HEPATOPROTECTIVE ACTIVITY OF BARKS EXTRACT OF SIX CINNAMOMUM SPECIES ON CARBON TETRACHLORIDE-INDUCED IN ALBINO RATS

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ABSTRACT

Hepatoprotective activity of crude methanolic extract of six Cinnamomum species were active against CCl₄ induced hepatic damage in albino rats was observed. In the present study, the therapeutic effects of six Cinnamomum species were studied by examining the prevention of CCl₄ induced hepatic damage in Albino rats. The results of CCl₄-induced hepatic damage were treated animals with C. walaiwarense, and C. travancoricum barks extracts were maximum reduction of liver weight and liver volume. Hepatoprotective active constituents were preliminary identified leukoanthocyanins, saponins, triterpenes, cardiotonic glycosides, steroids, anthraquinones and cyanogenic glycosides form barks of Cinnamomum species.

Keywords: Barks, Cinnamomum sulphuratum, Cinnamomum walaiwarense, Cinnamomum travancoricum, Cinnamomum malabatrum, Cinnamomum filipedicellatum and Cinnamomum wightii, Hepatoprotective activity, Albino rats, CCl₄

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INTRODUCTION

The liver is one the prime organ concerned with several states of metabolic and physiologic homeostasis of the human beings. About 20,000 deaths found every year due to liver disorders. Hepatocellular carcinoma is one of the ten most common tumors in the world with over 2,500,000 new cases each year (Ajay & Neelam 2006). There is no specific cure for several diseases such as cirrhosis, fatty liver, and chronic hepatitis.

The modern medicines of interferon, colchicine, penicillamine, and corticosteroids were efficiency of the treatment of inconsistent and more side effects (Luper 1998). There is the need of effective therapeutic agents. Traditional herbal medicine has been established over thousands of years and is based on experience and practice. For these reasons, developing drugs for liver diseases from plants used in traditional medicine, may lead to improve therapies (Roy et al. 2006).

In India, about 40 polyherbal commercial formulations reputed to have hepatoprotective action are being used. It has been reported that 160 phytoconstituents from 101 plants have hepatoprotective activity. Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthines. About 25 plants have been reported to cure liver diseases (Ajay & Neelam 2006).

The Lauraceae is a family of about 2000- 2200 species of mostly tropical trees (Chaverri & Ciccio 2005). The family is an important component of higher elevation forests in India where they occur in high abundance and diversity. This family is recognized by the simple, alternate, stiff and aromatic elliptic to obovate leaves, and by the fruits often borne in a cup.

The genus Cinnamomum is comprised of over 250 species of trees and shrubs from East and South East Asia to Australia that are aromatic with glossy, leathery leaves, clusters of very small flowers, and berry like fruits. Most species are aromatic and some are used as spices. About 26 species occur in India (Anonymous 1992).

Twelve Cinnamomum species are endemic to Peninsular India, of which nine are endemic to Southwestern Ghats, one of the mega centers of endemism in India (Nayar 1996). They were used as most of the Cinnamomum species are viz. Cinnamomum sulphuratum, Cinnamomum walaiwarense, Cinnamomum travancoricum, Cinnamomum malabatrum, Cinnamomum filipedicellatum and Cinnamomum wightii were ethno botanical uses for to treat wound, backache and urinary problems.
The present study was drug development from ethno-botanical information of bark extracts of *Cinnamomum* species were screened for carbon tetrachloride (CCl₄) induced liver toxicity in albino rats.

### MATERIALS AND METHODS

*Cinnamomum sulphuratum, Cinnamomum walaiwarense, Cinnamomum travancoricum, Cinnamomum malabatrum, Cinnamomum filipedicellatum* and *Cinnamomum wightii* barks were collected from Inzhikuzhi, Karaiyar region, Kalakad Mundanthurai - Tiger Reserve (KMTR) region, Tirunelveli District, Tamil Nadu, South India. 500gms of air-dried and powdered barks of *Cinnamomum sulphuratum, Cinnamomum walaiwarense, Cinnamomum travancoricum, Cinnamomum malabatrum, Cinnamomum filipedicellatum* and *Cinnamomum wightii* were extracted with methanol at 45°C. The methanol extract was evaporated to dryness. Qualitative tests for barks involving coloration, and precipitation reactions, were performed to detect active principles like alkaloids, leukoanthocyanins, saponins, triterpenes, glycosides, steroids, and anthraquinones. These tests were performed according to the method described by Domínguez (1973).

