Protective Responce of Methnolic Extract of *Garcinia Indica* Fruits on CCl₄ Induced Liver Damage

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**ABSTRACT**

*Garcinia indica* commonly known as “kokum” is widely used in different parts of India for the treatment of obesity. The present study was to evaluate the protective response of methanolic extract of *Garcinia indica* fruits on CCl₄ induced liver damage. Chronic liver diseases commonly result in liver fibrosis. Carbon tetra chloride (CCl₄) is widely used for experimental induction of liver fibrosis. It is a potent hepatotoxin producing centrilobular necrosis which causes liver injury. Five groups each of 6 rats were used. First group (normal control) was given 1 ml of liquid paraffin /kg b.wt. daily for 60 days and kept as normal control. Rats of the second group were given the same dose of liquid paraffin, in addition CCl₄ (30% in liquid paraffin) was given in a single oral dose of 1 ml /kg b.wt, for every 72 hours. Rats of the third, fourth and fifth groups were pre-treated orally with 200, 400 mg/kg of methanolic extract of *Garcinia indica* suspended in 1% CMC and 25 mg/kg b.wt of silymarin Serum was separated and used for various biochemical estimations. Liver and kidney were collected in ice-cold containers, washed with saline, homogenized with appropriate buffer and used for the estimation of protein, liver enzymes (SGPT & SGOT), ALP, hepatic TBARS, Glycogen content, catalase activity, liver Na⁺-K⁺ ATPase activity and glutathione. Results of this study revealed that *Garcinia indica* could afford a significant protection in the alleviation of CCl₄ induced hepatocellular injury.

**Key words:** *Garcinia indica*, hepatoprotective activity, silymarin and ccl₄

**INTRODUCTION**

Liver is the most vital organ concerned with the biochemical activities in human body. The main role is to detoxicate the toxic substances.[1] Liver diseases remain one of the serious health problems. The rate of hepatotoxicity has been reported to be much higher in developing countries like India (8 - 30%) compare to that of developed countries (2 – 3 %) with a similar dose schedule.[2] Medicinal plants are backbone of Indian traditional system of medicine. However only a small portion of hepatoprotective plants as well as formulations used in traditional of medicine of pharmacologically evaluated for their safety and efficacy.[3] In India, about 40 polyherbal commercial formulations are reputed to have hepato protective action it has been reported that 160 phytoconstituents from 101 plants have hepatoprotective activity. Herbal drugs are prescribed widely even when their biologically active components are unknown because of their effectiveness, fewer side effects relatively low cost.[4]

*Garcinia indica*, also known as kokum, is a plant native to tropical Asian, African and polynesian countries.[5] kokum is an underexploited fruit tree species found in tropical rain forests of Western Ghats of India, Konkana, North Kanara, South Kanara, Bombay, Goa and Coorg.[6] The extract obtained from *Garcinia indica* fruits is an herbal preparation that has been reported to have many medicinal properties[7] including antiulcer activity. This gastroprotective effect seems to be related an ability to decrease acidity and increase mucosal defence.[8] Additionally the extract is reported antioxidant,[8,9] anticancer, antibiotic, suppressed colonic aberrant crypt foci formation,[10] induction of apoptosis in human leukemia HL-60 cells and anti inflammatory[8] in experimental animals. It is traditionally home remedy in case of flatulence, heart strokes, liver disorders and infections.[11] *Garcinia indica* or kokum contains other compounds with potential anti oxidant properties. These include citric acid, malic acid, polyphenols, carbohydrates,[8] ascorbic acid and anthocyanin pigments.[13] In view of the reported hepatoprotective activity of *Garcinia indica* and traditionally claims the fruits of *Garcinia indica* fruit...
was evaluated against CCl₄ induced hepatic damage in rats with the aim of developing a natural protective drug.

MATERIALS AND METHODS

Materials: Fresh kokum (Garcinia indica) fruits were procured from the orchards near Mangalore in the month of April 2009 and identified and authenticated by Botanical Science of India, Coimbatore, and Tamil Nadu and voucher submitted for the herbarium.

Preparation of photochemical extract: The fresh fruits are washed and cut into four equal pieces (runds) parallel to the major axis, then ground after the removal of seeds. Then fruits were dried under sun shade for 6-7 days and coarsely powdered. The powder was extracted using soxhelt apparatus with methanol 2000 ml. The methanol was distilled condensed using rotatory vacuum evaporator and stored in desicator. The powder of the extract was suspended in appropriate solvent system and was subjected for qualitative phyto constituents and indicated the presence of carbohydrates, flavonoids, citric acid and malic acid.

