Hepatoprotective activity of whole Plant Extract of Catharanthus Pusillus (Murr.) G.Don (Apocynaceae)

Yokeswari Nithya, P Ananthi, S Mohan, V.R

Abstract: CCl₄ intoxicated rats showed significant elevation in serum enzymes, bilirubin and lipid per oxidation of the liver tissues and reduction in serum total protein, superoxide dismutase, catalase, reduced glutathione and glutathione peroxidase activity. Treatment with ethanol extract of Catharanthus pusillus whole plant altered the above parameters to the levels of near normal. All the above results were comparable with the standard drug silymarin (100mg/kg) treated group. Thus the present study ascertains that the ethanol extract of Catharanthus pusillus whole plant possesses significant hepatoprotective activity.

Keywords: Catharanthus pusillus, CCl₄, silymarin, bilirubin

I. INTRODUCTION

Liver disease has become a global concern worldwide. Liver is often abused by environmental toxins, poor eating habits and alcohol, that damage and weaken the liver leading to important public health problems like hepatitis, cirrhosis and alcoholic liver diseases (Treadway, 1998). India, numerous medicinal plants and their formulations are used for treating liver disorders in traditional systems of medicine. Herbal drugs play an important role in treatment of various ailments including liver problems. Therefore, many folk remedies from plant sources are being tested for their potential hepatoprotective activity in experimental animal models (Ganga Rao et al., 2012).

Catharanthus pusillus of Apocynaceae family is known with various names in India and all over the world. It is widely used as various treatments of diseases and traditionally used as herbal medicine (Don, 1999). The roots, leaves and latex of these plants are used to treat skin and liver diseases, leprosy, dysentery, worms, ulcers, tumor and ear aches. The leaf powder of C.pusillus were mixed with coconut oil and used for treat the antidiarrheal activity and also used to kill the lice (Rajakaruna,2002). The main purpose of this study was to know about the hepatoprotective activity of whole plant extract of C.pusillus on CCl₄ induced liver toxicity in rats.

II. MATERIALS AND METHODS

Plant Material:
The whole plant of Catharanthus Pusillus were agglomerated from Pechiparai, Kanayakumari District, Tamil Nadu. With the help of local flora, voucher specimens were identified and preserved in the Ethnopharmacology unit, Research department of Botany, V.O.Chidambaram College, Thoothukudi, Tamil Nadu for further references.

Preparation of plant extract:
The whole plant was shade dried and then powdered to obtain a coarse powder, which was then subjected to extraction in a Soxhlet apparatus using ethanol. The extract was tested for the identification of various phytochemical constituents as per standard procedures (Brinda,1981&Lala, 1993,). The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract were used for study.

Experimental animal

Adult male wistar albino rats weighing about 180 – 240 g body weight were selected for this work. They were abide under standard environmental conditions at room temperature (25±2°C) in a well-ventilated animal house with constant 12 h of darkness and 12 h of light schedule. The rats were fed with standard pellet diet (Goldmohar brand, Hindustan Lever Ltd., Mumbai, India) and water ad libitum.

Acute Toxicity Studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class...
method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study (OECD 2002.).

III. EXPERIMENTAL PROTOCOL

Induction of hepatotoxicity

Carbon tetrachloride (CCl₄) 2.5 ml/kg body weight was dissolved in 7.5 ml of paraffin and administered intraperitoneally.

Grouping of animals

A total of 30 rats were taken and were divided into six groups of 5 rats each, of which, five groups contained CCl₄ hepatic toxicity induced rats and the remaining one group contained normal rats.

Group – I: Rats received normal saline (0.9%), by using an intragastric catheter tube (IGC)- Normal control

Group – II: Liver injured rats received 2.5 ml/kg body weight of normal saline (0.9%) for 14 days, by using IGC, -CCl₄ hepatic toxicity induced control.

Group – III: Liver injured rats received C. pusillus whole plant ethanol extract at the dose of 150 mg/kg body weight for 14 days, by using an IGC.

Group – IV: Liver injured rats received C. pusillus whole plant ethanol extract at the dose of 300 mg/kg body weight for 14 days, by using an IGC.

Group – V: Liver injured rats received C. pusillus whole plant ethanol extract at the dose of 500 mg/kg body weight for 14 days, by using an IGC.

Group – VI: Liver injured rats received silymarin orally at the dose of 100 mg/kg body weight for 14 days, by using an IGC.

