Screening of Hepatoprotective Drugs against Carbon Tetrachloride Induced Hepatotoxicity by Non-invasive Method

V. Surendra*, Uday Raj Sharma* and Divakar Goli**
*Department of Pharmacology. ** Department of Pharmaceutical Biotechnology.
Acharya & B.M.Reddy College of Pharmacy, Soldevarahalli, Bangalore – 560 090

Abstract: The study was designed to evaluate the hepatoprotective activity of pre-treatment with Ethanolic extract of fruits of Gardenia jasminoides against CCl₄-induced hepatotoxicity in Wister rat model. Liver damage in Wister rats was induced by administering CCl₄ (0.7 ml/kg, ip) on alternative days for one week. Gardenia jasminoides (80, 200 and 400 mg/kg, po.) was given for one week. Silymarin (100 mg/kg, po.) was used as a reference drug. Silymarin and Ethanolic extract of Gardenia jasminoides (Shown to be hepatoprotective substances) prevented the CCl₄ induced reduction of ascorbic acid extraction in urine. The results indicate that the measurement of ascorbic acid excretion can be used as a non-invasive test for screening protective substances against CCl₄ induced hepatotoxicity in rats.

Key Words: Urine ascorbic acid, Gardenia jasminoides, Silymarin, Hepatoprotection.

Introduction

Numerous medicinal plants and their formulation are used for liver disorders in medical practice as well as traditional system of medicine in India. Herbs play a vital role in the management of various liver disorders. In the absence of a reliable liver protective drug in the modern medicine, a number of medicinal preparations in ayurveda are recommended for the treatment of liver disorders. The present work was taken up to evaluate the effects of ethanol extract of the fruit of Gardenia jasminoides against carbon tetrachloride induced hepatic damage in rats. The fruits of Gardenia jasminoides have been included in traditional medicine formulations for the treatment of hypertension, fever, edema (1). Several biochemical parameters such as levels of SGOT, SGPT, ALP and serum bilirubin are used to assess the hepatic function. These methods required invasive blood sampling procedure and are coupled with expensive analytical techniques.

A non-invasive method avoiding withdrawal of blood and employing a simple analytical procedure is very much preferred for regular screening to assess hepatoprotective activity of substances. The present work was done to find whether urinary excretion of ascorbic acid can be used to detect hepatotoxicity action of Silymarin and Gardenia jasminoides extract, used in ayurvedic formulations and shown beneficial against hepatotoxicity in CCl₄ treated rats (2, 3).

Materials and Methods

Collection of Plant Materials: The fruits of Gardenia jasminoides were collected in the month of October 2007 from China through distributors, and authenticated by Dr. D.Venugopal reddy, Botanist, V R Science College, Nellore, Andhra Pradesh. The voucher specimen of the plant material was deposited in the ABMRCP, Bangalore.

Preparation of Extract: The fruits were dried in shade at room temperature. The dried fruits were powdered by using mixture; coarse powder was packed into soxhlet column and extracted
with Petroleum ether (60-80°C) for 24 h. The same marc was successively extracted with Chloroform and Ethanol for 24 h. The extracts were concentrated using water bath. The yield of Petroleum ether extract, Chloroform extract, Ethanol extract were 30, 25, 55 gm/kg respectively. The dried extracts were stored in airtight container and placed in refrigerator. These extracts were subjected to preliminary Phytochemical testing for detection of major chemical groups (4, 5).

**Preliminary Phytochemical Screening:** The preliminary phytochemical investigation of the different extract of showed that: Petroleum ether contains fatty acids, Chloroform contains, Carbohydrates. Ethanol extract contains glycosides (mainly geniposide and gardenoside) carboxydrates, proteins, flavonoids, chlorogenic acid, ursolic acid, and iridoid glycosides (1).

