Hepatoprotective and antioxidant effect of *Polygala chinensis* L. whole plant against CCl$_4$ induced hepatotoxicity in rats

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Abstract

**Background and objectives:** Carbon tetrachloride (CCl$_4$) is one of the most commonly used hepatotoxins in the experimental study of liver diseases. Liver diseases are still a world wide health problem. Unfortunately, conventional and synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. There are numerous plants and polyherbal formulations claimed to have hepatoprotective activity. The ethanol extract of whole plant of *Polygala chinensis* protect the liver from CCl$_4$ hepatotoxins.

**Methods:** The CCl$_4$ intoxicated adult male albino rats were treated with the ethanol extract of whole plant of *Polygala chinensis* in doses of 100 and 200 mg/kg orally for 14 days. The rats were sacrificed at the end of the 14 days. Biochemical parameters and antioxidant activities were carried out in the serum of control, CCl$_4$ intoxicated and drug treated rats.

**Result:** CCl$_4$ intoxicated rats showed significant elevation in serum enzymes, bilirubin and lipid peroxidation of the liver tissues and reduction in serum total protein, superoxide dismutase, catalase, reduced glutathione and glutathione peroxidase activity. Treatment with ethanol extract of *Polygala chinensis* whole plant altered the above parameters to the levels of near normal. All the above results were comparable with the standard drug silymarin (100 mg/kg) treated group.

**Conclusion:** The present study ascertains that the ethanol extract of *Polygala chinensis* whole plant possesses significant hepatoprotective activity.

**Keywords:** *Polygala chinensis*, Hepatoprotective activity, Antioxidant, CCl$_4$, Bilirubin, Silymarin

1. Introduction

Liver is a versatile organ of the body that regulates internal chemical environment. Liver injury induced by various hepatotoxins has been recognized as a major toxicological problem for years. Because of its unique metabolic functions and relationship to the gastrointestinal tract, liver is an important target of toxicity to xenobiotics, oxidative stress, ethanol and toxic chemicals (antibiotics, chemotherapeutics, aflatoxins, carbon tetrachloride, chlorinated hydrocarbons, etc.). There are numerous plants and polyherbal formulations claimed to have hepatoprotective activity. Nearly 150 phytocoustituents from 101 plants have been claimed to possess liver protecting activity $^1$.

*Polygala* was traditionally used by Native Americans to treat snake bites$^2$ and as an expectorant to treat cough and
bronchitis. In traditional Chinese medicine, *Polygala* is used for a variety of purposes including the promotion to sleep and calming the spirit. *Polygala* considered as a powerful tonic herb that can help to develop the mind and aid in creative thinking. Biological activities such as antidiabetic, anti-inflammatory and antioxidant activities were reported. However, so far there is no systematic study on hepatoprotective activity has been reported in the literature. Hence, the aim of the present study was to investigate the hepatoprotective activity of *P. chinensis* whole plant extract on CCl₄ induced liver toxicity in rats.

2. Material and Methods

2.1. Plant material

The well grown whole plant of *Polygala chinensis* L. was collected from Vadavalli, Coimbatore, Tamil Nadu. The collected plants were identified by the Botanical Survey of India, Coimbatore. A voucher specimen (VOCB 1348) was retained in Ethnopharmacology unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin for further reference.

2.2. Preparation of plant extracts for phytochemical screening and hepatoprotective studies

The whole plant was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder, which was then subjected to extraction in a Soxhlet apparatus using ethanol. The extract was subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures. The ethanol extracts were concentrated in a rotary evaporator. The concentrated extract was weighed to calculate the yield of ethanol (10.20%). The concentrated ethanol extract were used for hepatoprotective studies.

2.3. Animals

Normal healthy male Wistar albino rats (180-240 g) were used for the present investigation. Animals were housed under standard environmental conditions at room temperature (25±2°C) and light and dark (12:12 h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water ad libitum. Study was carried out as per IAFC approval no:82/PARMA/SCRI, 2010.

2.4. 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. The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50, 100 and 2000 mg/kg body weight.

2.5. Experimental Design

In the investigation, a total of 25 rats (CCl₄ hepatic toxicity induced rats and 5 normal rats) were taken and divided into five groups of 5 rats each.

**Group I:** Rats received normal saline was served as a normal control.

**Group II:** CCl₄ hepatic toxicity induced control: Rats received 2.5ml/kg body weight of CCl₄ for 14 days.

**Group III:** Liver injured rats received ethanol extract of whole plant of *P. chinensis* at the dose of 100mg/kg body weight for 14 days.

