those of an earlier study suggests that the conceptual model can indeed serve as a simple coupled ocean–atmosphere model with general ocean thermodynamics.

Krishnamurthy et al. have earlier demonstrated that the aperiodicity in the interannual variability in the tropics is due to the interaction between the low-frequency mode and the nonlinear higher-frequency modes. In general, aperiodicity arises due to nonlinear interaction between more than one modes of the system. One way of studying the nonlinear interaction between different modes is through the determination of bispectrum. The bispectrum of the conceptual model for the coupled case ($\alpha = \beta = 0.1$) gave rise to additional modes with time scales very similar to those found in the earlier study of Selvarajan and Goswami, who employed a simple coupled ocean–atmosphere system with general ocean thermodynamics, in which the atmospheric heating is determined by sea surface temperature anomalies as well as the convergence feedback mechanism. This study further establishes the fact that the conceptual model does exhibit important characteristics of variability of the tropics.

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Hepatoprotection by Phyllanthus amarus and Phyllanthus debilis in CCl4-induced liver dysfunction

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The present investigation compares the hepatoprotective action of Phyllanthus amarus and Phyllanthus debilis in the treatment of liver damage in rats exposed to carbon tetrachloride. The evaluation has been carried out using liver function marker enzymes in plasma, liver tissue biochemical supported by liver histopathology. The extent of recovery has been compared with the natural liver regeneration after CCl4 damage.

Both the plant species have been found to be effective in the treatment of liver damage induced by CCl4. P. debilis has been found to be a better hepatoprotective agent than P. amarus.

Phyllanthus species form an important part of folklore medicines. Almost all species of Phyllanthus found in India are used medicinally, especially in the treatment of jaundice. No work, however, has been carried out on these species to assess their individual potential as liver tonic. The two species most commonly found in India are Phyllanthus amarus and Phyllanthus fraternus. In this communication we report our preliminary findings on the efficacy of P. amarus and P. debilis as liver tonics for CCl4-induced liver damage in rats. We had earlier reported TLC and UV spectral characteristic of these plants.

Whole plants of P. amarus and P. debilis were collected from Trichur, carefully segregated and dried at 45°C for two days and powdered. One sample of each species was sent to Raw Material Herbarium and Museum, Publication & Information Directorate, New Delhi, for authentic confirmation of the identification of the species. The voucher numbers assigned to them are P. amarus – 1726 and P. debilis – 1728. The plant material was powdered and sieved through a sieve (B.S.S. mesh no. 85). The sieved plant powder was suspended in distilled water (D/W) and administered to rats orally in volumes of 2 ml through gavage. The dose of plant administered was 0.66 g/kg in each rat. The administration was done in the morning. The dose of plant slurry was ascertained by a pilot study over a range of doses varying from 0.165 to 2.64 g/kg. The pilot study was carried out using three animals per dose group. In all, five dose groups were employed. The drug was administered for two days after CCl4 damage. The dose of CCl4 was fixed from published reports. Literature survey showed that earlier studies had used CCl4 at a concentration of 0.7 ml/kg as a low-dosage administration for inducing reversible liver damage in rats. The results of the pilot study (enzyme assays and histopathology) indicated that the animals administered 0.66 g/kg of plant slurry showed maximum hepatoprotection. Albino male Wistar rats were procured from Haffkine Institute, Parel, Bombay. The animals were housed in polyurethane cages and were maintained on standard rat pellets (12 mm) containing 20–21% crude protein and 4–5% ether-soluble fraction. Water was supplied ad libitum.

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The animals were divided into 5 groups of 6 rats each for the present study.

Group I served as control and was administered 2 ml D/W orally for 3 days along with i.p. administration of liquid paraffin on the first day.

Group II animals were treated with CCl₄ only. These rats were administered 2 ml D/W orally for 3 days along with i.p. administration of CCl₄ (0.7 ml/kg) in liquid paraffin, only on the first day.

Group III animals were administered P. amarus slurry (0.66 g/kg) orally along with i.p. CCl₄ (0.7 ml/kg) on the first day and only P. amarus slurry (0.66 g/kg) on successive days.

Group IV animals were administered P. debilis slurry (0.66 g/kg) orally along with i.p. CCl₄ on the first day and only P. debilis on successive days.

Group V animals were treated with CCl₄ only. These rats were administered 2 ml D/W orally along with i.p. administration of CCl₄ (0.7 ml/kg) in liquid paraffin on the first day. On remaining days the animals were administered only 2 ml D/W orally. These animals served as recovery group. All animals were sacrificed on day 4 (72 h after CCl₄ administration) except group V, which was sacrificed on day 7 (six days after CCl₄ treatment).

During sacrifice, blood was collected under light ether anaesthesia by cardiac puncture. Enzyme analysis was carried out in plasma. Liver was excised out of animal, rinsed in saline, blotted and weighed. Group V animals were sacrificed on the seventh day after a recovery period of 6 days subsequent to the CCl₄ administration. Microscopic examinations on liver were carried out to support the enzymological observations. A part of the liver was preserved in neutral buffered formalin for histopathological studies. Tissues fixed in neutral formalin were dehydrated in alcohol, cleared and embedded in paraffin wax. Seven-micron sections were cut and stained with hematoxylin and eosin. The rest of the liver after fixation for histopathology was processed for tissue biochemistry. DNA was analysed by the method of Burton³, and RNA was analysed by the method of Ceriotti⁴. