Evaluation of antioxidant and hepatoprotective potential of Cinnamomum tamala leaves in rats

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INTRODUCTION

The liver is an organ that has fascinated mankind; Galen considered liver as “principal instrument” of the body.[1] Since the liver is involved in the biochemical conversion of various endogenous and exogenously administered substances, there is a possibility of generation of free radicals. However, the liver has an integral system like the glutathione (GSH), etc., to scavenge them off. In spite of this the free radicals generated by some hepatotoxins, like paracetamol (PCM), may overpower the protective mechanism of the liver and cause hepatic damage.[2] Liver disorders are still a worldwide health problem.[3] The liver is continuously and variably exposed environmental toxins and abused by poor drug habits and alcohol and prescribed and over-the-counter drug which can eventually lead to various liver ailment like hepatitis cirrhosis and alcoholic liver disease.[4] Treatment options for common liver diseases such as cirrhosis, fatty liver, and chronic hepatitis are problematic. The effectiveness of treatment such as interferon colchicine, penicillamine, and corticosteroid are inconsistent at best, and incidence of side effect is profound through the treatment is worse than the disease.[3] Recent studies point out that oxidative stress might be a crucial originating factor in the pathogenesis of liver diseases along with deficiency of GSH.[5] Due to enormous side effects of allopathic medicine, today the world is returning toward the natural options for diseases treatment. Nutraceuticals may provide micronutrients and nonnutritive phytochemicals that have established potential beneficial effects.[7]

Context: Health is one of the commonly mentioned motivations behind food choices, and functional foods offer promising health benefits. Aims: In this study, the folklore claim of Cinnamomum tamala leaves as hepatoprotective functional food is evaluated. Settings and Design: Prophylactic and curative hepatoprotective properties of selected functional food was carried out against commonly used analgesic antipyretic paracetamol (PCM). Subjects and Methods: Prophylactic and curative hepatoprotective property of suspension of C. tamala leaves (SCT) was studied against PCM-induced hepatic damage in albino rats. Pre- and post-treatment with SCT, before and after PCM treatment, reduced the biochemical markers of hepatic damage like serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, serum alkaline phosphatase, serum cholesterol, total and direct bilirubin, and tissue glutathione (GSH). Histopathological studies also revealed that pre- and post-treatment with SCT, before and after PCM treatment protected the liver from PCM-induced liver damage. In addition, an antioxidant study was carried out by in vitro lipid peroxidation. Statistical Analysis Used: Statistical analysis was performed with one-way analysis of variance (one-way) followed by Tukey test. Results: In this study, it has been found that prophylactic study showed better hepatoprotection than the curative study. Conclusions: In the prophylactic study, lipid peroxidation and protection of GSH may be the reason behind better hepatoprotection and C. tamala possess hepatoprotective activity possibly because of its antioxidant potential.

Key words: Antioxidant, Cinnamomum tamala, hepatoprotection, paracetamol

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Bay leaf, is a small evergreen tree. Traditionally, the leaves are used as a tonic to the brain, anthelmintic, diuretic; good for the liver and spleen; useful in inflammation.\(^\text{[9]}\) The leaves valuable in cardiac disorders, inflammations, helminthiasis, dyspepsia, colic, diarrhea, hepatopathy, and splenopathy.\(^\text{[9]}\) In India, the leaves are used in a cough, digestion, and diabetes.\(^\text{[10]}\) Flavonoids like quercetin, polyphenols, and essential oil are reported from leaves.\(^\text{[11,12]}\) Hence, this study was undertaken to evaluate the antioxidant and hepatoprotective potential of \textit{C. tamala} leaves.

**SUBJECTS** AND **METHODS**

**Drugs** and **chemicals**

PCM and silymarin were gift samples from Leben Laboratories, Akola, India and Microlabs, Bengaluru, India, respectively. Kits for the estimation of selected biochemical parameters such as serum glutamic pyruvic transaminase (SGOT), serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), total and direct bilirubin (TDB), and cholesterol were of Erba Diagnostics, Germany purchased from local suppliers. All other chemicals used in this study were of analytical grade.