All the treatments were given between 9.30 and 10.00 in the morning. After 24h of last treatment, the final body weight was noted and the animals were sacrificed by decapitation. Blood was collected from each group of rats. Serum from the blood was separated by centrifugation at 3000g for 10 min and stored at -20°C until used for various biochemical assays. Estimation of GGT, Protein, Serum Glutamate Oxalo Transaminase (SGOT or AST), Serum Glutamate Pyruvate Transaminase (SGPT or ALT) and Alkaline Phosphate (ALP) was done as per the standard procedures.

Estimation of Total, Conjugated and Unconjugated Bilirubin

Total bilirubin and conjugated bilirubin were determined as described by Balistrei and Shaw (1987). The concentration of unconjugated bilirubin was the difference between total and conjugated bilirubin concentrations.

Estimation of Serum MDA, SOD, CAT, GPx and GRD

Quantitative estimation of MDA formation was done by the method proposed by Okhawa et al. (1979) and it was done by determining the concentration of thio barbituric acid reactive substance (TBARS) in serum. Enzymatic antioxidants, superoxide dismutase (SOD) (Mishra,1972), Catalase (Aebi, 1974), non enzymatic antioxidant glutathione peroxidase (GPx) (Paglia and Valentine, 1967), glutathione reductase (GRD) (Goldberg and Spooner, 1983) and reduced glutathione (GSH) were also assayed in serum.

Biochemical Analysis

The animals were betrayed at the end of experimental period of 14 days by decapitation. Blood was collected, sera separated by centrifugation at 3000g for 10 minutes. Serum protein and serum albumins was determined quantitatively by colorimetric method using bromocresol green.( Lowry et al., 1951). The total protein minus the albumin gives the globulin. Serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), serum alkaline phosphatase (ALP), total, conjugated bilirubin, unconjugated bilirubin were determined as per the standard procedures (Shanmugasundaram et al.,2010 & Thangakrishnakumari et al., 2012) . Liver homogenates (10%W/V) were prepared in ice cold 10mM tris buffer (pH7.4). Quantitative estimation of MDA formation was done by determining the concentration of thiobarbituric acid reactive substances (TBARS) in 10% liver homogenates by the method of Pal et al (Pal et al.,2011). Antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRD) were also assayed in liver homogenates as per the standard procedures (Anitha et al., 2010 & Suky et al., 2011)

Statistical Analysis

The data were expressed as the mean ± S.E.M. The difference among the means has been analyzed by one-way ANOVA. p<0.05and p<0.01 were considered as statistical significance using SPSS Software.
IV. RESULTS

Body weight

The administration of CCl₄ caused a significant (p<0.01) decrease in the body weight of rats as compared with the control rats. The animals treated with whole plant extract of C.pusillus (150 mg/kg, 300 mg/kg and 500 mg/kg) also gained weight during the experimental period (Table 1).

Table 1: Effect of whole plant ethanol extract of C.pusillus on the body weight in the normal, liver damaged and drug treated rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Initial Body weight (Gm)</th>
<th>Final Body weight (Gm)</th>
<th>Mean weight Gain (Gm)</th>
<th>% of Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0.9% Saline</td>
<td>220.65±0.34</td>
<td>220.65±0.36</td>
<td>0.01</td>
<td>0.06</td>
</tr>
<tr>
<td>Group II</td>
<td>0.9% Saline</td>
<td>220.65±0.34</td>
<td>220.65±0.36</td>
<td>0.01</td>
<td>0.06</td>
</tr>
<tr>
<td>Group III</td>
<td>150 (mg/kg)</td>
<td>220.65±0.34</td>
<td>220.65±0.36</td>
<td>0.01</td>
<td>0.06</td>
</tr>
<tr>
<td>Group IV</td>
<td>300 (mg/kg)</td>
<td>220.65±0.34</td>
<td>220.65±0.36</td>
<td>0.01</td>
<td>0.06</td>
</tr>
<tr>
<td>Group V</td>
<td>500 (mg/kg)</td>
<td>220.65±0.34</td>
<td>220.65±0.36</td>
<td>0.01</td>
<td>0.06</td>
</tr>
<tr>
<td>Group VI</td>
<td>100 (mg/kg)</td>
<td>220.65±0.34</td>
<td>220.65±0.36</td>
<td>0.01</td>
<td>0.06</td>
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</tbody>
</table>

Values are mean ± SD of 5 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test. *P <0.05; **P <0.01 as compared with Normal Control to liver damaged control: aP <0.05; aaP <0.01, aaaP <0.001 as compared with liver damaged control to drug treated animal ns: not significant.