**Animals:** Wister albino rats (150-200 gm), Mice (20-25 gm) of either sex were used for the hepatoprotective study. The animals were housed in polypropylene cages and maintained at 24°± 2 °C under 12 h light / dark cycle. They were supplied with standard diet and water ad libitum, one week before and during the experimental period. They were initially acclimatized for the study and the study protocol was approved by the Institutional Animal Ethical Committee as per the requirements of Committee for the Purpose of Control and Supervision on Animals (CPCSEA), New Delhi.

**Chemicals:** Carbon tetrachloride was procured from S.D. Fine Chemicals (India) Ltd. Mumbai; Silymarin was obtained as gift sample from Micro lab (India) Ltd. Bangalore.

**Pharmacological Experimentation:** Rats were divided into 5 groups of 6 animals each. Group I served as vehicle control and received normal saline (5 ml/kg). Group II was administered with Carbon tetrachloride and olive oil (1:1, v/v, 0.7 ml/kg, and ip on alternate days). Animals in Group III, IV and V received Ethanolic extract (80, 200 and 400 mg/kg, po respectively daily for 7 days) simultaneously with toxicant (Carbon tetrachloride and olive oil). Group VI was administered with reference standard drug, Silymarin (100 mg/kg, po) simultaneously with toxicant. The dose selection of *Gardenia jasminoides* is based on the privies references.

**Estimation of Ascorbic Acid in Urine:** Wister albino rats of either sex were divided into five groups each consisting of six animals. They were kept in metabolic cages for collection of urine. They were supplied with standard diet and water ad libitum. One week before and during the experimental period, urine sample were collected separately for each group in 5 ml of 10% oxalic acid solution and analyzed for ascorbic acid by the method described by Roe and Kuether and their average value were taken as normal controls(6). The rats of Group I were treated with 0.7 ml/kg, orally of CCl₄. Groups II, III and IV were treated with Ethanolic extract of *Gardenia jasminoides* at doses of 80, 200 and 400 mg/kg, orally, Group V served as Silymarin treated. After 1 h extract and Silymarin administration groups were challenged with CCl₄ (0.7 ml/kg, po). The collection of 24 h urine samples was continued for all groups and the sample were analyzed for ascorbic acid at 7th day.

**Statistical Analysis:** Mean values ± SEM were calculated for each parameter. For the determination of significant inter group differences, each parameter was analyzed separately and one way analysis of variance (ANOVA) was carried out. Individual comparisons of group means were done by multiple group comparisons (Turkey’s test). Percent protection in each parameter was calculated by the formula:

\[
\text{Percent protection} = \left( \frac{\text{Mean value of toxin-treated group} - \text{Mean value of toxin + Test substance treated group}}{\text{Mean value of toxin-treated group} - \text{Mean value of normal group}} \right) \times 100
\]

**Results**

The daily excretion of ascorbic acid by different groups of rats before and after treatment is summarized in table 1. a dose of 0.7 ml/kg Carbon tetrachloride produced significant reduction in ascorbic acid excretion. Ethanolic extract of *Gardenia jasminoides* increased Carbon tetrachloride induced reduction in ascorbic acid excretion. Higher dose of Ethanolic extract (400 mg/kg) and Silymarin prevented Carbon
Carbon tetrachloride induced reduction in ascorbic acid significantly \((P<0.001)\) as summarized in Table 1.

