PRELIMINARY PHARMACOLOGICAL SCREENING OF SIMAROUBA GLAUC A DC LEAF EXTRACTS FOR HEPATOPROTECTIVE ACTIVITY.

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ABSTRACT

Simarouba glauca DC, (SG) also known as Paradise tree is indigenous to South or Central America. The bark and leaf extract of Simarouba is well known for its different types of pharmacological properties. The ethanolic and chloroform extract of Simarouba glauca DC were tested for their hepatoprotective activity against Paracetamol induced hepatic damage. The degree of protection was measured by using biochemical parameters like SGPT, SGOT and ALP. Pretreatment with Silymarin (100 mg/kg, p.o.), Chloroform extract (200 mg/kg and 400 mg/kg) and ethanol extract (200mg/kg and 400mg/kg) of leaves of S.glauca for 5 days has significantly reduced the elevated serum enzyme level when compared with that of the hepatotoxic control group. Both chloroform and ethanol extracts of leaves of S.glauca reduced the histological changes caused by paracetamol. Also it could be concluded that the hepatoprotective potential may be due to its potent antioxidant activity.

KEYWORDS: Simarouba glauca, SGOT, SGPT, ALP, Hepatoprotectivity.

INTRODUCTION

Liver is a vital organ that plays a major role in metabolism and excretion of xenobiotics from the body. Liver injury or liver dysfunction is a major health problem that challenges not only health care professionals but also the pharmaceutical industry and drug regulatory agencies. The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. And it functions as a centre of metabolism of nutrients such as carbohydrates, proteins and lipids.
and excretion of waste metabolites. The bile secreted by the liver has, among other things, plays an important role in digestion. Therefore, maintenance of a healthy liver is essential for the overall well being of an individual. Liver cell injury caused by various toxicants such as certain chemotherapeutic agents, carbon tetrachloride, thioacetamide, chronic alcohol consumption and microbes are common. Acetaminophen (AAP) overdoses and the resulting hepatotoxicity is an important clinical problem. In addition, AAP is widely used as a prototype hepatotoxin to study mechanisms of chemical-induced cell injury and to test the hepatoprotective potential of new drugs and herbal medicines. Because of its importance, the mechanisms of AAP-induced liver cell injury have been extensively investigated and controversially discussed for about 30 years. Thus this present study is also based upon the paracetamol induced hepatotoxicity.\cite{1,2}

**Simarouba glauca** DC is a species of flowering tree that is native to Florida in the United States, South America, and the Lesser Antilles. Common names include Paradise Tree, Aceituno, and Bitter wood. Species from the Simaroubaceae family, known for their medicinal properties, are used traditionally for the treatment of various disorders.\cite{3,4} Although this plant extracts are widely shown to present antioxidant properties,\cite{5} hepatoprotective effect of *S.glauca* DC has not been explored. In view of this the present study was aimed at evaluating the hepatoprotective activity of *Simarouba glauca* DC against Paracetamol induced hepatotoxicity.

**MATERIALS AND METHODS**

**PLANT MATERIALS**

**Selection, collection and identification of plant material**

*S. glauca* leaves were collected from distinct region of Alappuzha district in Kerala, India. The plant was identified by Dr .G.V.S Murthy, Scientist ‘F’& Head of Office, Botanical survey of India, Coimbatore with BSI/SRC/5/23/2015/Tech/1476 as voucher number.

**Preparation of plant material**\cite{6}

Fresh leaves were collected and dried at room temperature. Dried leaves were powdered mechanically. Powdered leaves were then packed in Soxhlet apparatus and extraction was done.
**Chloroform:** 60 gm of dry powder was subjected to Soxhlet extraction with 300 ml chloroform, extraction was carried out for 3 hrs, 9 cycles and temperature was maintained at 65°C. Colour of extract was green.

**Ethanol:** 60 gm of dry powder was subjected to Soxhlet extraction with 300 ml Ethanol, extraction was carried out for 3 hrs, 10 cycles and temperature was maintained at 65°C. Colour of extract was dark green.