Chemicals and Drugs: Silymarin was purchased from Micro labs, Hosur, Karnataka, India. Carbon tetra chloride (CCl₄), 1-chloro-2, 4-dinitrobenzene (CDTNB), Di thio bis-2-nitrobenzoic acid (DTNB), Trichloro acetic acid (TCA) were purchased from SICCO Research Laboratory, Bombay, India. Bovine serum albumin (BSA) was purchased from Sigma Chemicals, St.Louis, USA and Thiobarbituric acid from Loba Chime Bombay, India.

Animals: Male wistar albino rats (200-250 gm) procured from the National Institute of Nutrition, Hyderabad. Housed in clean poly propylene cages and maintained at standard environmental conditions. They were fed with standard pellet diet (Hindustan liver, Bangalore) and water ad libitum during quarantine period. All procedures completed with the norms of the Animal Ethics Committee of our institution.

Toxicity studies: Garcinia indica in the dose range 200-2000 mg/kg were administered orally to different groups of rats comprising of 6 rats in each group. Mortality was observed after 72 hrs. Acute toxicity was determined according to the method Litchfield and Wilcoxon.

Carbon tetra chloride induced hepato toxicity: The experiment was carried out following the method with some modifications. The rats were divided into five groups (n = 6). First group (normal control) was given 1 ml of liquid paraffin /kg b.wt. daily once in every 72 hrs for 60 days were administered in animals from Group II-V.

Group II served as CCl₄ and was not treated with any drug methanolic extract of Garcinia indica (MEGI) at the dose of 200 and 400 mg/kg once daily were administered orally to the animals in Group III and Group IV respectively for 60 days. Standard drug Silymarin at the dose of 25 mg/kg was administered similarly to the animals in Group V. After 24 hrs of the last dose blood was collected from retro orbital plexus under ether anesthesia. The blood samples were allowed to clot and the serum was separated by centrifugation at 2500 rpm at 37°c and used for the assay of bio chemical marker enzymes. Immediately after collecting blood the animals were sacrificed and liver dissected out for biochemical studies SGOT, SGPT, alkaline phosphate (ALP) and bilirubin were determined by using commercially available kids (Span diagnostic limited, surat, India). Liver tissues were analyzed for content of glutathione, level of catalase activity, glycoprotein content, Total protein, Liver Na⁺ - K⁺ ATPase Activity and Thiobarbituric acid reactive substances.

Histopathological studies: The tissues of liver were fixed in 10% formalin and embedded in paraffin wax. Sections of 4-5 microns and stained with haematoxylin. Eosin and histopathological observations were made under light microscope.

Statistical analysis: The results are expressed as mean ± S.D. The difference between experimental groups were compared by one-way ANOVA (Toxic control Vs treatment. Bonferroni’s method; using Jandal Scientific, Sigmasstat statistical software, version 1.0) and were considered statistically significantly when p< 0.005.

RESULTS

The acute oral toxicity study of methanolic extract Garcinia indica showed no mortality up to 2000 mg/kg.

The effect of MEGI on serum transaminases (SGOT), serum phosphates (SGPT), alkaline phosphatase (ALP), bilirubin, total serum protein and TBARS level in CCl₄ intoxicated rats are summarized in Table 1. The effect of MEGI on Glutathione (GLY), catalase activity, Na⁺ - K⁺ ATPase activity and Glutathione content (GSH) were summarized in Table 2.

Histological studies also provided supportive evidence for biochemical analysis. Histology of the liver section of normal control animals showed normal hepatic cells each with well preserved cytoplasm, prominent nucleus and nucleolus and well bought out central vein (Figure 1). The liver sections of CCl₄ intoxicated mice showed massive fatty changes, necrosis, ballooning...