**Plant material and suspension preparation**

Indian systems of medicine consider that complex diseases can be treated with a complex combination of botanicals, unlike in the West, with single drugs. Whole foods are, hence, used in India as functional foods rather than supplements.\(^\text{[13]}\) The leaves of \textit{C. tamala} were purchased from the local market of Akola, India. The leaves are identified by the Department of Botany, Shri Shivaji Science College, Akola, India, where a voucher is deposited in the Herbarium. The dried leaves were pulverized in a grinder and passed through mesh no. 80. The sieved powder of the leaves was suspended in 2% gum acacia aqueous solution, which is referred as suspension of \textit{C. tamala} (SCT), prior to administration.

**Animals**

Albino Wister rats (150–250 g) and mice (25–35 g) were housed under standard conditions of constant temperature and lighting (12 h light/dark cycle). They had free access to standard pellet diet (Gold Mohur Lipton Indian Ltd.,) and water \textit{ad libitum}. The Institutional Animal Ethical Committee of A. U., College of Pharmaceutical Sciences, Andhra University, Visakhapatnam approved by CPCSEA with registration number 515/01/a/CPCSEA approved the study.

**Antioxidant activity**

As the PCM-induced hepatotoxicity is due to lipid peroxidation and lowering of GSH. \textit{In vitro} lipid peroxidation and \textit{in vivo} GSH estimation are carried out to evaluate antioxidant activity of SCT.

**In vitro lipid peroxidation**

Inhibition of lipid peroxidation was determined by the method developed by Ohkawa \textit{et al.}\(^\text{[14]}\) Rat liver tissue weighing 10 g was homogenized with a Polytron homogenizer in ice-cold Tris-HCl buffer to produce a 25% w/v homogenate. Then it was centrifuged at 4000 rpm for 10 min. An aliquot of supernatant 0.1 ml was mixed with 0.1 ml of SCT at different concentrations, followed by addition of 0.1 ml of potassium chloride (30 mM), 0.1 ml of ascorbic acid (0.06 mM) as standard, and 0.1 ml of ammonium ferrous sulfate (0.16 mM) and were incubated for 1 h at 37°C. The reaction mixture was treated with 0.2 ml of sodium dodecyl sulfate (8.1%), 1.5 ml of thiobarbituric acid (0.8%), and 1.5 ml of 20% acetic acid (pH 3.5). The total volume was then made up to 4 ml by adding distilled water and kept in an oil bath at 100°C for 1 h. After the mixture had been cooled, 1 ml of distilled water and 5 ml of 15:1 v/v butanol-pyridine mixture were added. Following vigorous shaking, the tubes were centrifuged at 4000 rpm for 10 min, and the absorbance of the organic layer containing the thiobarbituric acid reactive substance was measured at 532 nm. A control was prepared using 0.1 ml of the respective vehicle in the place of SCT/ascorbic acid. The experiment was performed in triplicate. The percentage inhibition of lipid peroxidation was as follows.

\[
\text{Percentage inhibition} = \frac{\text{-Average of test sample OD}}{\text{Average of the control OD}} \times 100
\]

**Tissue glutathione measurement**

GSH measurements were performed using a modification of the Ellman procedure.\(^\text{[15]}\) After hepatotoxicity study tissue samples were homogenized in ice-cold trichloroacetic acid (TCA) (1 g tissue plus 10 ml 10% TCA) in an ultraturrax tissue homogenizer. Briefly, after centrifugation at 3000 rpm for 10 min, 0.5 ml supernatant was added to 2 ml of 0.3 M disodium hydrogen phosphate solution. A 0.2 ml solution of dithiobis nitrobenzoate (0.4 mg/ml in 1% sodium citrate) was added, and the absorbance at 412 nm was measured immediately after mixing.

**Acute toxicity study**

The acute toxicity was performed in a group of 3 mice who were administered 2000 mg/kg body weight of SCT orally through an oral feeding tube. The animals were observed for 24 h for any sign of toxicity and lethality.\(^\text{[16]}\)

**Hepatoprotective activity**

**Preventive study**

Albino Wister rats of either sex were selected and divided into five groups each containing six rats. PCM, powder of the \textit{C. tamala} and silymarin were dissolved in 2% gum acacia suspension. The treatment protocol was planned to study the effect of SCT in the preventive aspect of PCM-induced hepatotoxicity.\(^\text{[17]}\) The treatment protocol is summarized and given below:

- **Group 1**: Normal control, 2% w/v gum acacia suspension orally, 1 ml/kg once daily for 3 days
- **Group 2**: PCM as toxicant 2 g/kg orally once daily for 3 days
- **Group 3**: SCT 200 mg/kg orally, 30 min prior to PCM
2 g/kg orally for 3 days
• Group 4: SCT 400 mg/kg orally, 30 min prior to PCM 2 g/kg orally for 3 days
• Group 5: Silymarin 100 mg/kg orally, 30 min prior to PCM 2 g/kg orally for 3 days.