*Simarouba glauca* were prepared on each day of the experiment by reconstituting a weighed quantity of the crude extract with 2% tween 80.

**Preliminary phytochemical screening**

The extracts were subjected to preliminary phytochemical testing to detect the presence of different chemical groups of compounds. *S. glauca* leaves extract were screened for the presence of alkaloids, flavonoids, carbohydrates, glycosides, phenolic compound, tannins, triterpenoids, cardinolides, saponins etc.\(^7\)

**EXPERIMENTAL ANIMALS**

The studies were carried out on male Sprague dawley (100–150 g). The rats were obtained from Animal house, Govt. Veterinary College, Mannuthy. They were fed with a standard pellet diet and water ad libitum. Before their use in the experiment, the rats were kept in standard environmental conditions, (temperature 25–28 °C and 12 h light/dark cycle). All procedures were performed according to CPCSEA guidelines after proper approval from the Institutional Animal Ethics Committee. (Proposal number: SJCP/IAEC/21/03/15/04)

**DRUGS AND CHEMICALS**

- Silymarin - Tab Silybon -70, Microlabs limited
- Paracetamol – Tab Paracip -500, Cipla
- Diclofenac – Tab Reactin -50, Cipla
- SGPT, SGOT, ALP Enzyme Kits-Agape diagnostic Pvt. Ltd, Kolenchery
- Tween 80 – Merck.

**EXPERIMENTAL DESIGN**

Ethanolic and Chloroform extract of *Simarouba glauca leaves* were evaluated for its hepatoprotective activity in Sprague-dawley rats.
Selection of dose of the extract
Dose of the extract was selected by acute oral toxicity studies. (Dr Shankara Sharma et al. 2014) and other researchers/literatures already conducted acute toxicity on S.glauca leaf extracts. They reported that S.glauca leaf extract was non toxic up to dose 1800 mg/kg body weight and extract did not produce any lethal effect. Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further studies.[6]

Paracetamol induced hepatotoxicity in rats
Animals were divided into 7 groups of 6 animals each.
Rats in group 1 (normal control) received normal saline (1 mL/kg b.w., p.o.) for 5 days;
Group 2 (toxic group) received saline (1mL/kg b.w., p.o.) for 5 days and paracetamol (2 g/kg received) on the 2nd and 3rd day.
Group 3 (standard group) received Silymarin (100 mg/kg p.o.) for 5 days and paracetamol (2 g/kg po) on the 2nd and 3rd day, 30 min after Silymarin administration.
Group 4 and 5 (test groups), received chloroform extract for (200 and 400 mg/kg, p.o.) 5 days and were administered paracetamol (2 g/kg, po) on the 2nd and 3rd day, 30 min after administration of the chloroform extract.
Group 6 and 7 (test groups), received were administered ethanolic extract (200mg /kg and 400 mg/kg, p.o.) for 5 days and paracetamol (2 g/kg, p.o.) on the 2nd and 3rd day, 30 min after ethanolic extract administration.

Animals were sacrificed and blood was collected directly through retro orbital plexus. Serum was separated after coagulating at 37°C for 30 min and centrifuged at 1200-1500 rpm for 15-20 m. The serum was used for the estimation of SGPT, SGOT, ALP and liver tissue collected were subjected to histopathology.

Histopathology
After draining the blood liver samples were excised washed with normal saline and processed separately for histopathological observations. Initially, the materials were fixed with 10% buffered neutral formalin for 48 h. Further the histopathological analyses were carried out according to the standardized procedures.[8]

STATISTICAL ANALYSIS
The results were expressed as mean ± SEM. Statistical analyses of all the data obtained were evaluated using one-way ANOVA followed by Tukey & Dunnett’s post–hoc multiple
comparison test with SPSS Program; Version 20. All the results were also expressed as graph by Graph Pad Prism software (v.5). P values < 0.05 were considered as statistically significant.