Biochemical parameters

The results of serum biochemical parameters are presented in Table 2. In the CCl₄ control group, significant (p<0.05; p<0.01) decrease in the levels of total protein, albumin and globulin was observed. But the group which received the drug of whole plant extract at the dose of 500 mg/kg body weight showed a significant (p<0.01) increase in the reduced levels of protein albumin and globulin. The elevation of SGOT, SGPT and ALP in CCl₄ intoxicated rat was significantly (p<0.01) high when compared to the normal. The elevated levels of SGOT, SGPT and ALP in groups IV and V rats (post treated with whole plant extract, 300 mg/kg and 500 mg/kg) were significantly (p<0.05; p<0.01) low as depicted in Table 2.

Table 2: Effect of whole plant ethanol extract of C.pusillus on the serum protein, albumin, globulin concentration and serum GOT, GPT and ALP enzyme activity in the normal, liver damaged and drug treated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Date</th>
<th>Total Bilirubin (mg/dl)</th>
<th>Conjugated (mg/dl)</th>
<th>Unconjugated (mg/dl)</th>
<th>GGTP (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0.9% saline</td>
<td>0.04±0.01</td>
<td>0.02±0.01</td>
<td>0.07±0.01</td>
<td>0.06±0.01</td>
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<tr>
<td>Group II</td>
<td>0.9% saline</td>
<td>0.04±0.01</td>
<td>0.02±0.01</td>
<td>0.07±0.01</td>
<td>0.06±0.01</td>
</tr>
<tr>
<td>Group III</td>
<td>150 mg/kg</td>
<td>0.04±0.01</td>
<td>0.02±0.01</td>
<td>0.07±0.01</td>
<td>0.06±0.01</td>
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<tr>
<td>Group IV</td>
<td>300 mg/kg</td>
<td>0.04±0.01</td>
<td>0.02±0.01</td>
<td>0.07±0.01</td>
<td>0.06±0.01</td>
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<tr>
<td>Group V</td>
<td>500 mg/kg</td>
<td>0.04±0.01</td>
<td>0.02±0.01</td>
<td>0.07±0.01</td>
<td>0.06±0.01</td>
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<tr>
<td>Group VI</td>
<td>100 mg/kg</td>
<td>0.04±0.01</td>
<td>0.02±0.01</td>
<td>0.07±0.01</td>
<td>0.06±0.01</td>
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Table 3: Effect of whole plant ethanol extract of C.pusillus on the serum total, conjugated, unconjugated bilirubin and GGTP levels in the normal control, liver injured and drug treated rats.
Hepatoprotective activity of whole plant extract of Catharanthus pusillus (Murr.) G.Don (Apocynaceae)

Values are mean ± SD of 5 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test. *P <0.05; **P <0.01 as compared with liver damaged control: a P<0.05; **damaged control to drug treated animal ns: not significant.

The effect of ethanol extract of whole plant of C. pusillus on lipid peroxidation (LPO), glutathione peroxide (GPx), glutathione reductive (GRD), superoxide dismutase (SOD), catalese (CAT) and reduced glutathione (GSH) activities are shown in Table 4. When compared to the normal control rats (Group I), the level of lipid peroxidation increased significantly (p<0.01) and the levels of glutathione peroxidase, superoxide dismutase, catalese and reduced glutathione decreased significantly (p<0.01) in CCl₄ intoxicated control rats (Group II). Treatment with the ethanol extract of whole plant of C. pusillus, at the dose of 500mg/kg decreased the elevated lipid peroxidation level significantly and restored the altered glutathione peroxidase, glutathione reductase, superoxide dismutase, catalese and reduced glutathione levels towards normal in a dose dependent manner. The results were well comparable with that of silymarin, the standard drug treated rats.

Table 4: Effect of whole plant ethanol extract of C. pusillus on serum LPO, GPX, GRD, SOD, CAT and GSH activity in the normal control, liver injured and drug treated rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>LPO (nmol MDA/mg protein)</th>
<th>GPX (mg/mg Protein)</th>
<th>SOD (mU/mg)</th>
<th>CAT (mU/mg)</th>
<th>GSH (mg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0.5% volume</td>
<td>2.56±0.073</td>
<td>4.11±0.130</td>
<td>0.43±0.16</td>
<td>0.28±0.09</td>
<td>4.16±0.31</td>
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<tr>
<td>Group II</td>
<td>0.5% volume</td>
<td>3.98±0.094**</td>
<td>1.84±0.011**</td>
<td>0.26±0.02**</td>
<td>0.19±0.16**</td>
<td>2.53±0.07**</td>
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<tr>
<td>Group III</td>
<td>100mg/kg</td>
<td>4.12±0.054**</td>
<td>2.88±0.148</td>
<td>0.30±0.18</td>
<td>0.35±0.02**</td>
<td>2.89±0.019</td>
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<tr>
<td>Group IV</td>
<td>100mg/kg</td>
<td>3.34±0.104**</td>
<td>1.98±0.062</td>
<td>0.19±0.11</td>
<td>0.22±0.013a</td>
<td>3.84±0.077a</td>
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<tr>
<td>Group V</td>
<td>100mg/kg</td>
<td>3.01±0.016a</td>
<td>4.96±0.072a</td>
<td>0.49±0.36</td>
<td>0.24±0.04a</td>
<td>4.96±0.04a</td>
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<tr>
<td>Group VI</td>
<td>100mg/kg</td>
<td>2.94±0.066a</td>
<td>3.98±0.132</td>
<td>0.41±0.39</td>
<td>0.25±0.05a</td>
<td>4.13±0.069</td>
</tr>
</tbody>
</table>

Values are mean ± SD of 5 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test. *P <0.05; **P <0.01 as compared with Normal Control to liver damaged control.

V. DISCUSSION

CCl₄ induced liver injuries are the best characterized system of xenobiotic – induced hepatotoxicity and commonly used models for the screening of antihapatotoxicity and or hepatoprotective activities of drug. (Clowson 1998; Lin et al 2002). Since the changes associated with liver damage induced by CCl₄ are similar to that of acute viral hepatitis. CCl₄ mediated hepatotoxicity was chosen as the experimental model. CCl₄ is accumulated in hepatic parenchyma cells and metabolically activated by cytochrome P₄₅₀ dependent monooxygenases to form a trichloromethyl radical (CCl₃). The CCl₃ radical alkylates cellular proteins and other macromolecules with simultaneous attack on polysaturated fatty acids, in the presence of oxygen, to produce lipid peroxides, leading to liver damage. Thus, antioxidant or free radical generation inhibition is important in production against CCl₄ induces liver lesions. Hepatotoxic compounds such as CCl₄ are known to cause marked elevation in serum enzymes and bilirubin levels. It causes marked reduction in total protein levels. Silymarin is used as standard hepatoprotective compound since it is reported to have a insulating effect on the plasma membrane of hepatocytes.

Normally, SGOT and SGPT are present in high concentration in liver mainly due to the hepatocyte necrosis or these enzymes are released from the cells and their levels in the blood increases. SGPT is a sensitive indicator of critical liver damage and upgrading of this enzyme in non hepatic diseases is unusual. SGPT is more selectively a liver parenchymal enzyme than SGOT (Shah et al; 2002). Assessment of liver function can be made by estimating the activities of serum SGPT, SGOT and ALP which are enzymes originally present in higher concentration in cytoplasm. When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage (Nkosi et al; 2005).

In the present study, the elevated level of these marker enzymes are observed in the group II CCl₄ treated rats. The elevations of these enzymes are due to the extensive liver damage induced by toxin. The reduced concentrations of SGPT, SGOT and ALP as a result of ethanol extract of C. pusillus whole plant administration was observed during the present study. The normalization of serum markers by ethanol extract of C. pusillus whole plant suggests that they are able to condition the hepatocytes so as to protect the membrane integrity against CCl₄ induced leakage of marker enzyme into the circulation. The above changes can be considered...
as an expression of the functional improvements of hepatocytes.

Alkaline phosphatase concentration is related to the functioning of hepatocytes, high level of alkaline phosphatase in the blood serum is related to the increased synthesis of its by cells lining bile canaliculi usually in response of cholestasis and increased biliary pressure (Graw et al., 1999). Increased level was obtained after CCl₄ administration and it was brought to normal level by C.pusillus whole plant extract treatment.

