highly reactive and are the basis for the generation of lipid peroxide products such as malondialdehyde, which are considered to be a cause for carcinogenesis.

Several plant products have been shown to exert a protective role against the formation of free radicals and playing a beneficial role in maintaining disease condition (Ajitha & Rajnarayana, 2001).
Enicostemma littorale Blume (Family — Gentianaceae) a glabrous herb commonly used as a bitter tonic and substitute for Swertia chirayita (Roxb. ex Flem.) Karst., is also called Chhotachirayta. It is also reported to possess antitumor (Dash et al, 2000), antiarthritic (Sahu et al, 2000), hypoglycaemic (Ravi et al, 2000) and antimalarial activities (Katewa & Arora, 2001). The anticancer activity of methanolic extract of the plant has been evaluated against Dalton’s ascetic lymphoma in Swiss albino mice (Kavimani & Manisenthilkumar, 2000). In the present study authors have evaluated the hypolipidemic and antilipid peroxidative activities of the plant against p-DAB induced hepatotoxicity in animals.

Experimental Work

Plant material and chemicals

The plant, E. littorale was collected from Chennai, Tamil Nadu in the month of December and was identified and authenticated by Dr T. Anandan; Research Officer, Central Research Institute for Siddha Medicine, Chennai. The aerial parts were air-dried and ground well to fine powder, and used as drug in the crude form along with physiological saline.

p-DAB was purchased from Sigma Chemical Company (St Louis, Mo, USA). All other reagents used were of analytical grade.

Animals

Wistar male albino rats having body weight between 150-200g were purchased from an authorized firm at Bangalore and were housed in spacious polypropylene cages and maintained under standard conditions of temperature 27 ±2°C, relative humidity of 60 ± 5%, 12 hours of light/dark cycle, and fed with pellet diet manufactured by Pranav Agro Industries Limited, Shangli, Maharashtra under the trade name “Amrut rat and mice feed” and had free access to sterilized water.

Experimental design

Animals were divided into four groups each group containing six animals. Group I served as normal animals, which received the vehicle 3% DMSO (5ml/kg body wt). Group II animals with hepatocellular damage induced by p-dimethylaminoazobenzene (1mg/kg body wt) in 3% DMSO was injected intraperitoneally (i.p.) once in every 3 days for 15 days. Group III animals with hepatocellular damage were induced as per group II and followed by last dosage were treated with the plant (1g/kg body wt) in crude form along with physiological saline twice a day for 15 days. Group IV animals with hepatocellular damage induced as per group II, after the last dose, vitamin E (30mg/kg body wt) was given orally in the suspended form with sunflower oil. Administration was done for twice a day for 15 days.

Biochemical estimations

The animals were fasted for 12 hours after the last dose of the drug treatment and were sacrificed by cervical decapitation. Blood was collected in tubes and serum was separated as per conventional methods. Liver samples were separated and was homogenized in 10% saline. The homogenate was centrifuged at 15,000 x g for 30 minutes at 4°C and the supernatant was used for lipid peroxidation analysis. The serum total cholesterol (TC) was estimated by the method of Zak’s (1957); triglycerides (TG) was estimated by method of Van Handel & Zilversmit (1957). High density lipoprotein cholesterol (HDL-c) was estimated by the method of Burstein et al (1970). Low-density lipoprotein cholesterol (LDL-c) was calculated from the above measurement by using the equation, LDL = TC - (HDL +TG), suggested by of Friendwald et al (1972).

The γ-glutamyl transpeptidase activity was estimated by the method of Orloswski & Meister (1965). Lipid peroxidative effect was evaluated through measuring the hepatic content of thiobarbituric acid reacting substances (TBARS), expressed as malondialdehyde (MDA) equivalents by the method of Niehaus & Samuelsson (1968).

Statistical analysis

Results were statistically evaluated using one-way analysis of variance (ANOVA) for repeated observation. Values of p< 0.05 were considered to be significant.

Results and Discussion

Table 1 emphases the effect of aerial part of E. littorale on total cholesterol, triglyceride, HDL-c, LDL-c, γ-Glutamyl transpeptidase (γ-GT) and Lipid peroxidation levels of p-DAB...
induced hepatotoxicity in animals. In the 
p-DAB induced animals, the level of total 
cholesterol (87.59 ± 5.57 mg/dl), triglycerides (153.47 ± 6.94 mg/dl), LDL-c (103.23 ± 3.45 mg/dl) and γ-GT (8.26 ± 1.05 IU/L) were significantly increased and a significant decrease in HDL-c (10.28 ± 1.62 mg/dl) in serum was observed compared to the normal group animals. Administration of aerial part of the plant significantly reduces the level of total cholesterol (46.79 ± 6.22 mg/dl), triglycerides (125.73 ± 76.54 mg/dl), LDL-c (93.59 ± 2.92 mg/dl), γ-GT (4.90 ± 0.48 IU/L) and increases HDL-c (12.25 ± 1.65 mg/dl) content in serum samples analyzed. Malondialdehyde content in the liver was enhanced in the p-DAB induced hepatotoxicity (168.80 ± 4.70 n mol/g) when compared to normal animals. The plant significantly reduced the malondialdehyde content in the liver (90.58 ± 3.18 n mol/g). Vitamin E treated animals also expressed the similar reduction of total cholesterol, triglycerides, LDL-c, γ-GT and lipid peroxides in liver and an increase in HDL-c content in serum samples.

