Hepatoprotective activity of Annona muricata Linn and Polyalthia cerasoides Bedd.

P. Padma\textsuperscript{a}, J.P.N. Chansouri\textsuperscript{b} and R.L. Khosa\textsuperscript{a}

\textsuperscript{a}Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi – 221 005.
\textsuperscript{b}Centre of Experiemental Medicine and Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi – 221 005, India.

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\textbf{ABSTRACT:} The hepatoprotective effect of Annona muricata and Polyalthia cerasoides (Annonaceae) were monitored by estimating the serum transaminases (SGOT and SGPT), serum alkaline phosphatase (SALP), liver and brain lipid peroxidation (LOP) and their total protein content. Both drugs at a dose of 100 µg/kg significantly prevented the increase in serum transaminases, SALP, liver and brain LOP and decrease in liver and brain total protein content following carbontetrachloride (CCI) induced hepatotoxicity in albino rats.

\textbf{Key words :} Annona muricata Linn., Polyalthia cerasoides Bedd.; Carbontetrachloride; lipid peroxidation; hepatoprotective activity.

\textbf{INTRODUCTION}

There is a progressive increase in the incidence of hepatic damage mainly due to the viral infection, hepatotoxic chemicals (alcohol), peroxides, toxin in food, pharmaceuticals, environmental pollutants and xenobiotics. There is hardly any remedy available in the modern system of medicine, including corticosteroids and immunosuppressive agents which bring about symptomatic relief supporting only the process of healing or liver regeneration (Handa et al., 1986; Hikino and Kiso, 1988). Hence increasing attention is being given to plants recommended for the treatment of hepatic disorders in the traditional system of medicine. A decoction of both these plant drugs are prescribed by practitioners in the Indian system of medicine for the treatment of liver disorders. In this context, we have assessed the hepatoprotective activity of alcoholic extracts of \textit{A.muricata} an \textit{P.cerasoides} against carbontetrachloride induced hepatotoxicity as its involvement in free radical mediated reactions to produce hepatic injury is very well established.

\textbf{MATERIALS AND METHODS}

\textbf{Plant material and extraction}

The stembarks of \textit{A.muricata} and \textit{P.cerasoides} were procured from Tirunelveli district (Tamilnadu, India) and authenticated by Dr. V. Chelladurai, Survey of Medicinal Plant Unit, Government Siddha Medical College, Tirunelveli. Voucher specimen PP/PH/01/95 and PP/PH/02/95 are preserved in the Department of Pharmaceutics, Banaras Hindu University, Varanasi. The plant materials were dried, reduced to moderately coarse powder and then materials (500 gm each) were defatted with petroleum ether (60-80°C, 31, 18hrs) in a...
soxhlet and thoroughly extracted with ethanol (95%, 21-36 hr) the ethanolic extract was dried (48gm and 24.2 gm respectively) in a heated vacuum desiccator and made into a suspension in water and propylene glycol (4:1) containing Tween 80 (0.08%) at the concentration of 200 mg/ml.

**Animals**

Adult albino rats (Charles- Foster strain, 150±20 gm, b.w.) were kept in polypropylene cages (3 in each cage) at an ambient temperature of 25±2°C with 55-65% relative humidity and 12±1 hr dark – light cycle. These animals had free access to water and normal laboratory diet (Lipton India Ltd.).

**Hepatoprotective Activity**

Animals were divided into 4 groups, group I and II served as control and carbon tetrachloride control, received the vehicle (Water : propylene glycol : 4:1) by gastric intubation, once daily for 7 days. Group III and IV were given, 1 ml each of suspension of alcoholic extract of *A. muricata* and *P.cerasoides* at a dose of 100 mg/kg, b.w., once daily for 7 days through gastric intubation.

On the 8th day, one hour after the administration of the last dose of drug, the animals of group II,III,IV were given an intraperitoneal injection of carbon tetrachloride 0.5 ml/kg, b.w. All the animals were then fasted for 24 hours after which they were anaesthetized and the blood was collected by cardiac puncture. The liver and brain were quickly dissected out and stored at -20°C. The blood samples were allowed to coagulate at room temperature for one hour. Serum was separated by centrifugation at 4°C, 12,000 rpm for 5 minutes.

**Biochemical Studies**

The activity of serum glutamic oxaloacetic transaminase (SGOT, AST) and glutamic pyruvic transaminase (SGPT, ALT) were estimated according to the method of Reitman and Frankel (1957). The serum alkaline phosphatase (SALP) levels were determined by the method of Bessey et al. (1946). Liver and brain lipid peroxidation level were estimated by the method of Ohkawa et al. (1979) and the total protein content in liver and brain were estimated by Lowry et al. (1951).