**Group IV:** Liver injured rats received ethanol extract of whole plant of *P. chinensis* at the dose of 200mg/kg body weight for 14 days.

**Group V:** Liver injured rats received standard drug silymarin at the dose of 100mg/kg body weight for 14 days.

2.6. Biochemical Analysis

The animals were sacrificed at the end of experimental period of 14 days by decapitation. Blood was collected, sera separated by centrifugation at 3000 g for 10 minutes. Serum protein and serum albumins was determined quantitatively by colorimetric method using bromocresol green. 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 \(^{12,13}\). 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 \(^{14}\). 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 \(^{15,16}\).

2.7. Statistical Analysis

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

3. Results

The ethanol extract of whole plant of *Polygala chinensis* subjected for phytochemical study showed the presence of
alkaloids, coumarin, glycosides, flavonoids, saponins, steroids, phenols, tannins and xanthoproteins. The ethanol extract did
not show any sign and symptoms of toxicity and mortality upto 2000 mg/kg dose. The effect of ethanol extract of *P.
chinensis* on serum total protein, albumin, A/G ratio, serum transaminases and alkaline phosphatases in CCl\(_4\) intoxicated rats are summarized in Table 1. There was a significant \((p<0.01)\) increase in serum GOT, GPT and ALP levels
in CCl\(_4\) intoxicated group (Group II) compared to the normal control group (Group I). The total protein and albumin levels
were significantly \((p<0.01)\) decreased to 6.18 g/dl and 3.28 g/dl in CCl\(_4\) intoxicated rats from the levels of 7.98 g/dl and
4.88 g/dl respectively in normal group. Ethanol extract of *P. chinensis* whole plant at the dose of 100 and 200 mg/Kg orally
significantly decreased the elevated serum marker enzymes and reversed the altered total protein and albumin to almost
normal level.

**Table 1. Effect of whole plant extracts of *Polygala chinensis* on the protein, albumin, globulin concentration and
c enzyme activity of serum GOT, GPT, and ALP in the normal, liver damaged and drug treated rats.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>T.Protein (mg/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
<th>A/G Ratio</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>I</td>
<td>7.98±0.81</td>
<td>4.88±0.34</td>
<td>3.1±0.11</td>
<td>1.5:1</td>
<td>19.56±1.36</td>
<td>26.16±0.93</td>
<td>143.29±5.32</td>
</tr>
<tr>
<td>II</td>
<td>II</td>
<td>6.18±0.34*</td>
<td>3.28±0.11*</td>
<td>2.9±0.23</td>
<td>1.1:1</td>
<td>40.11±1.21</td>
<td>43.19±1.08</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>III</td>
<td>7.21±0.18**</td>
<td>4.46±0.16</td>
<td>2.75±0.12*</td>
<td>1.6:1</td>
<td>24.91±1.14</td>
<td>28.31±1.53</td>
<td>146.22±6.16</td>
</tr>
<tr>
<td>IV</td>
<td>IV</td>
<td>7.96±0.19**</td>
<td>4.37±0.14*</td>
<td>3.59±0.11*</td>
<td>1.2:1</td>
<td>20.27±1.08</td>
<td>30.44±1.09</td>
<td>151.93±5.18</td>
</tr>
<tr>
<td>V</td>
<td>V</td>
<td>7.48±0.11*</td>
<td>4.51±0.31*</td>
<td>2.97±0.16</td>
<td>1.5:1</td>
<td>21.33±1.19</td>
<td>27.06±1.33</td>
<td>146.55±6.94</td>
</tr>
</tbody>
</table>

Each Value is SEM± 5 individual observations *\(P<0.05\); **\(P<0.01\) Compared normal control vs liver injured rats \(P<0.05\); aa \(P<0.01\) Compared liver injured rats vs drug treated

The effect of ethanolic extract of *P. chinensis* on total, conjugated and unconjugated bilirubin is shown in Table 2.