Both sex of Albino rats (75-100gm) were used in these experiments. The animals were housed in standard cages (48 cm x 35 cm x 22 cm) with food and water ad libitum, at room temperature (27 ±2°C) with artificial light from 7.00 am to 7.00 pm. The approximate LD₅₀ was determined in albino mice according to the method of Smith (1960).

Albino rats of both sex weighing 75-100gms were divided into each group of ten animals. Two groups served as controls. Carbon tetrachloride was given orally to all groups (except the blank control) in doses of 0.2 ml/100 g with an equal quantity of liquid paraffin.

The drugs were dissolved in propylene glycol and given orally in doses of 300 mg/kg one hour prior to the CCl₄ administration. The blank control group received propylene glycol only. Forty eight hours after CCl₄ treatment, the animals were counted in order to calculate the percent mortality, the remaining animals were sacrificed. The liver was weighed and its volume was measured in a measuring cylinder by the displacement method (Singh et al. 1978).

All data were collected and statistically analyzed for differences between individual groups by the use of student’s $t$-test. Values for $p<0.05$ were considered statistically significant.

### RESULTS

Using standard methods of preliminary phytochemical analysis of six *Cinnamomum* species of bark samples of the drug suggested that the positive results of leukoanthocyanins, saponins, triterpenes, cardiotonic glycosides, steroids, anthraquinones and cyanogenic glycosides and also tests for alkaloids was negative (Table- 1).

No mortality was observed the bark extracts of *Cinnamomum* species but 10% mortality was observed in ascorbic acid and 70% died on CCl₄-induced animals. As seen table- 2 shows the results on hepatotoxicity effects of orally administered barks extracts of six *Cinnamomum* species on carbon tetrachloride (CCl₄) induced liver weight and liver volume prevented in Albino rats.

Mean liver weight in untreated (control) animals was found to be about 1.85g/100g of body weight. Carbon tetrachloride treatment markedly increased the weight to about 2.78 ± 0.10/100g of body weight. Pretreatment of CCl₄-treated animals with *C. walaiwarense, C. travancoricum* barks extracts were maximum reduction of liver weight 1.96±0.23 and 2.11±0.41/100g of body weight, and also liver volume 2.12 ± 0.40 and 2.46 ± 0.10ml/ body weight respectively.

Moderately reduced in the liver weight and liver volume was treated with extract of *C. malabatrum, C. sulphuratum, C. filipedicellatum* and *C. wighti* barks (Seen in table-2). These results were suggested that the positive effect of *Cinnamomum* extract to give protection against CCl₄ induction of liver injury.

### DISCUSSION

Carbon tetrachloride is one of the most commonly used hepatotoxin. It is well documented that carbon tetrachloride is bio transformed under the action of cytochrome P- 450 in the microsomal compartment of liver to trichlomethyl radical which readily reacts with molecular oxygen to form trichloromethyloeroxy radical. In the present study, six *Cinnamomum* species bark extracts were evaluated for its protective effects on hepatocellular damage in CCl₄-intoxicated rats.
Table 1. Metabolites in barks of *Cinnamomum* species

<table>
<thead>
<tr>
<th>Test</th>
<th>Bioactive constituents (Present/Absent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid method of Web for alkaloids</td>
<td>C. sulphuratum -</td>
</tr>
<tr>
<td>Method of Cain for alkaloids</td>
<td>C. walaiwarense -</td>
</tr>
<tr>
<td>Method of Cain for leukoanthocyanins</td>
<td>C. travancoricum +</td>
</tr>
<tr>
<td>Method of Cain for saponins</td>
<td>C. malabatrum +</td>
</tr>
<tr>
<td>Method of Cain for triterpenes</td>
<td>C. filipedicellatum +</td>
</tr>
<tr>
<td>Method for cardiotonic glycosides</td>
<td>C. wightii +</td>
</tr>
<tr>
<td>Method for steroids</td>
<td></td>
</tr>
<tr>
<td>Method for anthraquinones</td>
<td></td>
</tr>
<tr>
<td>Method for cyanogenic glycosides</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Effect of barks of *Cinnamomum* species on CCl$_4$ induced changes in liver weight and volume of albino rats