**RESULTS AND DISCUSSION**

The results are given in the Tables 1,2 and 3. The hepatotoxicity of CCl₄ has been shown to depend on its metabolism by cytochrome P-450 to produce free radicals which can initiate the damaging process through enhancement of membrane lipid peroxidation, covalent binding to macromolecules, protein and metabolic enzymes (Poli, 1993). Carbontetrachloride treated group (hepatotoxin only Group II) had shown leakage of transaminases culminating in the liver injury. In liver and brain, there was a significant increase in LPO levels whereas their protein level showed a significant decrease suggesting a secondary mechanism involved in CCl₄ metabolism capable of affecting distant loci. It is very well established that the CCl₄ induced hepatotoxicity can be divided into three sequences viz. initial events, secondary evoked mechanism and end-stage pathological consequences, finally altering physiochemical properties of the membranes leading to its functional and structural disruption (Recknagel, 1983; Feher et al., 1986).
The alcoholic extract of *A.muricata* and *P.cerasoides* (Tables 1 & 2) have afforded and overall protection against CCl4 induced toxicity, the activity being slightly less in the case of *P.cerasoides*. LPO (in both liver and brain) and SALP levels were reduced significantly by both drugs (Tables 1 & 2) suggesting that the plant drug scavenges free radicals produced by CCl4 metabolism which could be the possible mode of action of these drugs as hepatoprotective agents. The percentage protection afforded by the two drugs against increase in SGOT, SGPT, SLP, liver and brain LPO levels and decrease in liver and brain protein levels are given in Table 3. All these data suggest that the plant drugs possess possible antihepatotoxic activity which are in tune with the fact that these drugs are prescribed by traditional medical practitioners in India to be administered in form of decoction against liver disorders.

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**REFERENCES:**

Bessey, O.A., Lowry, O.H. and Bros, M.J. (1946) A method for the rapid determination of alkaline phosphatase with five cubic millimeter of serum *Journal of Biological Chemistry* 164, 321-329.

Feher, J., Csomos, G. and Vereckei, A. (1986) *Free radical reactions in Medicine.* Springerverlag, Berlin, p.107.

Handa, S.S., Sharma, A and Chakraborti, K.K. (1986). Natural products and plants as liver protecting drugs. *Fitoterapia* 57, 307 – 351.

Hikino, H and Kiso, Y. (1988) *Natural O.products for liver diseases.* In: H.Wagner, H. Hikino and N.R. Farnsworth (Eds.), *Economic and Medicinal Plant Research.* Academic Press, London, p.39.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein measurement with the Folin’s phenol reagent, *Journal of Biological Chemistry* 193, 265-275.

Ohkawa, H., Ohishi, N. and Yagi, K. (1979) Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry* 95, 351-358.

Poli, G. (1993) Liver damage due to free radicals. *British Medical Bulletin* 49, 604 – 620.

Recknagel, R.O. (1983) Carbontetrachloride hepatotoxicity: status quo and future prospects. *Trends in Pharmaceutical Sciences* 4, 129-131.
Reitman, S. and Frankel, S. (1957) A colorimetric method for the determination of serum glutamic oxaloacetic acid, glutamic pyruvic transaminases. *American Journal of Clinical Pathology* 28, 56-58.

**TABLE 1**

| Sl. No. | Groups                  | SGPT (ALT) U/ml | SGOT (AST) U/ml | Alkaline Phosphatase (KA units) |
|--------|-------------------------|-----------------|-----------------|---------------------------------|
| 1      | Control                 | 35.56±6.12 (10) | 118.60±17.80(10) | 14.57±1.40(7)                  |
| 2      | Carbon Tetrachloride control | 1700.00±114.82 (10) | 2010.00±67.47(10) | 74.77±9.98(7)                  |
| 3      | A.muricata treated      | 765.00±91.86(7) | 1243.30±115.75(7) | 26.19±1.81(7)                  |
| 4      | P.cerasoides treated    | 1197.14±118.92 (7) | 1425.71±196.33(7) | 40.71±3.87(7)                  |

*P<0.001; P<0.01; P<0.02 when compared to carbon tetrachloride control. Figures in paranthesis indicate the number of observations.

**TABLE 2**

| Sl. No. | Group                  | Protein mg/g tissue | Lipid peroxidation MDA nmole/mg |
|---------|------------------------|---------------------|---------------------------------|
|         |                        | Liver | Brain | Liver | Brain | Liver | Brain |
| 1       | Control (7)            | 117.07±6.81 | 37.49±1.01 | 422.37±28.0 | 178.34±14.46 |
| 2       | Carbon tetrachloride Control (7) | 62.80±3.29 | 18.39±0.88 | 889.20±22.48 | 634.70±25.27 |
| 3       | A.muricata treated (7) | 101.70±4.02       | 28.20±1.71a    | 492.00±18.72a | 210.50±29.71a |
| 4       | P.cerasoides treated (7) | 98.20±3.97a       | 22.40±2.01    | 639.00±29.97a | 357.00±30.71a |

*P<0.001 when compared to carbon tetrachloride control.

**TABLE 3**

| Treatment Group | SGPT U/ml | SGOT U/ml | SALP KA units | Lipid peroxidation nmoles/mg tissue | Total Protein mg/g tissue |
|-----------------|-----------|-----------|---------------|-----------------------------------|--------------------------|
|                 |           |           |               | Liver | Brain | Liver | Brain | Liver | Brain |
| A.muricata treated | 56.20 | 40.54 | 80.70 | 85.09 | 92.30 | 71.68 | 33.71 |
| P.cerasoides treated (7) | 30.21 | 30.89 | 56.58 | 53.60 | 56.91 | 65.23 | 13.79 |

Value from drug treatment – Value from CCl4 treated

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\% \text{ Mean protection} = \frac{\text{Value from vehicle treated} – \text{Value from CCl4 treated}}{\text{Value from vehicle treated}} \times 100
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