ABSTRACT

Objective: To study the potential Hepatoprotective effect of methanolic extract of Quassia indica leaves in Carbon tetrachloride induced Liver injury.

Method: Wistar albino rats were divided into 7 groups. In two group one with vehicle CMC (p.o) control and other with ccl4 (i.p). The remaining four group treated with methanolic extract of Quassia indica for fourteen days (p.o) and compared with the serum biochemical parameters and histopathological evaluation with the standard silymarin treated group.

Results: Methanolic leaf extract of Quassia indica restored the increased level of serum biochemical markers and showed hepatic globular architecture normalized in histopathological evaluation.

Conclusion: Leaf extract shows good hepatoprotective activity in CCl4 induced liver damage in Wistar albino rats.
INTRODUCTION

Herbs play a protective role in the management of various liver disorders. Number of commercial preparations are available all over the world, which were claimed for liver protection. In India about 33 patent herbal formulations are available for liver ailments and these preparations represents a variety of combination out of 100 Indian medicinal plants belonging to about 40 families\(^{1,2,3}\). Some of the polyherbal formulations like Liv52, Livol, Hopotomed, Jigrine, Tefroli, Stimuliv, Icterine and Hepacure available in Indian market for treatment of various liver diseases. \textit{Quassia indica} is one such bitter plant which is abundantly grown and used as important medicinal plant. However much of its medicinal importance is not assessed. A moderate size tree grows up to 20 meters in height and which is smooth in texture. Bark is pale, wood is light and soft. Leaves are large, oblong-lanceolate, acuminate entire. Flowers numerous, pinkish yellow in long dropping axillary umbels. Fruits large flat pear shaped and compressed. By literature survey it is found that the leaves of the plant contain main phytochemical constituents such as quassinoids, samaderin b, samaderin c\(^{6,7}\). It also contains phenols, flavonoids, alkaloid, carbohydrates and tannins. This plant contains flavonoids hence we have planned to study its hepatoprotective property\(^{8,9,10}\). Earlier studies shows that plant of same family (Simarubiaceae) \textit{Ailanthus excelsa} leaves shows hepatoprotective activity, due to the presence of flavonoids\(^{5,4}\).

\begin{center}
\textit{Quassia indica} leaves
\end{center}

MATERIALS AND METHOD

Collection And Extraction

The fresh leaves of \textit{Quassia indica} was collected from the locally growing area of Kottayam district, Kerala in February 2012. The plant was identified and authenticated by Dr. K.V.George, Department of Botany, C.M.S College Kottayam, Kerala. A herbarium
specimen is deposited in our college museum UCP/MGU/RIMSR/herb9. The leaves were shade dried at room temperature. The dried samples were finely powdered, 100 gm were soaked in 500ml of methanol for 3-5 days with intermittent shaking. At the end of the extraction, it was passed through Whatman filter paper. This filtrate was concentrated under reduced pressure on rotary evaporator. The yield was 32.4 % w/w and subjected to phytochemical screening to identify the various phytoconstituents.

**Experimental animals**

Female *Wistar albino* rats of weighing 150-200gms and female albino mice weighing between 20-25gms were obtained from the animal house of UCP, Regional institute of medical sciences and housed in polycarbonate cages. The animals were housed under standard conditions. Approved at the Institutional Animal Ethics Committee (IAEC) of CPCSEA NO –1702/PO/C/12/CPCSEA of UCP DPS RIMSR was taken for conducting hepatoprotective activities. After procurement, all the animals were divided into different groups and were left for one week for acclimatization.

**Acute toxicity study**

This was performed for the extracts to ascertain safe dose by the acute oral toxic class method by the Organization of Economic Cooperation and Development (OECD) 423 guidelines. Swiss Albino Mice weighing between 20 and 25 g and between age eight and twelve weeks were procured for the experimental trial. Up to 2000mg/kg is given and doses were selected, 2000 mg/kg is cut off dose.

**Hepato-protective activity in CCl₄ induced hepatotoxicity**

The Wistar albino rats were divided into 7 groups, each group had six animals.

**Procedure**

Group I, the normal control group animals were administered p.o. single daily dose of carboxy methyl cellulose (1%w/v) on all 14 days. Group II, the CCl₄ control group animals were administered carbon tetrachloride (1 ml /kg body weight, i.p. 1:1 v/v mixture of CCl₄ and liquid paraffin) was given for every 72 hr for 14 days and were administered single daily dose of CMC (1%w/v) for every days. Group III, the standard group animals were administered Silymarin at a dose of 100 mg/kg p.o. on all 14 days and a single dose of CCl₄ (1 ml/kg) i.p., was given for every 72 hr for 14 days after Silymarin administration. Group IV animals were administered methanolic extract MEQI (200 mg/kg) p.o on all 14 days and a
single dose of CCl₄ (1 ml/kg) i.p. was given for every 72 hr for 14 days, 30 min after MEQI administration. Group V animals were administered methanolic extract MEQI (400 mg/kg) p.o, on all 14 days, and a single dose of CCl₄ (1 ml/kg) i.p. was given for every 72 hr for 14 days after 30 min after MEQI administration. Group VI animals were administered methanolic extract MEQI (800 mg/kg) p.o, on all 14 days, and a single dose of CCl₄ (1ml/kg) i.p. was given for every 72 hr for 14 days after 30 min after MEQI administration. Group VII animals were administered methanolic extract MEQI (1600 mg/kg) p.o. on all 14 days, and a single dose of CCl₄ (1 ml/kg) i.p. was given for every 72 hr for 14 days after 30 min after MEQI administration. Animals were sacrificed 48 hrs after the last dose of the drug. The liver samples were dissected and blood was collected. Blood samples were collected for evaluating the serum biochemical parameters and liver was dissected out, blotted off blood, washed with saline and stored in 10% formalin and preceded for histopathology to evaluate the details of hepatic architecture in each group microscopically\textsuperscript{(11,12)}.