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Weight (gm/100 gm body weight ± SE)</th>
<th>'p' value</th>
<th>Volume (ml/100 gm body weight ± SE)</th>
<th>'p' value</th>
<th>Mortality (48 hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black control (Normal)</td>
<td>1.85 ± 0.10</td>
<td>-</td>
<td>1.96 ± 0.12</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Control (CCl$_4$)</td>
<td>2.78 ± 0.21</td>
<td>&lt;0.05</td>
<td>3.87 ± 0.21</td>
<td>&lt;0.05</td>
<td>70</td>
</tr>
<tr>
<td>C. sulphuratum</td>
<td>2.06 ± 0.16</td>
<td>&lt;0.05</td>
<td>2.65 ± 0.11</td>
<td>&lt;0.05</td>
<td>10</td>
</tr>
<tr>
<td>C. walaiwarense</td>
<td>2.16 ± 0.30</td>
<td>&gt;0.05</td>
<td>2.52 ± 0.28</td>
<td>&gt;0.05</td>
<td>0</td>
</tr>
<tr>
<td>C. travancoricum</td>
<td>1.96 ± 0.23*</td>
<td>&lt;0.01</td>
<td>2.12 ± 0.40</td>
<td>&lt;0.05</td>
<td>0</td>
</tr>
<tr>
<td>C. malabatrum</td>
<td>2.11 ± 0.41</td>
<td>&gt;0.05</td>
<td>2.46 ± 0.20</td>
<td>&gt;0.05</td>
<td>0</td>
</tr>
<tr>
<td>C. filipedicellatum</td>
<td>2.15 ± 0.20</td>
<td>&gt;0.05</td>
<td>2.45 ± 0.31</td>
<td>&gt;0.05</td>
<td>0</td>
</tr>
<tr>
<td>C. wightii</td>
<td>2.36 ± 0.12</td>
<td>&lt;0.05</td>
<td>2.78 ± 0.30</td>
<td>&lt;0.01</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>2.56 ± 0.32</td>
<td>&gt;0.05</td>
<td>3.25 ± 0.19</td>
<td>&gt;0.05</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2. Effect of barks of *Cinnamomum* species on CCl$_4$ induced changes in liver weight and volume of albino rats

**All drugs were given orally in doses of 10mg/100gm body weight followed by 0.2 ml/100 g body weight of CCl$_4$.**

According to Tsukamoto et al. (1990) reported that CCl$_4$ is one of the most widely used toxicant for experimental induction of liver fibrosis in laboratory animals. The present study was observed that the animals were sacrificed for assessing the treatment of barks extract of *Cinnamomum* species on CCl$_4$-induced changes in liver weight and volume.

Earlier studies of several synthetic has been shown to protect the liver against carbon tetrachloride-induced hepatotoxicity and ethanol-induced hepatic damage (Subbarao & Gupta 1974, Subbarao 1975, Patrao 1957, Sule et al. 1956). According to Dhawan and Srimal, (1998) previous reported that the plant extract was calculated the internally accepted therapeutic dosage of humans was extrapolated to mice.

The observed protective effect of the plant extract against carbon tetrachloride may be attributed to the presence of flavonoids, ascorbic acid, carotenoids, tannis and lignins among the plant constituents (Gilani & Janbaz 1995). The results obtained are very interesting as they provide evidence in favour of the protective effect. The present study observed that the positive results of barks extract of *Cinnamomum sulphuratum, Cinnamomum walaiwarense, Cinnamomum travancoricum, Cinnamomum malabatrum, Cinnamomum filipedicellatum* and *Cinnamomum wightii* against carbon tetrachloride-induced hepatotoxicity in Albino rats.

Many compounds known to be beneficial against carbon tetrachloride-mediated liver injury exert their protective action by toxin-mediated lipid peroxidation either via a decreased production of carbon tetrachloride derived free radicals or through the antioxidant activity of the protective agents themselves. (Thabrew et al.1987, Jayatilaka et al.1990).

The results of the present study indicated that the barks extract of *Cinnamomum* species present in the may be active constituents were leukoanthocyanins, saponins, triterpenes, cardiotonic glycosides, steroids, anthraquinones and cyanogenic glycosides is against hepatoprotective effects on carbon tetrachloride induced liver damage in albino rats.
CONCLUSION

In conclusion, the present study demonstrated that methanolic extract of Cinnamomum species has hepatoprotective effect in CCl₄ induced liver damage. However, it is necessary to determine other parameters such as oxidative stress markers and molecular biology assays to confirm our findings. Further study will be needed to isolate and purify the active principle involved in the hepatoprotective activity of Cinnamomum sulphuratum, Cinnamomum walaiaiwarense, Cinnamomum travancoricum, Cinnamomum malabatrum, Cinnamomum filipedicellatum and Cinnamomum wightii and to determine its mechanism of action.

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REFERENCES