RESULTS

Phytochemical screening of plant extract
The preliminary phytochemical studies of extract were carried out to find out the presence of carbohydrate, steroids, terpenoids, flavanoids, saponins, tannins, alkaloids and cardiac glycosides. The obtained results were shown in Table I

Table I

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Tests</th>
<th>Chloroform Extract</th>
<th>Ethanol Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Mayer’s test</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Dragendorff’s test</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Hager’s test</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Wagner’s test</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Alkaline reagent test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Shinoda Test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Molisch’s test, Fehling’s test and Benedict’s test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Borntragar’s test</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>FeCl₃ test, lead acetate test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>FeCl₃ test</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Steroids/Terpenoids</td>
<td>Salkowski test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Liebermann – Burchard test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Liebermann test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam test</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ (positive) means Present, - (negative) means absent

Paracetamol induced hepatic damage
In this study the biochemical parameters such as SGOT, SGPT, and ALP levels where compared with the positive control.

Table II: Effect of SG on SGPT, SGOT and ALP levels

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Groups</th>
<th>SGPT</th>
<th>SGOT</th>
<th>ALP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>32.70±1.22</td>
<td>69.98±3.41</td>
<td>37.53±1.62</td>
</tr>
<tr>
<td>2</td>
<td>+ve Control</td>
<td>115.74±3.46</td>
<td>138.94±1.43</td>
<td>120.44±2.72</td>
</tr>
<tr>
<td>3</td>
<td>Standard(Silymarin, 100 mg/kg p.o)</td>
<td>48.41±1.31***</td>
<td>93.55±1.09***</td>
<td>52.06±1.14***</td>
</tr>
<tr>
<td>4</td>
<td>Chloroform ext 200 mg/kg.p.o</td>
<td>89.36±1.36***</td>
<td>121.85±1.94***</td>
<td>83.27±1.15***</td>
</tr>
<tr>
<td>5</td>
<td>Chloroform ext 400 mg/kg.p.o</td>
<td>70.96±2.77***</td>
<td>103.44±2.28***</td>
<td>65.83±9.47***</td>
</tr>
<tr>
<td>6</td>
<td>Ethanol ext 200 mg/kg.p.o</td>
<td>63.41±1.33***</td>
<td>99.97±9.63***</td>
<td>70.60±8.73***</td>
</tr>
<tr>
<td>7</td>
<td>Ethanol ext 400 mg/kg.p.o</td>
<td>53.23±1.11***</td>
<td>94.40±9.42***</td>
<td>55.46±1.06***</td>
</tr>
</tbody>
</table>
Results are expressed as Mean ± S.E.M. The difference between experimental groups was compared by One-way Analysis of Variance (ANOVA), followed by Tukey. The results were considered statistically significant when *P<0.05, **P<0.01, ***P<0.001. Compared with Positive Control.

Effect of SG on SGPT, SGOT, and ALP values

<table>
<thead>
<tr>
<th>Parameters</th>
<th>IU/L</th>
</tr>
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<tbody>
<tr>
<td>SGPT</td>
<td></td>
</tr>
<tr>
<td>SGOT</td>
<td></td>
</tr>
<tr>
<td>ALP</td>
<td></td>
</tr>
</tbody>
</table>

![Graph showing SGPT, SGOT, and ALP values](image)

Test 1 – Chloroform extract

Test 2- Ethanol extract.

Histopathological evaluation

Normal control rat shows normal hepatocytes and central vein whereas paracetamol treated group found to cause hepatocellular degeneration, with small nucleus confirming hepatotoxicity. Compared to paracetamol intoxicated group, administration of low and high dose of chloroform and ethanolic extracts of *Simarouba glauca* reduced such changes of cells and central vein in the histology of liver.