On the 0th day (1 day before the start of dosing) and on the 4th day (next day of the last dosing) blood was collected by a retro-orbital puncture from all the animals. Serum was separated by centrifugation (3000 rpm for 15 min) and subjected to estimation of biochemical parameters such as SGPT, SGOT, ALP, serum cholesterol, and bilirubin. Then on the 4th day, the animals were sacrificed, and a small piece of fresh liver is used for GSH estimation from all the above groups.

Curative study
The treatment protocol was planned to study the curative effect of SCT in PCM-induced hepatotoxicity.\(^\text{[17]}\) The treatment protocol is summarized and given below:
• Group 1: Normal control, 2% w/v gum acacia suspension orally, 1 ml/kg once daily for 10 days
• Group 2: PCM as toxicant 2 g/kg orally once daily for 3 days followed by 1 ml/kg 2% gum acacia suspension from 4th day to 10th day
• Group 3: PCM 2 g/kg orally for 3 days followed by SCT 200 mg/kg orally from 4th day to 10th day
• Group 4: PCM 2 g/kg orally for 3 days followed by SCT 400 mg/kg orally from 4th day to 10th day
• Group 5: PCM 2 g/kg orally for 3 days followed by silymarin 100 mg/kg orally from 4th day to 10th day.

On the 0th day (1 day before the start of dosing) and on the 11th day (next day of the last dosing) blood was collected by retro-orbital puncture from all the animals. Serum was separated by centrifugation (3000 rpm for 15 min) and subjected to estimation of biochemical parameters such as SGPT, SGOT, ALP, cholesterol, and bilirubin.

Statistical analysis
Results were expressed as a mean ± standard error of the mean, \((n = 6)\). Statistical analysis was performed with one-way analysis of variance (one-way) followed by Tukey test carried out by using Graphpad prism. \(P < 0.05\) was considered to be statistically significant. \(^\text{**}P < 0.01\), \(^\text{*}P < 0.05\), and \(^\text{**}P < 0.001\) when compared with toxicant group.\(^\text{[18]}\) Fifty percentage of inhibition concentration (IC\(_{50}\)) for \(in vitro\) lipid peroxidation activity has been calculated by Microsoft Excel.