**Discussion**

Carbon tetrachloride is a pharmacological tool used to produce liver damage in animal models; its hepatotoxicity action begins with changes in endoplasmic reticulum which results in loss of metabolic enzymes located in the intracellular structure \((7-9)\). Ascorbic acid is formed as a metabolite of glucose and galactose in rat liver microsomes *via* the glucoronic acid pathway and is excreted in urine. The enzyme UDP glucose dehydrogenase and UDP glucuronide transferase are responsible for its formation in the liver microsomes. Its formation and excretion is altered by several drugs and substances that affect the drug metabolizing enzyme systems \((10-12)\). Our results showing reduction in ascorbic acid excretion in \(\text{CCl}_4\) treated rats may reflect the inhibition of such enzymes. An earlier report that orally administered \(\text{CCl}_4\) increased hepatic ascorbic acid level during 30-180 minutes and later reduced to minimum at 12 hours lends support for our observation \((13)\). Alteration in urinary ascorbic acid excretion appears to be reflecting ascorbic acid levels in liver. Hence, the reduction in urinary ascorbic acid excretion can be used as an index for \(\text{CCl}_4\) produced hepatotoxicity. Prior administration of Silymarin is reported to reverse \(\text{CCl}_4\) induced prolongation of hexobarbital sleeping time in rats, by protecting hepatic metabolizing enzymes from the effect of \(\text{CCl}_4\). In our studies also it antagonized the \(\text{CCl}_4\) induced reduction of ascorbic acid excretion in rats. So it might antagonise \(\text{CCl}_4\) produced inhibition of enzymes responsible for ascorbic acid formation, the alcoholic extract of *Gardenia jasminoides* also antagonized \(\text{CCl}_4\) effect on urinary ascorbic acid excretion similar to Silymarin. Silymarin is a mixture of silybin, sildianin and silychristin which are flavanolignans. Geniposide, gardenoside, flavonoids, chlorogenic acid, ursolic acid, and iridoid glycosides are isolated from fruits of *Gardenia jasminoides*. The results of the study indicate that reduction in urinary ascorbic acid excretion can be used as an index for \(\text{CCl}_4\) induced hepatotoxicity and protection from such effect can be used as a guide for hepatoprotective activity of substances. The methods available earlier require withdrawal of blood samples from tail vein or heart puncture in rats. The method developed is simple and involves only collection of 24 hour samples of excreted urine and estimation of ascorbic acid by a simple colorimetric method. It is more convenient to be followed as a regular screening test for hepatotoxicity or hepatoprotective of substances. However, studies are to be conducted on more number of animals and also with other hepatotoxicity and hepatoprotective substances with simultaneous estimation of blood parameters and urinary ascorbic acid to clearly establish the benefit of this method \((14-20)\).

**Table 1**

Effects of Ethanolic Extract of Fruits of *Gardenia jasminoides* on Ascorbic Acid in \(\text{CCl}_4\)-induced Hepatotoxicity in Rat

<table>
<thead>
<tr>
<th>Treatment and dose</th>
<th>Mean (µg/ml) ± SEM</th>
<th>Prior to treatment</th>
<th>During treatment (on 7th day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group ((5 \text{ ml/kg, po}))</td>
<td>125.75 ± 3.30</td>
<td>120.15 ± 6.16</td>
<td></td>
</tr>
<tr>
<td>(\text{CCl}_4) control ((0.7 \text{ ml/kg, sc}))</td>
<td>122.22 ± 4.23</td>
<td>60.24 ± 5.24</td>
<td></td>
</tr>
<tr>
<td>Extract + (\text{CCl}_4) ((80 \text{ mg/kg, po}))</td>
<td>128.22 ± 4.30</td>
<td>86.25 ± 6.06 (^*)</td>
<td>43.41%</td>
</tr>
<tr>
<td>Extract + (\text{CCl}_4) ((200 \text{ mg/kg, po}))</td>
<td>123.30 ± 3.30</td>
<td>88.50 ± 4.86 (^{**})</td>
<td>47.17%</td>
</tr>
<tr>
<td>Extract + (\text{CCl}_4) ((400 \text{ mg/kg, po}))</td>
<td>120.00 ± 3.45</td>
<td>100.20 ± 5.42 (^{***})</td>
<td>66.70%</td>
</tr>
<tr>
<td>Silymarin + (\text{CCl}_4) ((100 \text{ mg/kg, po}))</td>
<td>130.22 ± 1.22</td>
<td>118.40 ± 4.22 (^{***})</td>
<td>97.07%</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM, \((n = 6 \text{ in each group})\). Figures in parenthesis are percent protection as compared to \(\text{CCl}_4\) control group was compared with normal group and all values were significantly different \((P< 0.01)\). Experimental groups were compared with \(\text{CCl}_4\) control: \(^*P<0.05\).

**References**


