ANTI-HEPATOTOXIC EFFECT OF FRESH CITRUS LEAVES EXTRACT ON CARBON TETRACHLORIDE INDUCED TOXICITY IN ALBINO RATS

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ABSTRACT
Fresh leaves extract of citrus given orally at exhibited significant anti hepatotoxic activity against carbontetrachloride (CCl₄)-induced hepatotoxicity in male albino rats. Hepatotoxicity and its prevention were assessed by serum markers viz. bilirubin, Cholesterol, triglycerides, alanine amino transferase, Aspartate transaminase and alkaline phosphatase.

KEYWORDS: Citrus lemon, Liver toxicity, Hepato protective, Carbon tetrachloride.

INTRODUCTION
Liver is the major organ playing a role in various divisions such as Metabolic function like Anabolism synthesis in carbohydrates, lipids and protein. The other functions are Storage function, Defense function (phagocyte & RES, Metabolism of Hormones, Detoxification of Drug & toxic substance. The liver can be affected by many problems such as Virus causes – hepatitis A, hepatitis B and hepatitis C, Scar form due to illness- cirrhosis, jaundice, Result of drugs, poisons or drinking too much alcohol and cancer and hemochromatosis (inherited disorder). The various drugs like paracetamol, amiodarone, allopurinol, ketoconazole and rifampicin may cause the liver damage in our body apart from some agents like carbon tetrachloride, cylindrospermopsin and amatoxin are also used to induce toxicity of liver. The high concentration of carbon tetrachloride affect the major organs like liver and kidney and its leads to liver damage this can result in painful swollen liver, hemorrhage, hepatic coma.
and death. Indian plants are playing a major role in food and medicines. Many plants and plant formulations are involved in the protective effect liver toxicity induced by various chemicals and drugs.[1-5]

CITRUS LEAVES

Taxonomy
Division: Magnoliophyta
Class: Magnoliopsida
Family: Rutaceae
Tribe: Aurantioieae
Genus: Citrus

![Figure 1: Lemon tree with leaves and green lemons.](image)

The species of citrus and various parts (figure 1) are having various pharmacological applications such as Fruits acts as an antiemetic, antitoxic remedy against plague, stomach tonic, Peel play an role in antitoxic, appetizer, cardiac tonic, digestive, elixir, stomachic and pulmonary sedative, Pulps in antihelmentic, antipyretic, appetizer, vascular stimulant, antiemetic, remedy against drunkenness, tonic, antitoxic, cures boils, throat and tonsils, Flowers in cardiac stimulant, digestive and stomachic, tonic, Wood in woodworm repellent, Leaves in digestive, insect repellent and etc.[6] Vitamin C and flavanoids are the major components of citrus and having various biomedical applications against coronary heart disease[7], colon cancer[8], human squamous cell carcinoma[9], leukemia[10], prostate cancer cells[11], colonic
epithelial abnormalities\textsuperscript{[12]} and chronic venous insufficiency.\textsuperscript{[13]} It involves the process of reduction of higher cholesterol level in blood leads to reduced heart diseases\textsuperscript{[14, 15, 16]}, antihyperglycemic effects in HepG2 cells,\textsuperscript{[17]} Mosquito larvicidal activity,\textsuperscript{[18]} Anti-obesity and anti-hyperglycemic effects\textsuperscript{[19]} and etc. The present study is carried out the investigation of the possible protective and toxic effects of fresh citrus leaves on CCl4 induced liver injury in rats. For this purpose the following parameters such as AST, ALT, ALP, Cholesterol, triglycerides and Bilirubin were studied.

**MATERIALS AND METHOD**
All chemical products used were of high purity and analytical grade. Carbon tetrachloride (CCl4) and other chemicals used in this study were purchased from Hi media laboratories, Mumbai.

**Plant and Extraction**
Fresh citrus leaves were procured from Adhiparasakthi agricultural college, Kalavai, South India. The 25 g of fresh leaves were thoroughly washed with distilled water and small pieces of leaves were boiled for 30 mins in 100 ml Millipore water containing conical flask. The boiled liquid parts are filtered and stored in sterilized container for toxicological studies.

**EXPERIMENTAL ANIMALS**
The rats weighed between 130-180g were used. The animals were housed in spacious polyurethane cages in a well ventiled animal house and had free access tap water all the time, fed with commercial pellet diet. The laboratory animal protocol used for this study was approved by the Institutional Animals Ethics Committee.

**EXPERIMENTAL DESIGN**
Rats were randomly divided in to 4 groups, each containing 6 animals. Totally 24 animals were used in this experiment.

**Group A**: About six Albino rats were maintained in normal condition with balanced food and saline water. They are not administered with any experimental chemical.

**Group B**: CCl4 injection about 0.5ml/kgbw/day dissolved in olive oil injected oral for days for three times.
**Group C**: The oral administration of *citrus leaves* extract (50mg/kgbw/day) dissolved in saline for 10 days.

**Group D**: The oral treated administration of *Citrus leaves* extract (50mg/kgbw/day) dissolved in saline to the CCl4 induced rats for 10 days.