RESULTS
SCT and ascorbic acid at different concentrations (12.5–400 µg) inhibited the lipid peroxidation in a dose-dependent manner. The amount needed for 50% inhibition of lipid peroxidation by SCT and ascorbic acid was found to be 375.18 µg and 179.66 µg, respectively [Table 1]. Three days treatment of 2 g/kg PCM drastically decreased tissue GSH levels \((P < 0.001)\) compared to the normal control group. The lower and higher doses of SCT lowered the elevated levels of tissue GSH by 81.25% (200 mg/kg) and 179.66% (400 mg/kg) of this cutoff dose have been selected for further \(in vivo\) studies. In PCM-induced hepatotoxicity, the lower dose of 2 g/kg body weight of PCM induced a significant increase in serum GPT, GOT, ALP, cholesterol, total bilirubin, and direct bilirubin levels when compared to the normal control group \((P < 0.001)\). Pretreatment of rats with SCT significantly prevented the elevation of serum GPT, GOT, ALP, cholesterol, total bilirubin, and direct bilirubin levels as compared to PCM-treated group (Group 2). However, the lower dose of SCT caused a nonsignificant decrease in serum ALP and direct bilirubin in the prophylactic study [Table 3]. Posttreatment of rats with SCT after PCM treatment in the curative study also prevented the elevation of serum GPT, GOT, ALP, cholesterol, total bilirubin, and direct bilirubin levels as compared to PCM-treated group (Group 2). However, the lower dose of SCT caused a nonsignificant decrease in serum GPT, ALP, cholesterol, and direct bilirubin. Even the higher dose of SCT caused a nonsignificant decrease in serum cholesterol and direct bilirubin in the curative study [Table 4]. Silymarin as standard drug diminished the higher dose of SCT caused a nonsignificant decrease in serum GPT, GOT, ALP, cholesterol, total bilirubin, and direct bilirubin levels as compared to PCM-treated group (Group 2). However, the lower dose of SCT caused a nonsignificant decrease in serum GPT, GOT, ALP, cholesterol, total bilirubin, and direct bilirubin levels as compared to PCM-treated group (Group 2). However, the lower dose of SCT caused a nonsignificant decrease in serum GPT, GOT, ALP, cholesterol, total bilirubin, and direct bilirubin levels as compared to PCM-treated group (Group 2). However, the lower dose of SCT caused a nonsignificant decrease in serum GPT, GOT, ALP, cholesterol, total bilirubin, and direct bilirubin levels as compared to PCM-treated group (Group 2). However, the lower dose of SCT caused a nonsignificant decrease in serum GPT, GOT, ALP, cholesterol, total bilirubin, and direct bilirubin levels as compared to PCM-treated group (Group 2). However, the lower dose of SCT caused a nonsignificant decrease in serum GPT, GOT, ALP, cholesterol, total bilirubin, and direct bilirubin levels as compared to PCM-treated group (Group 2). However, the lower dose of SCT caused a nonsignificant decrease in serum GPT, GOT, ALP, cholesterol, total bilirubin, and direct bilirubin levels as compared to PCM-treated group (Group 2). However, the lower dose of SCT caused a nonsignificant decrease in serum GPT, GOT, ALP, cholesterol, total bilirubin, and direct bilirubin levels as compared to PCM-treated group (Group 2).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Quantity (µg)</th>
<th>IC(_{50}) value (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12.5</td>
<td>25</td>
</tr>
<tr>
<td>Cinnamomum tamala</td>
<td>0.124±0.02</td>
<td>0.132±0.01</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.138±0.01</td>
<td>0.161±0.01</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are the mean±SEM, \(n=3\), values in parenthesis indicate percentage inhibition. SEM: Standard error of mean, SCT: Suspension of Cinnamomum tamala, IC\(_{50}\): Fifty percentage inhibition concentration.
compared to the PCM-treated group. The percentage change in serum enzyme levels from 0th day to 4th day in the prophylactic study [Figure 1] and on the 11th day in the curative study [Figure 2] was also calculated. This percentage of protection was inferior in the curative study compared to the prophylactic study. The prophylactic study with SCT showed better results than the curative study.

**DISCUSSION**

PCM is an extensively used analgesic and antipyretic drug and, though safe when used at therapeutic doses, is associated with significant hepatotoxicity when taken in overdose. Under normal conditions, PCM is primarily metabolized in the liver by glucuronidation and sulfation. A small proportion of the drug is metabolized by several of the cytochrome P450 enzymes into the reactive intermediate N-acetyl-p-benzoquinoneimine (NAPQI), which is normally detoxified by GSH both nonenzymatically and enzymatically. In overdose, sulfation and glucuronidation become flooded and GSH is depleted by NAPQI. Excess of NAPQI causes oxidative stress and binds covalently to liver proteins.[19,21]

In living systems, the liver is considered to be highly sensitive to toxic agents. The study of different enzyme activities such as SGOT, SGPT, serum ALP, total bilirubin, and cholesterol...

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**Table 2: Influence of SCT on GSH level in PCM-induced hepatotoxicity**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Absorbance (mean±SEM)</th>
<th>Percentage increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2% gum acacia (1 ml/kg)</td>
<td>0.93±0.0573*</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>PCM (2 g/kg)</td>
<td>0.32±0.0731**</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>PCM + SCT (2 g/kg + 200 mg/kg)</td>
<td>0.38±0.0132†</td>
<td>18.40</td>
</tr>
<tr>
<td>4</td>
<td>PCM + SCT (2 g/kg + 400 mg/kg)</td>
<td>0.41±0.0361**</td>
<td>26.68</td>
</tr>
<tr>
<td>5</td>
<td>PCM + silymarin (2 g/kg)</td>
<td>0.58±0.0341**</td>
<td>79.75</td>
</tr>
</tbody>
</table>