Hepatotoxicity is associated with biochemical, histological and functional changes and progressive increase in the chance of liver damage and death of an organ. Liver damage due to increased level of lipid profile and lipid peroxidation appears to be an important determinative mechanism of hepatotoxicity studies (Satturwar et al, 2003; Sharma et al, 2003). The extract contains several alkaloids, flavanoids and reducing sugars and exhibited hypolipidemia and antioxidant properties in the present study similar to the studies carried out by Murali et al (2002) and Kavimani & Manisenthkumar (2000). Animals that received a subcutaneous implantation of the p-DAB displayed cancerous hyperlipidemia characterized by increased serum cholesterol (hypercholesterolemia) and triglyceride (hypertriglyceridemia) levels (Irikura et al, 1985; Kawasaki et al, 1998; Komatsu et al, 1998). The hypercholesterolemia in the hepatoma-bearing animals showed a highly atherogenic lipoprotein profile, that is, a notable increase in the cholesterol and significant decrease in the HDL fraction (Irikura et

<table>
<thead>
<tr>
<th>S. No</th>
<th>Experiment design</th>
<th>Total Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>HDL-Cholesterol (mg/dl)</th>
<th>LDL-Cholesterol (mg/dl)</th>
<th>γ-Glutamyl transpeptidase (IU/L)</th>
<th>Lipid Peroxides (n mol TBARS /g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group I Normal animals</td>
<td>33.57 ± 3.105</td>
<td>61.61 ± 4.74</td>
<td>16.78 ± 1.02</td>
<td>47.48 ± 2.25</td>
<td>1.11 ± 0.23</td>
<td>79.01 ± 2.43</td>
</tr>
<tr>
<td>2</td>
<td>Group II DAB induced animals</td>
<td>87.59 ± 5.57</td>
<td>153.47 ± 6.94</td>
<td>10.28 ± 1.62</td>
<td>103.25 ± 3.45</td>
<td>8.26 ± 1.05</td>
<td>168.80 ± 4.70</td>
</tr>
<tr>
<td>3</td>
<td>Group III DAB induced + E. littorale drug treated</td>
<td>46.79 ± 6.22</td>
<td>125.73 ± 6.97</td>
<td>12.25 ± 1.62</td>
<td>93.59 ± 2.92</td>
<td>4.90 ± 0.48</td>
<td>90.58 ± 3.18</td>
</tr>
<tr>
<td>4</td>
<td>Group IV DAB induced + Vitamin E treated</td>
<td>41.25 ± 4.12</td>
<td>111.75 ± 5.15</td>
<td>14.62 ± 1.25</td>
<td>87.4 ± 3.62</td>
<td>3.25 ± 0.25</td>
<td>85.45 ± 2.25</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. Group III & IV are compared to Group II (*p<0.001).
al, 1985). In general, vitamin E inhibits ROS induced generation of lipid peroxyl radicals, thereby protecting cells from peroxidation of PUFA in membrane phospholipids from oxidative damage of plasma very low-density lipoproteins (Topinka et al, 1989). Thus, administration of vitamin E reduces the lipid profile significantly.

In the present study, *E. littorale* significantly reduces the serum cholesterol level in hepatoma-bearing rats on par with vitamin E treated groups, compared with the p-DAB treated animals as shown in Table 1. As to the hepatoma-induced hypercholesterolemia, a component of the plant enhances cholesterol catabolism, with the help of cholesterol acyl transferase by esterification of free cholesterol in the HDL. Cholesterol acyl transferase along with HDL plays an important role in the transport of cholesterol from the tissue to the liver for its catabolism, establishes that it has hypocholesterolemic action in the hepatobearing state. The inhibition of hepatoma by this plant may have been partially responsible for the decreased hepatoma-induced hypertriglyceridemia, although this decrease may also be attributable to other mechanisms such as the activation of lipoprotein lipase in the adipose tissue (Kawasaki et al, 1996, 1998).

In addition, the level of serum γ-GT was significantly increased in p-DAB induced hepatoma animals (Velanganni & Balasundaram, 2003). This trend was reverted to normal on the administration of aerial part of *E. littorale*. p-DAB and its metabolites induce lipid peroxidation products (Gerez et al, 1998), such as malondialdehyde. The reduction in level of malondialdehyde proves the antioxidative property of this plant. With vitamin E, similar observations were made on both hyperlipidemia and antilipid peroxidative property.

**Conclusion**

Currently research studies are focused on the exploitation of natural products. As per the present study, recovery of liver damage through natural sources is of high importance to retrieve back the liver functions without any side effects. Though the experiments were carried out with animal models by inducing the toxicity, the results of this study would be useful to evaluate the potentiality of *Chhotachirayta* for its hypolipidemic and antilipid peroxidation effect.

**References**

13. Kavimani S and Manisenthilkumar KT, Effect of methanolic extract of *Enicostemma littorale* on Dalton's


17. Labuc GE and Blunk JM, Metabolic activation of the hepatocarcinogen 3'-methyl-4-dimethylaminooazobenzene by a rat liver cell-free system, Biochem Pharmacol, 1979, 28, 2367-2373.


37. WHO, Monographs on the evolution of carcinogenic risk of chemicals to man 1975, 8.