**DISCUSSION**

Carbon tetrachloride is one of the most widely used chemicals for the screening of hepatoprotective drugs.²⁵ It is well documented that carbon tetrachloride is biotransformed under the action of cytochrome P₄₅₀ in the microsomal compartment of liver to trichloro methyl radical (CCl₃·).²⁶,²⁷ Radical which readily reacts with molecular oxygen to form trichloro methyl peroxo radical attack the cell membrane and leads to membrane damage, alteration in the structure and function of cellular membrane by forming covalent bonds with macro molecules and induce peroxidative degradation, broad infiltration of lymphocytes and kuffer cells around the central vein and the loss of cellular boundaries (Figure 2). The animals treated with 200 mg/kg dose of methanolic extract of *Garcinia indica* exhibited only mild to moderate necrosis and lymphocyte infiltration (Figure 3). However, moderate accumulation of fatty globules (Figure 4) was noticed in the sections of animals treated with 400 mg/kg dose of methanolic extract of *Garcinia indica*. The sections of liver taken from the animals treated with standard drug silymarin showed the hepatic architecture, which was similar to that of control group (Figure 5).

**Table 1: Effect of MEGI on some serum biochemical parameters of CCl₄ intoxicated rats.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal group</th>
<th>CCl₄ treated group</th>
<th>MEGI (200 mg/kg)</th>
<th>MEGI (400 mg/kg)</th>
<th>Silymarin (25 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver wt (gm)</td>
<td>6.95 ± 0.3</td>
<td>5.78 ± 0.5</td>
<td>6.30 ± 0.2</td>
<td>6.57 ± 0.32</td>
<td>7.01 ± 0.9</td>
</tr>
<tr>
<td>SGOT (IU/l)</td>
<td>61.13 ± 10.03</td>
<td>210.56 ± 14.87</td>
<td>164.00 ± 13.37</td>
<td>106.02 ± 12.49</td>
<td>92.28 ± 10.96</td>
</tr>
<tr>
<td>SGPT (IU/L)</td>
<td>48.26 ± 8.53</td>
<td>110.16 ± 11.19</td>
<td>84.05 ± 8.79</td>
<td>69.76 ± 7.92</td>
<td>60.08 ± 6.57</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>129.18 ± 8.62</td>
<td>392.49 ± 12.29</td>
<td>223.51 ± 14.89</td>
<td>197.52 ± 12.62</td>
<td>163.25 ± 10.08</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>0.90 ± 0.24</td>
<td>2.91 ± 0.38</td>
<td>1.62 ± 0.25</td>
<td>1.13 ± 0.46</td>
<td>1.05 ± 0.22</td>
</tr>
<tr>
<td>Total protein</td>
<td>7.03 ± 0.60</td>
<td>5.93 ± 0.72</td>
<td>7.10 ± 0.88</td>
<td>7.25 ± 0.99</td>
<td>7.49 ± 0.85</td>
</tr>
</tbody>
</table>

Values are mean ± S.D (n = 6). CCl₄ control group compared with all the treatment groups: p<0.005.

**Table 2: Effect of MEGI on Glycogen, Lipid peroxidation, Catalase activity, Na⁺-K⁺ATPase and glutathione content of CCl₄ intoxicated rats.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal group</th>
<th>CCl₄ treated group</th>
<th>MEGI (200 mg/kg)</th>
<th>MEGI (400 mg/kg)</th>
<th>Silymarin (25 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen (mg/g wet tissue)</td>
<td>69.98 ± 2.6</td>
<td>52.21 ± 2.16</td>
<td>55.15 ± 6.21</td>
<td>57.76 ± 0.32</td>
<td>66.29 ± 1.29</td>
</tr>
<tr>
<td>Lipid peroxidation (µ moles MDA/g liver)</td>
<td>40.75 ± 4.34</td>
<td>115.29 ± 10.86</td>
<td>59.84 ± 6.95</td>
<td>49.84 ± 5.85</td>
<td>40.93 ± 4.38</td>
</tr>
<tr>
<td>Catalase activity (Unit/g liver)</td>
<td>2.28 ± 0.31</td>
<td>0.92 ± 0.29</td>
<td>1.62 ± 0.71</td>
<td>2.96 ± 0.28</td>
<td>2.10 ± 0.23</td>
</tr>
<tr>
<td>Na⁺-K⁺ATPase (µ/mg protein)</td>
<td>7.96 ± 0.45</td>
<td>5.21 ± 0.91</td>
<td>6.01 ± 2.92</td>
<td>6.92 ± 0.15</td>
<td>7.62 ± 0.90</td>
</tr>
<tr>
<td>GSH(µg/g of liver)</td>
<td>22.56 ± 4.65</td>
<td>10.16 ± 1.28</td>
<td>13.92 ± 1.89</td>
<td>29.79 ± 3.61</td>
<td>39.76 ± 16.58</td>
</tr>
</tbody>
</table>

Values are mean ± S.D (n = 6). CCl₄ control group compared with all the treatment groups: p<0.005.