**Statistical analysis**

Results were expressed as mean ± SEM, (n=6). Statistical analysis were performed with one way analysis of variance (ANOVA) followed by Dunnett’s ‘t’test. P value less than <0.05 was considered to be statistically significant. *P<0.05, **<0.01 and ***<0.001, when compared with control and toxicant group as applicable.

**RESULT AND DISCUSSION**

The preliminary phytochemical analysis of the leaf extracts revealed the presence of alkaloids, tannins and phenolic compounds, triterpenes, carbohydrate, steroids, proteins and flavanoids in methanolic extract.

Acute toxicity studies for methanolic extracts of *Quassia indica* belonging to the family Simarubiaceae were conducted as per OECD guidelines 423 using albino Swiss mice. The extracts were found to be safe up to 2000 mg/kg body weight since no death was observed and 2000mg/kg was cut off dose and 200 mg/kg,400 mg/kg,800mg/kg and 1600mg/kg of this dose were selected for and hepatoprotective activities.
Table 1: Hepatoprotective effects of *Quassia indica* methanolic extract on serum biochemical parameters in CCl₄ intoxicated rats.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>BIOCHEMICAL PARAMETERS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SGOT</td>
</tr>
<tr>
<td>CONTROL</td>
<td>74.0±3.3 4</td>
</tr>
<tr>
<td>CCl₄</td>
<td>331.0±11.53</td>
</tr>
<tr>
<td>SILYMARIN</td>
<td>115.0±7.590***</td>
</tr>
<tr>
<td>MEQ1200±CCl₄</td>
<td>254.9±12.438***</td>
</tr>
<tr>
<td>MEQ1400±CCl₄</td>
<td>163.35±9.829***</td>
</tr>
<tr>
<td>MEQ1800±CCl₄</td>
<td>128.47±1.849***</td>
</tr>
<tr>
<td>MEQ11600±CCl₄</td>
<td>114.48±9.213***</td>
</tr>
</tbody>
</table>

The methanolic leaf extract of *Quassia indica* showed good hepatoprotective effect when administered 200,400,800,1600 mg/kg orally and effect was dose dependent. Treatment with methanolic extract has decreased the elevated levels of biochemical markers like SGPT, SGOT, ALP, total bilirubin. Rats were treated with CCl₄ (Group II) showed a significant increase in serum SGOT, SGPT, ALP and total bilirubin levels compared to control animals (Group I). The increase in the SGOT, SGPT, ALP, total bilirubin levels in CCl₄ treated group were restored to 1600mg/kg methanolic extract of *Quassia indica* leaves which is near to the effect of 100mg/kg silymarin. The higher dose 1600mg/kg and the silymarin 100mg/kg produced a significant reduction in serum biochemical markers (p<0.001), lower doses produce effect but it is less effective.

**Figure (1-7):** Histopathological studies of the liver in CCl₄ induced hepatotoxicity.

*Fig 1: Architecture of normal liver*  
*Fig 2: CCl₄ treated liver*
Section of liver treated with vehicle control group shows liver parenchyma with intact architecture which is the normal appearance. Histopathological profile of liver from CCl4 (positive control group) intoxicated rats reveals hepatic globular architecture disrupted, hepatic cells has shown various degree of fatty degeneration, infiltration of lymphocytes and proliferation of kupffer cells. Silymarin treated group shows liver parenchyma with intact architecture. Some of the central veins show congestion with diffuse congestion of sinusoids. Section of liver in test drug treated groups (800 & 1600mg/kg) shows intact architecture, few regenerative hepatocytes, and scattered mononuclear inflammatory cells which is similar to silymarin treated group.
CONCLUSION
Carbon tetrachloride is a hepatotoxin, the effect which is due to the generation of free radicals. Free radicals involved in the process of lipid peroxidation are considered to play a cardinal role in numerous chronic pathologies, such as cancer and cardiovascular diseases, hepatic disorders among others, and are implicated in the ageing process. Histopathological observations shows that hepatic globular architecture was normalized; fewer lymphatic infiltrations were seen, kupffer cells proliferation appears to be normal, and from the observations of the serum biochemical parameters suggested that the plant extract pocesess hepatoprotective activity against CCl4 challenge.
We can assume that methanolic extract of leaves of Quassia indica possess hepatoprotective activity may be due to the presence of flavonoids and antioxidant principles. Future studies are focusing on the isolation of active constituent responsible for this activity formulate and compare with the commercially available preparations.
REFERENCES