**Table 2. Effect of whole plant extracts of *Polygala chinensis* on the serum Total, conjugated and unconjugated
bilirubin levels in the normal control, liver injured and drug treated rats.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Total Bilirubin (µmol/L)</th>
<th>Conjugated (µmol/L)</th>
<th>Unconjugated (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>I</td>
<td>0.68±0.03</td>
<td>0.24±0.01</td>
<td>0.44±0.02</td>
</tr>
<tr>
<td>II</td>
<td>II</td>
<td>3.69±0.43*</td>
<td>1.49±0.03*</td>
<td>2.20±0.06**</td>
</tr>
<tr>
<td>III</td>
<td>III</td>
<td>1.16±0.05*</td>
<td>0.12±0.01</td>
<td>1.04±0.06*</td>
</tr>
<tr>
<td>IV</td>
<td>IV</td>
<td>0.81±0.03**</td>
<td>0.22±0.03**</td>
<td>0.59±0.02**</td>
</tr>
<tr>
<td>V</td>
<td>V</td>
<td>0.88±0.01**</td>
<td>0.20±0.01*</td>
<td>0.68±0.3**</td>
</tr>
</tbody>
</table>

Each Value is SEM± 5 individual observations *\(P<0.05\); **\(P<0.01\) Compared normal control vs liver injured rats \(P<0.05\); aa \(P<0.01\) Compared liver injured rats vs drug treated
A significant elevation of total, conjugated and unconjugated bilirubin in the serum of CCl₄ intoxicated group (Group II) when compared to normal control (Group I). The ethanol extract of P. chinensis at the dose 100 and 200 mg/kg reduced the levels of total, conjugated and unconjugated bilirubin (Group III and Group IV). The decreases in the concentration of total bilirubin, conjugated bilirubin and unconjugated bilirubin were found to be greater in standard silymarin (Group V) followed by Group IV and Group III (Table 2).

The effects of ethanol extract of P. chinensis on lipid peroxidation (LPO), Glutathione peroxidase (GPx), Glutathione reductase (GRD), Superoxide dismutase (SOD) and Catalase (CAT) activity is shown in Table 3.

Lipid peroxidation level was significantly (p<0.01) increased and glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase activity were significantly (p< 0.01) decreased in CCl₄ intoxicated rats when compared with those of the animals in normal control group. Rats treated with ethanol extract of P. chinensis at the doses of 100 and 200 mg/kg significantly decreased the elevated lipid peroxidation levels and restored the altered glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase levels towards the normal levels in a dose dependent manner. The results are well comparable with silymarin (standard drug) treated group.

Table 3. Effect of whole plant extracts of Polygala chinensis on liver LPO, GPX, GRD, SOD and CAT in the normal control, liver injured and drug treated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LPO (n mole of MDA/mg protein)</td>
</tr>
<tr>
<td>I</td>
<td>0.82±0.03</td>
</tr>
<tr>
<td>II</td>
<td>3.16±0.54**</td>
</tr>
<tr>
<td>III</td>
<td>1.32±0.21*</td>
</tr>
<tr>
<td>IV</td>
<td>0.94±0.17**</td>
</tr>
<tr>
<td>V</td>
<td>0.89±0.11**</td>
</tr>
</tbody>
</table>

Each Value is SEM± 5 individual observations * P<0.05; ** P<0.01 Compared normal control vs liver injured rats a P<0.05; aaP<0.01 Compared liver injured rats vs drug treated

4. Discussion

It is well established that CCl₄ induces hepatotoxicity by metabolic activation; therefore it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. CCl₄ is bio-transformed by the cytochrome P₄₅₀ system in the endoplasmic reticulum to produce trichloromethyl free radical (CCl₃). Trichloromethyl free radical then combined with cellular lipids and proteins in the presence of oxygen to form a trichloromethylperoxyl radical, which may attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloromethyl free radicals lead to elicit lipid peroxidation, the destruction of Ca²⁺ homeostasis and finally, results in cell death. These results in changes of structures of the endoplasmic reticulum and other membrane, loss of enzyme, metabolic enzyme activation, reduction of protein synthesis and loss of glucose-6-phosphate activation, leading to liver damage. Hepatotoxic compounds like CCl₄ are known to cause marked elevation in serum enzyme activities. In the present study, treatment with P. chinensis whole plant extract attenuated the increase in the activities of SGOT, SGPT and ALP produced by CCl₄ indicating that Polygala chinensis whole plant extract protects liver injury induced by CCl₄ towards normalization. Silymarin, a prototype hepatoprotective agent also showed similar changes.