**Figure 1:** Hepatocytes of the normal control group showed a normal lobular architecture of the liver.

**Figure 2:** Hepatocytes of the CCl₄ treated group showed liver cell necrosis and inflammation also observed in the centrilobular region with portal triaditis.
Degradation of the lipids of endoplasmic reticulum rich in poly unsaturated fatty acids. This leads to the formation of lipid peroxide followed by pathological changes such as depression of protein synthesis, elevated levels of serum marker enzymes such as SGPT, SGOT, ALP and bilirubin and released into circulation after cellular damage. Depletion of glutathione content, catalase activity and increased in lipid peroxidation is a better markers for the hepato cellular damage.

The significant of SGPT, an enzyme found primarily in liver, is far greater enhanced and released into the blood stream is the result of liver abnormality. If therefore serve as a fairly specific indicator of liver status and it’s elevated levels in serum indicates liver damage. MEGI reduces the SGPT levels indicating its protective effect over liver and important in liver functional efficiency.

SGOT is an enzyme found primarily in the cells of the liver, heart, skeletal muscles, kidneys, and pancreas and to a lesser extent in red blood cells. Its serum concentration is in proportion to the amount of cellular leakage or damage. It is released into serum in larger quantities when any one of these tissue is damaged. Its increased levels are usually associated with liver disease or heart attacks. MEGI decreased the SGOT level, which is an indication of the protective effect on liver and heart.

The raise in the levels of serum bilirubin is most sensitive and confirms the intensity of jaundice. MEGI decreased the serum bilirubin level. It is an indication that MEGI fruit has liver protective response.

Liver is damaged with CCl₄ indicates that increase the level of lipid peroxidation values because free radicals induced peroxidation. MEGI decreases the level of lipid peroxide values when compared to CCl₄ toxicated rats. The content of lipid peroxidation value is increased in MEGI extract treated groups when compare to silymarin treated group.

An increased level of ALP indicates bones disease, liver disease or bile tract blockage. Increase in serum ALP is due to increased synthesis, in presence of increasing biliary pressure in CCl₄ toxicated rats. MEGI has reduces the level of increased of serum ALP.

CCl₄ causes the decreases in the number of hepatocytes which in turn may result into decreased hepatic capacity to synthesize protein, glycogen and consequently reduces in liver weight. But, when the MEGI was given along with CCl₄ the significant increase in total protein and glycogen was observed indicating the hepato protection in the liver.
The activities of Na\(^+\)-K\(^-\) ATPase are decreased in CCl\(_4\) induced animals. MEGI prevented this effect of CCl\(_4\). Therefore; MEGI may be useful agent for normalization of CCl\(_4\) induced impaired membrane function and adrenal cortex.\(^{[37]}\)

Glutathione is an important endogenous antioxidant system that is found in particularly high concentration in liver and it is known to have key functions in protective processes. The reduced form of GSH becomes readily oxidized to GSSG on interacting with free radicals. Excessive production of free radicals resulted in the oxidative stress, which leads to damage of macromolecules e.g. lipids, and can induce lipid peroxidation \textit{in-vivo}. In our study, CCl\(_4\) treatment produced the depletion in liver and it is known to have key functions in protective processes. The reduced form of GSH becomes readily oxidized to GSSG on interacting with free radicals. The increased concentration of GSH. These results suggest that the hepato protective action of MEGI might be due to the presence of antioxidants like Polyphenols.

However, this is also proved by measuring catalase activity in different groups. MEGI at the dose of 400 mg/kg not only shows better improvement in catalase activity than the silymarin treated group but also increase catalase activity even more than the normal animals (Table 2).

A comparative histopathological study of the liver from different groups further corroborated the hepatoprotective potential.

Possible mechanism that may be responsible for the protection of CCl\(_4\) induced liver damage by free radical scavenger intercepting those radicals involved in CCl\(_4\) metabolism by microsomal enzymes.

CONCLUSION

MEGI is a promising hepato protective agent. The hepatoprotective action combined with antioxidant activity has a synergistic effect to prevent the process of initiation, oxidative stress and progression of hepatocellular damage.\(^{[31]}\)

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