HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF ALTHAEA OFFICINALIS LINN AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY ON ALBINO WISTAR RATS

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Article Received on: 10/10/11 Revised on: 21/11/11 Approved for publication: 16/12/11

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ABSTRACT
In Indian traditional system of medicine, herbal remedies are prescribed for the treatment of various diseases including liver diseases. The present study was aimed to investigate the hepatoprotective activity of the ethanolic extract of Althaea officinalis against Carbon tetrachloride induced hepatotoxicity in rats. Liver function were assessed by the determination of SGPT and SGOT studies. The serum biochemical analysis results suggested that the use of ethanolic extract of Althaea officinalis exhibited significant protective effect from hepatic damage in CCl₄ induced hepatotoxicity model.

Key Words: Althaea officinalis, Carbontetra chloride, hepatotoxicity.

INTRODUCTION
Liver is the important vital organ, regulating various physiological processes such as, metabolism, secretion, storage and detoxification of toxic substances. Therefore, damage to the liver inflicted by hepatotoxic agents is of great concern. The hepatotoxicity mainly caused by toxic chemicals such as CCl₄ alcohol, drugs such as paracetamol and antidiabetic drugs. The use of plant extracts for the treatment for liver diseases are now on the increase. Plants that were once considered of no value are now being investigated, evaluated and developed in to drugs with no side effects. One of such plants is Althaea officinalis commonly known as Gulkhairo belongs to the family Malvaceae and is widely distributed throughout the world as a useful medicinal plant. Althaea officinalis is found in the Himalayan region from Kashmir to Punjab and cultivated in Kullu valley in Himachal Pradesh. The roots counteract excess of stomach acid, and are prescribed to treat peptic ulcer, gastritis and intestinal problems including ileitis, colitis, diverticulitis and irritable bowel syndrome. An ointment prepared from the roots is applied to cure inflammatory tumors, burns, boils and abscesses. A root decoction is utilized as mouth wash to relieve inflammation, bruises, sprain, as an expectorant in cough hoarseness of voice, bronchitis and whooping cough. The present study was undertaken to investigate the effects of extract on liver cells of albino wistar rats.

MATERIALS AND METHOD

Plant material
The dried roots of Althaea officinalis were purchased from Khari Baoli market of Delhi. The authenticity of the material was established by Prof. M.P. Sharma, the Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi. A voucher specimen No-PRL/ JH/08/38 in deposited in the herbarium of the Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi.

Preparation of extract
The dried plant material (250 g) was crushed and extracted exhaustively with alcohol in a Soxhlet apparatus. The solvent was removed under reduced pressure. The yield of the extract was 5.6% in terms of starting material. The dried alcoholic extract was fractionated by heating successively with petroleum ether and water soluble and water insoluble and the solvents were removed as to give "PET (88 g)"  "WS (50 g)" and "WIS (13 g)", respectively. Healthy albino rats of either sex (180-200 g) were divided into five groups of six animals each. The same quantity of food in the form of pellets was given to all animals. The plant extracts were administrated in the form of suspension.

Experimental Animals
Albino rats of wistar strain 180-200 g used in the experiments were obtained from the Central Animal House, Jamia Hamdard. The animals were kept under standard laboratory condition 28 ±1 °C. The animals were fed with standard diet.

Administration of hepatotoxins
Carbon tetrachloride was mixed with liquid paraffin in a ratio of 1:1 and 1.5 ml/kg mixture was given orally to each rat. The effect of the toxicant was ascertained by measuring biochemical parameters.

Administration of drug fractions
The drug treatment was followed for 7 days after CCl₄ paraffin administration. The treatment was given orally in the form of suspension (1% Tween). On the last day, two rats from each group were sacrificed and serum from 5 animals was taken for biochemical evaluation. The blood was taken out from the retro-orbital sinus, allowed to coagulate at 37°C for 30 min and serum was separated by centrifugation at 3500, rpm for 10 min.

Experimental Design
Animals were divided into five groups, consisting of six animals in each group. Group I served as vehicle control which received 5% Tween 80 (10ml/kg) body weight, Group II received CCl₄ (1ml/ kg), Group III received CCl₄ (1ml/ kg) and PET(100 mg/kg) body weight, Group IV received CCl₄ (1ml/ kg) and WS (100 mg/kg) body weight, Group V received CCl₄ (1ml/ kg) and WIS (100 mg/kg) body weight, simultaneously for 7 days.

Estimation of serum SGOT, SGPT
On last day the blood samples were collected by cardiac puncture and serum was separated and used for the determination of biochemical parameters of SGPT and SGOT. Colorimetric method was followed to determine the Serum glutamate pyruvate transminase (SGPT) level by using the method described by Reitman et al, (1957) King and king(1954) method was employed(ALP).

Statistical Analysis
The data of biochemical estimation was reported as ± S.E.where n=5. For determining the statistical significance one way analysis of variance (ANOVA) and Dunnett’s test were used. P- values of less than 0.05 were considered significance.