**Biochemical Analysis**

Animals were sacrificed taken by anesthesia with chloroform and the blood was collected from the carotid vein centrifuge tube. From that, serum was collected by maintained in centrifugation method at 3000rpm for 10mins. Diagnosis of SGOT, SGPT, ALP, Cholesterol, triglycerides and Bilirubin from the collected serum. AST catalysis the transfer an amino group from dL-aspartate (dL ASP) to alpha-ketoglutarate to yield oxaloacetate and L-glutamate. The liberated oxaloacetate reacts with 2, 4-dinitrophenyl hydrazine to form 2, 4-dinitrophenylhydrazone, which is measured at 540nm. ALT catalyses the transfer of an amino group from dL-Alanine to alpha-ketoglutarate to yield pyruvate and L-glutamate. The liberated pyruvate reacts with 2, 4-dinitrophenyl hydrazine to form 2, 4-dinitrophenylhydrazone, which is measured at 540nm. Serum alkaline phosphatase estimated using disodium phenyl phosphate as the substrate. Disodium phenyl phosphate is hydrolyzed by alkaline phosphate with the liberation of phenol, which reacts under alkaline condition with Folins phenol reagent to form blue color, which was estimated calorimetrically at 680nm. Serum Bilirubin was estimated by Van Den Berg reaction. It was based on the formation of purple coloredazobilirubin when Bilirubin reacts with iazotisedsulphanilic acid.

**Statistical Analysis**

The difference of biochemical parameters were measured using the statistical method of Analysis of Variance (ANOVA). Analysis of Variance refers to the examination of difference among the samples. A value of <0.05 was considered to indicate a significant difference between groups. Value sharing a common superscript o not differ significantly with each at p<0.05 and p<0/0.01.

**RESULTS AND DISCUSSION**

Plant leaves are the very good sources used for hepatoprotective activity induced by various drugs and chemicals.[1-3] The hepatotoxic effect of carbon tetrachloride clearly shown in this present investigation. Our result shows significant increase of AST, ALT, ALP, Cholesterol, triglycerides and Bilirubin in this changes prevented by administration of fresh *Citrus leaves*
extracts. As indicated in tables changes taken among different groups of rats. The Table-1 showed changes in AST, ALT and ALP. Group-A is normal rats value used as control. When CCl4 induced values went to high level in Group-B. These increased level when melatonin carried decrease in value in Group-C also in Group-D is treated with the value of Group-C.

Table 1. Changes in the level of AST, ALT and ALP of normal and experimental groups values are expressed as mean± SD for six animals in each group.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Parameters</th>
<th>Normal (Group-A)</th>
<th>CCl4 Induced (Group-B)</th>
<th>Citrus Leaves Test Control (Group-C)</th>
<th>Citrus Leaves Treated (Group-D)</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AST</td>
<td>60.43±4.3205</td>
<td>117.05±5.310</td>
<td>68.7±4.342</td>
<td>70±6.121</td>
<td>P&lt;0.05(A&amp;C)</td>
</tr>
<tr>
<td>2</td>
<td>ALT</td>
<td>21.65±2.804</td>
<td>64.5±4.4609</td>
<td>29.4±6.172</td>
<td>27±4.583</td>
<td>P&lt;0.05(A&amp;C)</td>
</tr>
<tr>
<td>3</td>
<td>ALP</td>
<td>85.162±3.544</td>
<td>178.31±3.266</td>
<td>93.012±5.017</td>
<td>93.165±3.763</td>
<td>P&lt;0.01(A&amp;C)</td>
</tr>
</tbody>
</table>

Table-2 expressed the Bilirubin value changes of various groups of investigation. Here also increased level (Group-B) is reduced to lower value by treating with fresh Citrus leaves extract. (Group-C & Group-D). In the case of Group-B, the Bilirubin level is high. The Group-C and Group-D were treated with fresh Citrus leaves extract and hence the parameters were decreased when compared to drug induced (Group-B). As indicated in table 2, Bilirubin is also one of the most functional clinical markers to the severity of necrosis, and it leads to increased level of unconjugated Bilirubin. Then it can compensated only by continual administration of citrus leaves extract resulted in significantly reduced. The plant leaves containing phytochemicals are the major sources for hepatoprotective effects.[1, 3]

Table 2. Changes in the level of Bilirubin if normal and experiment group’s values are expressed as mean ±SD for six animals in each group.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Normal (Group-A)</th>
<th>CCL4 Induced (Group-B)</th>
<th>Citrus Leaves Test control (Group-C)</th>
<th>Citrus Leaves Treated (Group-D)</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bilirubin</td>
<td>0.481±0.0765</td>
<td>1.397±1.1233</td>
<td>0.6233±0.0237</td>
<td>0.5597±0.0371</td>
<td>P&lt;0.01(A&amp;C)</td>
</tr>
</tbody>
</table>

Changes in the Level of Serum Total Cholesterol and Triglycerides
Lemon juices are very well known Hypocholesterolemic agents because of its phytochemicals.[14-16] Table 3 present the changes in the level of serum cholesterol and serum triglycerides for the four groups. Here, CCL4-induced liver damage was characterized by increased level of total cholesterol and triglycerides and they return to normal level after the treatment with fresh citrus leaves.
Table 3: Changes in the level of total cholesterol and triglycerides in control and experimental rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal (Group-A)</th>
<th>CCL4 Induced (Group-B)</th>
<th>Citrus Leaves Test control (Group-C)</th>
<th>Citrus Leaves Treated (Group-D)</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>121.67 ± 5.62</td>
<td>349.50±15.6</td>
<td>115.00± 4.23</td>
<td>155.00±5.24</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>108.50 ± 5.14</td>
<td>219.83±10.20</td>
<td>112.33± 5.40</td>
<td>135.00±6.02</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

**CONCLUSION**

The present work reports for the hepatoprotective potential of aqua-alcoholic extract of citrus fresh leaves against CCl4 induced liver injury. The various phyto nutrients present in the fresh citrus leaves may attribute to its hepatoprotective potential. The results indicated the possible of the plant extracts to offer protection against the hepatotoxicity induced by CCl4 is maybe due to the flavonoids and other medicinally valued compounds. Further studies are in development to better understand the mechanism of action of bioactive compounds in fresh citrus leaves, responsible for its hepatoprotective effects.

**REFERENCES**