Bilirubin is the main bile pigment that is form the breakdown of heme in the red blood cells. It is transported to the liver where it is secreted by the liver into the bile. Conjugation of bilirubin is a prerequisite for its excretion into the bile. Malfunctioning of the liver was evidenced by the significant increase (p< 0.01) in the level of unconjugated bilirubin in the serum of the group treated with only CCl₄ when compared to normal control. Increase in the level of unconjugated bilirubin in the blood may result from a defect in the function of the liver to conjugate the bilirubin being produced. The significant reduction (p< 0.05) of unconjugated bilirubin level in the serum when CCl₄ was simultaneously administrated with the
ethanol extract of *P. chinensis* when compared with the administration of CCl₄ alone indicates that the conjugating function of the liver was improved. The reduction of the unconjugated bilirubin level by the ethanol extract suggest that the extracts may activate the constitutive androstane receptor (CAR) which is a key regulator in bilirubin clearance in the liver. The primary function of CAR is the bilirubin clearance pathway is to direct coordinate response to elevated levels of bilirubin by increasing the hepatic expressive of each component of the pathway.

The ability of simultaneous administration of CCl₄ with ethanol extract of *P. chinensis* to significantly reduce (p<0.01) the level of serum total bilirubin when compared with that of the CCl₄ treated group suggests the potential of the extract is clearing bilirubin from the serum when its level elevated. Since the results obtained for the serum total protein and albumin concentrations followed the same trend, it thus implicated the same mechanism by which the ethanol extract of *P. chinensis* exerts its effect on these parameters. The administration of CCl₄ alone may adversely interfere with protein metabolism probably by inhibiting the synthesis of proteins. Administration of ethanol extract of *P. chinensis* whole plant reversed these changes may be by increasing protein synthesis. This indicates the hepatoprotective activity of *P. chinensis* whole plant against damage by CCl₄. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism which accelerates regeneration of cells.

Intake of CCl₄ results in excessive generation of free radicals. Free radicals are the reactive oxygen species (ROS) which are known to cause oxidative damage to number of molecule in cell, including membrane- lipids, proteins and nucleic acids. In the present study the hepatic cellular injury might be due to increased oxidative stress and thereby leading to lipid peroxidation. The level of lipid peroxidation in the CCl₄ treated rats was assessed by measuring the levels of TBARS in the liver tissues.

The increased TBARS levels in the liver of CCl₄ treated animals indicate enhanced lipid peroxidation leading to tissue injury. The cellular antioxidant defense mechanism, which includes scavenging activities of enzymes viz., SOD, CAT and GPx plays an important role in scavenging toxic intermediates of reactive oxygen species. During hepatotoxicity these enzymes might be functionally impaired due to excess generation of free radicals creating oxidative imbalance.

SOD is metalloprotein catalyzing the dismutation of superoxide anion to hydrogen and oxygen. Numerous studies have shown the importance of SOD in protecting cells against oxidative stress. The SOD activity could be decreased in tissue during CCl₄ injection. This decrease could be due to the feedback inhibition or oxidative inactivation of enzyme protein due to excess ROS generation.

CAT, heme protein, catalyzes the reduction of hydrogen peroxides, acts as preventive antioxidant and plays an important role in protection against the deleterious effects of lipid peroxidation. The activity levels of catalase in tissue decreased in CCl₄ treated animals might be due to the inhibition of CAT activity, which is suggestive to enhanced synthesis of O₂⁻ is a powerful inhibitor of catalase.

GPx is an enzyme with selenium in the form of selenocysteine and can catalyze the reduction of hydrogen peroxide and hydroperoxidases to non toxic products: GPx has a well-established role in protecting cells against oxidative injury. GPx is non-specific for H₂O₂ and lipid peroxide generated during CCl₄ treatment which is efficiently scavenged by GPx activity. The depression of this enzyme activity reflects perturbations in normal oxidative mechanism during CCl₄ treatment.

The cellular antioxidant defense enzymes viz., SOD, CAT and GPx were significantly reduced in the CCl₄ treated rats. This might lead to decreased antioxidant defense and increased oxidative stress and thereby the tissue injury occurs. Similar studies also indicate the failure of cellular antioxidant defense system during hepatotoxicity was recorded.

In conclusion, the results of this study demonstrate that the ethanol extract of *Polygala chinensis* whole plant have a potent hepatoprotective action aqueous CCl₄ induced hepatic damage in rats. Its mode in affording the hepatoprotective activity against CCl₄ induced liver damage may be due to cell membrane stabilization, hepatic cells regeneration and enhancement of antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase production. The hepatoprotective and antioxidant potential of whole plant extract could have been brought about by various phytochemical principles i.e. flavonoids, alkaloids, phenolics and tannins present in *P. chinensis* whole plant. So results of this study demonstrated that the *P. chinensis* has significantly protection on CCl₄ induced hepatotoxicity.
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References