The liver failure results the drastic come down of total protein and albumin levels of serum because of the hepatotoxicity. The normalized values of total protein and albumin were seen in the group III, IV and V which was because of the rejuvenating or repairing of liver. Ethanol extract of C.pusillus whole plant may have the capability to reform the liver cells by increasing protein values of serum.

The most sensitive tests employed in the diagnosis of hepatic diseases is serum bilirubin. Excessive heme destruction and blockage of biliary tract leads to Hyperbilirubinemia. Due blockage of the biliary tract, there is a mass inhibition of the conjugation reaction and release of unconjugated bilirubin from damaged and dead hepatocytes (Wolf et al., 1997). Ethanol extract of C.pusillus whole plant decreased the level of bilirubin and increased the level of protein suggesting that it offered protection.

The activity of serum γ-glutamyl transferase is generally increased as a result of liver disease, since γ-glutamyl transferase is a hepatic microsomal enzyme. Serum γ-glutamyl transferase is the most useful enzyme in the diagnosis of liver disease. Change in γ-glutamyl transferase is parallel to those of amino transferase. The critical damage induced by CCl₄ elevated the γ-glutamyl transferase level but, the same attains the normal after treatment with the whole plant of C.pusillus due to its antioxidant potential.

Lipid peroxidation has been identified as the cause for the destructive process of liver injury due to CCl₄ administration. In the present study, increased MDA levels in liver indicated increased lipid peroxidation induced by CCl₄ (GroupII). This enhanced lipid peroxidation finally triggered hepatic tissue damage and failure of antioxidant defense mechanisms to prevent formation of extravagant free radicals. Treatment with ethanol extract of whole plant of C.pusillus significantly reversed these changes. Hence, it may be possible that the mechanism of hepatoprotection by ethanol extract of whole plant of C.pusillus is due to its antioxidant effect.

Pivotal components of the antioxidant defense system in the body are cellular antioxidant enzymes (SOD and glutathione), which are involved in the reduction of reactive oxygen species (ROS) and peroxides produced in the living organisms as well as in the detoxification of certain compounds of exogenous origin. Thus are cellular antioxidant enzymes playing a primary role in the maintenance of a balanced redox status (Michiels et al., 1994). Hence, they can serve as a potential marker of susceptibility, early and reversible tissue damage, and of decrease in antioxidant defense. (Packer et al., 1994). SOD plays an important role in the elimination of ROS and protects cells against the deleterious effects of super oxide anion derived from the peroxidative process in liver and kidney tissues (Fridovich et al., 1975). In the present study, it was observed that the ethanol extract of C.pusillus whole plant significantly increased the SOD activity in CCl₄ intoxicated rats there by diminished CCl₄ induced oxidative damage.

Glutathione peroxide (GPX) is a selenoenzyme and it protects the cells from damage due to free radicals like hydrogen and lipid peroxides (Zaltzber et al., 1999). It catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphides and reaction product of the hydroperoxides .Glutathione (GSH) extensively found in cells, protects cells from electrophilic attacks provided by xenobiotics such as free radicals and peroxides. GSH deficiency leads to cellular damage in kidney, muscle, lungs, colon, liver, lymphocytes and brain. (Orhan et al., 2007). In the present study, treatment with the whole plant extract of C.pusillus increased the activities of GPx and GSH in CCl₄ induced liver damaged rats.

VI. CONCLUSION

This study finally confirms C.pusillus having hepatoprotective effect on hepatic damage induced by CCl₄. Thus it can be finalized that mechanism of hepatoprotective activity of C.pusillus whole plant may be due to its free radical scavenging, antioxidant activity and synergistic effect of the phenolics and flavonoids present in the whole plant. The GC-MS analysis of the whole
Hepatoprotective activity of whole plant extract of Catharanthus pusillus (Murr.) G.Don (Apocynaceae)  

plant of *C. pusillus*, confirmed the presence of 9, 10-Secocholesta-5,7,10 (19)-triene-3,21,25-triol, (3p,5z,7e)-4-Chloro-17-hydroxy-(17β) Androst-4-en-3-one, α-Tocopherol and Campersterol. The presence of these compounds might be responsible for the hepatoprotective activity.

**ACKNOWLEDGEMENT**  
The authors are thankful to Dr.R.Sampathraj, Honorary Director, Samsun Clinical Research Laboratory, Thirupur for providing necessary facilities to carry out this work.

**REFERENCES**