Values are the mean±SEM of six rats/treatment, ***P<0.001, **P<0.01, *P<0.05 compared to PCM treatment on 4th day in prophylactic study, #P<0.001 compared to control group (Group 1). SEM: Standard error of mean, SCT: Suspension of *Cinnamomum tamala* GSH: Glutathione, PCM: Paracetamol

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**Table 3: Influence of SCT on biochemical parameters in rats for PCM-induced hepatotoxicity (prophylactic study)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGPT (0th day)</td>
<td>36.10±2.88</td>
<td>33.09±0.99</td>
<td>32.68±1.71</td>
<td>32.22±1.26</td>
<td>30.95±0.8</td>
</tr>
<tr>
<td>SGPT (4th day)</td>
<td>36.85±2.22</td>
<td>130.0±3.91*</td>
<td>98.0±5.84*</td>
<td>70.0±8.74***</td>
<td>33.0±3.30***</td>
</tr>
<tr>
<td>SGOT (0th day)</td>
<td>81.91±4.47</td>
<td>82.30±1.83</td>
<td>83.94±2.0</td>
<td>82.12±1.04</td>
<td>80.5±2.04</td>
</tr>
<tr>
<td>SGOT (4th day)</td>
<td>83.00±3.46</td>
<td>450.3±16.68*</td>
<td>333.2±7.48***</td>
<td>218.0±3.93***</td>
<td>90.1±4.74***</td>
</tr>
<tr>
<td>ALP (0th day)</td>
<td>182.4±5.84</td>
<td>180.3±4.28</td>
<td>181.5±2.97</td>
<td>181.5±2.98</td>
<td>182.3±2.66</td>
</tr>
<tr>
<td>ALP (4th day)</td>
<td>184.2±4.99</td>
<td>722.1±14.56*</td>
<td>667.6±28.80 NS</td>
<td>434.6±103.2NS</td>
<td>201.2±18.08 NS</td>
</tr>
<tr>
<td>Cholesterol (0th day)</td>
<td>100.8±1.37</td>
<td>99.9±1.76</td>
<td>100.9±1.63</td>
<td>101.1±3.73</td>
<td>101.5±3.1</td>
</tr>
<tr>
<td>Cholesterol (4th day)</td>
<td>102.2±5.72</td>
<td>245.1±13.53*</td>
<td>185.0±8.67**</td>
<td>155.0±9.21***</td>
<td>108.0±5.33**</td>
</tr>
<tr>
<td>TB (0th day)</td>
<td>0.89±0.075</td>
<td>0.82±0.04</td>
<td>0.93±0.06</td>
<td>0.91±0.05</td>
<td>0.92±0.04</td>
</tr>
<tr>
<td>TB (4th day)</td>
<td>0.90±0.05</td>
<td>3.13±0.37*</td>
<td>1.9±0.1***</td>
<td>1.73±0.2***</td>
<td>1.00±0.04**</td>
</tr>
<tr>
<td>DB (0th day)</td>
<td>0.23±0.03</td>
<td>0.14±0.02</td>
<td>0.14±0.02</td>
<td>0.17±0.03</td>
<td>0.16±0.04</td>
</tr>
<tr>
<td>DB (4th day)</td>
<td>0.23±0.03</td>
<td>0.23±0.04</td>
<td>0.22±0.06 NS</td>
<td>0.25±0.03NS</td>
<td>0.17±0.02NS</td>
</tr>
</tbody>
</table>

Values are the mean±SEM of six rats/treatment, ***P<0.001, **P<0.01, *P<0.05, NS: Not significant compared to PCM treatment (Group 2), #P<0.001 compared to normal control group (Group 1). SCT: Suspension of *Cinnamomum tamala* PCM: Paracetamol, SGPT: Serum glutamic pyruvic transaminase, SGOT: Serum glutamic oxaloacetic transaminase, ALP: Alkaline phosphatase, TB: Total bilirubin, DB: Direct bilirubin, SEM: Standard error of mean