CONTROL

![Low Resolution](image) ![High Resolution](image)

A- Normal Central Vein, B- Radially arranged Hepatocytes.
POSITIVE CONTROL

Fig 3: Low Resolution
A-Hepatocellular changes with small nucleus

Fig 4: High Resolution

STANDARD

Fig 5: Low Resolution
A – Normal Central Vein, B – Normal Hepatocytes

Fig 6: High Resolution

CHLOROFORM EXTRACT LOW DOSE

Fig 7: Low Resolution
A – Normal Central Vein, B – Congested Hepatocytes

Fig 8: High Resolution
CHLOROFORM EXTRACT HIGH DOSE

Fig 9: Low Resolution
A- Normal Hepatocytes with mild congestion

Fig 10: High Resolution

ETHANOL EXTRACT LOW DOSE

Fig 11: Low Resolution
A- Normal Hepatocytes, B-Portal tract

Fig 12: High Resolution

ETHANOL EXTRACT HIGH DOSE

Fig 13: Low Resolution
A – Normal Central Vein, B – Normal Hepatocytes

Fig 14: High Resolution
The present study shows that there is a significant increase in the activity of serum enzymes, SGPT, SGOT and ALT in the case of Positive Control (Paracetamol Treated Group) when compared with that of other groups. Pretreatment with Silymarin (100 mg/kg, p.o.), Chloroform extract (200 mg/kg and 400 mg/kg) and ethanol extract (200mg/kg and 400mg/kg) of leaves of S. glauca for 5 days has significantly reduced the elevated serum enzyme level when compared with that of the hepatoxic control group. Both chloroform and ethanol extracts of leaves of S. glauca reduced the histological changes caused by paracetamol.

**DISCUSSION**

This present study mainly aimed to explore the hepatoprotective activities of Simarouba glauca D.C leaves extracts. Simarouba glauca DC (family-Simaroubaceae) has a long history in herbal medicine in many countries. Although a lot of work has been carried out on the various parts of the plant to best our knowledge, no scientific studies have been reported for the Hepatoprotective activities of Simarouba glauca DC leaves. Therefore this present study includes the screening for hepatoprotective activity using two extracts (chloroform and ethanol) by paracetamol induced hepatic damage.

The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ.

The hepatotoxicity of acetaminophen appears to occur by a complex mechanistic sequence. These events include: (1) CYP metabolism to the reactive metabolite NAPQI which depletes glutathione by a conjugation reaction and covalently binds to proteins; (2) loss of glutathione causing an increased oxidative stress response (decreased detoxification of reactive oxygen and nitrogen species); (3) increased oxidative stress, possibly associated with alterations in calcium metabolism, initiation of signal transduction responses and mitochondrial permeability transition; (4) mitochondrial permeability transition occurring with an even larger increase in oxidative stress, loss of mitochondrial membrane potential, and loss of the ability of the mitochondria to synthesize ATP; and (5) loss of ATP which causes necrosis. During hepatic damage, cellular enzymes like SGPT, SGOT, and ALP will leak into the serum resulting in elevation of their serum concentrations. Pretreatment with Silymarin (100 mg/kg, p.o.), Chloroform extract (200 mg/kg and 400 mg/kg) and ethanol extract (200mg/kg and 400mg/kg) of leaves of S. glauca for 7 days has significantly reduced the
elevated serum enzyme level when compared with that of the hepatotoxic control group. From the study it is evident that the ethanolic extract at a dose of 400 mg/kg shows an activity similar to that of standard. The SGPT, SGOT, ALP values of 400 mg/kg of ethanolic extract of *Simarouba glauca* were 53.23±1.11***, 94.40±.942***, 55.46±1.06***

Both chloroform and ethanol extracts of leaves of *S.glauca* reduced the histological changes caused by paracetamol. The possible mechanism of action may be associated with the antioxidant property of leaves of *Simarouba glauca*.

**CONCLUSION**
The plant *Simarouba glauca* DC was traditionally claimed for its large number of pharmacological action and medicinal use.

The present study reveals that both the chloroform and ethanolic extract possess the hepatoprotective activity whereas the ethanolic extract exhibits the maximum hepatoprotective potential. The results substantiate its Antioxidant property. It can be concluded from present study that *S.glauca* leaf extract can be used for the development of a new remedy for the hepatoprotective activity and it requires further phytochemical studies to isolate the active compounds responsible for these pharmacological activities and to explore its exact mechanism of action.

**REFERENCE**