RESULT AND DISCUSSION
Increased level of serum SGOT and SGPT were observed in disease induced control groups, but in different dose levels of test drug treated groups, significant (P< 0.001) reduction SGOT, SGPT was observed.
Carbon tetrachloride (CCl₄) is a potent hepatotoxin and a single exposure to it can rapidly lead to an increase in the Level of several enzymes, centrilobular necrosis and steatosis. The hepatotoxicity induced by CCl₄ is due to its metabolite CCl₃⁺, a free radical which bind to lipoprotein and leads to endoplasmic reticulum. The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms, which have been distributed by a hepatotoxin, is in index of its protective effects. The lowering of enzymes level is a definite indication of the hepatoprotective action of the enzymes due to altered membrane permeability. Although the doses of Althaea officinalis offer hepatoprotection, the dose of PET (100mg/kg b.w) is more effective (Table 2) depending on the type of cell and the membrane involved, lipoperoxidation due to CCl₄ result in hemolysis. The Althaea officinalis may be used in the acute condition of jaundice. Simultaneously treatment of ethanolic extract with CCl₄ produces lesser degree of damage to the animas treated with CCl₄ alone. The section of the liver treated with ethanolic extract (100mg/kg b.w) and CCl₄ level better showed hepatoprotective activity almost similar to the control group. The transport function of the hepatocytes is distributed in hepatic injury, causing the leakage. Their oxidative decomposition of the lipid is initiated and organic peroxides are formed after reacting with O₂ (lipid peroxidation). The reaction is autocatalytic in that new radicals are formed from the peroxide radicals themselves. CCl₄ induced liver cell injury is both severe and extremely rapid in onset, within less than 30 min, there is a decline in hepatic protein synthesis; within 2 hrs, there is swelling of smooth endoplasmic reticulum and dissociation of ribosomes from the rough endoplasmic reticulum. Lipid export from the hepatocytes is reduced, resulting fatty liver of CCl₄ poisoning. Mitochondrial injury then occurs and this is followed by progressive swelling of the cells due to increased permeability of the plasma membrane. Finally cell death following massive influx of calcium ions. The Althaea officinalis may be used in the acute condition of jaundice. Simultaneously treatment of ethanolic extract with CCl₄ produces lesser degree of damage to the animas treated with CCl₄ alone. The section of the liver treated with ethanolic extract (100mg/kg b.w) and CCl₄ reveal better hepatoprotective activity almost similar to the control group. The petroleum ether and water soluble fractions and water insoluble fraction of the alcoholic extract of the A. officinalis roots showed decrease in SGOT and SGPT levels in carbon tetrachloride induced hepatotoxicity in rat models. There was slight variation of SGOT levels when the rats were treated with these fractions. However, the level of SGPT decreased more effectively with the petroleum ether fraction than aqueous fractions. The total alcoholic extract decreased SGPT and SGOT levels significantly on the rat models.

REFERENCES

Table 1: Effect of the alcoholic extract of Althaea officinalis roots on SGOT and SGPT in rat model

<table>
<thead>
<tr>
<th>Group</th>
<th>SGOT</th>
<th>% changes</th>
<th>% inhibition</th>
<th>SGPT</th>
<th>% changes</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>65±6.2</td>
<td>0%</td>
<td>-</td>
<td>32±4.0</td>
<td>0%</td>
<td>-</td>
</tr>
<tr>
<td>Toxicant(CCl₄), (1 ml/kg)</td>
<td>89±2.0</td>
<td>100%</td>
<td>-</td>
<td>69±2.0</td>
<td>100%</td>
<td>-</td>
</tr>
<tr>
<td>Toxicant(CCl₄)+ (alcoholic extract)(1ml/kg+100 mg/kg)</td>
<td>68±2.0</td>
<td>23.5%</td>
<td>76.5%</td>
<td>42±2.0</td>
<td>39.1%</td>
<td>60.9%</td>
</tr>
</tbody>
</table>

Table-2. Effect of the various fractions of Althaea officinalis roots on SGOT and SGPT in rat model

<table>
<thead>
<tr>
<th>Group</th>
<th>SGOT</th>
<th>% changes</th>
<th>% inhibition</th>
<th>SGPT</th>
<th>% changes</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.6±1.0</td>
<td>-</td>
<td>-</td>
<td>0.3±0.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Toxicant (CCl₄), (1ml/kg)</td>
<td>0.9±0.08</td>
<td>100%</td>
<td>-</td>
<td>0.8±2.04</td>
<td>100%</td>
<td>-</td>
</tr>
<tr>
<td>PET+Toxicant (CCl₄), 100+1 ml/kg</td>
<td>0.77±0.1</td>
<td>11.7%</td>
<td>88.3%</td>
<td>0.5±2.03</td>
<td>35.6%</td>
<td>64.4%</td>
</tr>
<tr>
<td>WS+Toxicant (CCl₄), 100+1ml/kg</td>
<td>0.71±0.08</td>
<td>17.1%</td>
<td>82.9%</td>
<td>0.71±0.08</td>
<td>9.02%</td>
<td>90.98%</td>
</tr>
<tr>
<td>WIS+Toxicant (CCl₄), 100+1 ml/kg</td>
<td>0.70±0.02</td>
<td>18%</td>
<td>82%</td>
<td>0.7±0.02</td>
<td>9.84%</td>
<td>90.16%</td>
</tr>
</tbody>
</table>

Fig. 1 Effect of the alcoholic extract of Althaea officinalis roots on SGPT in rat model
Fig. 2 Effect of the alcoholic extract of *Althaea officinalis* roots on SGOT in rat model

Fig. 3 Effect of petroleum ether soluble, water soluble, and water insoluble of ALC extract of *Althaea officinalis* on SGOT in carbon tetrachloride induced hepatotoxicity in rat model

Fig. 4 Effect of petroleum ether soluble, water soluble, and water SGPT in carbon tetrachloride induced hepatotoxicity in rat model

Source of support: Nil, Conflict of interest: None Declared