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**Table 4: Influence of SCT on biochemical parameters in rats for PCM-induced hepatotoxicity (curative study)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGPT (0th day)</td>
<td>33.7±1.95</td>
<td>33.21±1.66</td>
<td>32.09±3.59</td>
<td>33.71±1.64</td>
<td>31.48±1.40</td>
</tr>
<tr>
<td>SGPT (11th day)</td>
<td>34.60±2.73</td>
<td>119.1±8.76*</td>
<td>101.1±7.02 NS</td>
<td>91.03±2.54***</td>
<td>35.37±3.71***</td>
</tr>
<tr>
<td>SGOT (0th day)</td>
<td>83.18±2.97</td>
<td>80.67±2.85</td>
<td>83.3±1.62</td>
<td>80.96±3.16</td>
<td>81.89±2.14</td>
</tr>
<tr>
<td>SGOT (11th day)</td>
<td>84.19±3.79</td>
<td>410.1±5.19*</td>
<td>333.1±22.51***</td>
<td>224.4±14.76***</td>
<td>96.06±4.29***</td>
</tr>
<tr>
<td>ALP (0th day)</td>
<td>181.9±4.35</td>
<td>180.3±3.46</td>
<td>183.4±3.08</td>
<td>182.4±2.95</td>
<td>182.6±2.37</td>
</tr>
<tr>
<td>ALP (11th day)</td>
<td>184.2±3.68</td>
<td>585.0±35.53*</td>
<td>580.3±10.59 NS</td>
<td>440.2±17.67***</td>
<td>219.3±13.55***</td>
</tr>
<tr>
<td>Cholesterol (0th day)</td>
<td>99.17±3.57</td>
<td>99.16±2.90</td>
<td>101.8±3.92</td>
<td>101.3±4.32</td>
<td>99.1±2.77</td>
</tr>
<tr>
<td>Cholesterol (11th day)</td>
<td>100.1±2.58</td>
<td>208.2±3.82*</td>
<td>194.2±3.94***</td>
<td>164.9±14.0NS</td>
<td>113.2±4.33***</td>
</tr>
<tr>
<td>TB (0th day)</td>
<td>0.84±0.02</td>
<td>0.86±0.02</td>
<td>0.83±0.03</td>
<td>0.89±0.01</td>
<td>0.88±0.03</td>
</tr>
<tr>
<td>TB (11th day)</td>
<td>0.85±0.02</td>
<td>2.95±0.08*</td>
<td>1.92±0.15***</td>
<td>1.81±0.06**</td>
<td>1.02±0.05**</td>
</tr>
<tr>
<td>DB (0th day)</td>
<td>0.16±0.02</td>
<td>0.18±0.03</td>
<td>0.23±0.03</td>
<td>0.2±0.02</td>
<td>0.25±0.03</td>
</tr>
<tr>
<td>DB (11th day)</td>
<td>0.16±0.01</td>
<td>0.42±0.05*</td>
<td>0.41±0.02 NS</td>
<td>0.32±0.03 NS</td>
<td>0.28±0.04 NS</td>
</tr>
</tbody>
</table>

Values are the mean±SEM of six rats/treatment, ***P<0.001, NS: Not significant compared to PCM treatment (Group 2), #P<0.001 compared to normal control group (Group 1). SEM: Standard error of mean, SCT: Suspension of *Cinnamomum tamala* PCM: Paracetamol, SGPT: Serum glutamic pyruvic transaminase, SGOT: Serum glutamic oxaloacetic transaminase, ALP: Alkaline phosphatase, TB: Total bilirubin, DB: Direct bilirubin
have been found to be of great value in the measurement of clinical and experimental liver damage.\[22,23\] The extent of the PCM-induced hepatotoxic effect is assessed by the level of released cytoplasmic enzymes - SGPT, SGOT, ALP, cholesterol, total bilirubin, and direct bilirubin in circulation. An elevated level of SGOT is known to indicate liver damage due to viral hepatitis, cardiac infarction, and muscle injury. SGPT, a better bio-indicator of liver injury than SGOT, catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. Serum ALP and bilirubin levels conversely are related to the function of the hepatic cell. The elevated level of serum ALP is due to increased synthesis, in the presence of increasing biliary pressure.\[24\]

If any drug lowered these elevated levels of enzymes due to hepatotoxic agents, it may be the result of stabilization of plasma membrane thereby preserving the structural integrity of the cell, as well as the repair of hepatic tissue damage caused by PCM.\[25\] In this study, both prophylactic and curative study showed that due to 3 days treatment with PCM-induced a sharp rise in all above-mentioned liver enzymes when compared to the normal control group (\(P < 0.001\)). This is an indicator of cellular leakage and loss of functional integrity of cell membrane in the liver.\[26\] This study showed that orally pre- and post-treatment of rats with SCT suppressed PCM-induced liver damage by lowering the elevated enzymes levels remarkably mainly by the higher dose of SCT, an indication of stabilization of plasma membrane, as well as repair of hepatic tissue damage. Effective control of bilirubin level and ALP activity points toward an early progress in the secretory mechanism of the hepatic cell. It has been indicated that microviscosity of a membrane increases noticeably with an increase in cholesterol level in plasma. PCM-induced toxicity in rats may have altered membrane structure and function as suggested by the increases in cholesterol. This result is an indication of membrane rigidity caused by PCM. Alteration of biomembrane lipid profile disturbs its fluidity, permeability, the activity of associated enzymes, and transportation system.\[27\] In this study, it has been observed that cholesterol levels have been increased due to PCM treatment. However, treatment of rats with SCT inhibited the alteration of lipid membranes and fluidity; hence, lowered cholesterol level in plasma. The percentage change on the 4\(^{th}\) day and 11\(^{th}\) day in prophylactic and curative studies, respectively, also showed better hepatoprotection in prophylactic study [Figures 1 and 2]. The higher dose showed better hepatoprotection compared to a lower dose of SCT.

Increased lipid peroxidation in hepatic tissues after administration of PCM could be expected owing to the depletion of GSH.\[28\] In our study, the in vitro lipid peroxidation of SCT showed percentage reduction comparable with standard ascorbic acid [Table 1]. The \(IC_{50}\) value for the SCT and standard ascorbic acid was found to be 375.18 \(\mu\)g and 179.66 \(\mu\)g, respectively. GSH is one of the most abundant tripeptide, nonenzymatic biological antioxidant present in the liver. Its functions are related with the removal of free radical species such as hydrogen peroxide, superoxide radicals, alkoy radicals, and maintenance of membrane protein thios and as a substrate for GSH peroxidase.\[29\] In our study, the lowered level of GSH due to PCM treatment (\(P < 0.001\)) was increased by SCT treatment in a dose-dependent manner [Table 2]. PCM toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome \(P_{450}\) or depletion of hepatic GSH is a requirement for PCM-induced hepatotoxicity.\[30\] Therefore, the antihepatotoxic activity of the SCT may be due to inhibition of cytochrome \(P_{450}\) promotion of glucuronidation, reduction in lipid peroxidation, stimulation of hepatic regeneration, or activation of the functions of the reticuloendothelial systems.

Today we realized that diets rich in bioactive phytochemicals reduce the risk of degenerative disorders. Foods containing these phytochemicals, and commonly known as functional
food, not only supply our diet with certain antioxidant vitamins like Vitamin C, Vitamin E, and pro-Vitamin A but also a complex mixture of other natural substances with antioxidant capacity.\(^{[28]}\) Flavonoids like quercetin and polyphenols are reported from leaves\(^{[31]}\) which are said to be good antioxidants. It has been reported that the leaves of \textit{C. tamala} possess good antioxidant activity.\(^{[31,32]}\) These previous findings support the antioxidant role of SCT in hepatoprotection. In the conventional cooking system, we generally overcook our foods and damaged their essential nutrients and also important antioxidants. It has also been proved that heat treatment affect the antioxidant activity of vegetables and in many cases has been observed lower antioxidant capacity in processed samples versus raw vegetables. Nutrient antioxidants may act together to reduce reactive oxygen species level more effectively than single dietary antioxidants because they can function as synergists.\(^{[33‑35]}\) Consuming raw vegetables, fruits, and nuts are more beneficial than cooking them harshly. Hence, this study conducted on SCT leaves without any extraction procedure.

**CONCLUSION**

The SCT possess antioxidant and hepatoprotective activity. In this study, it has been found that prophylactic study showed better hepatoprotection than curative activity which may further support the antioxidant mechanism behind hepatoprotection. In prophylactic study, may be lipid peroxidation and protection of GSH are the reason behind better hepatoprotection but in the curative study already the lipid peroxidation and depletion of GSH will be produced before the commencement of SCT administration.

Although further studies are needed to rule out the possibilities of other mechanism of action.

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**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**


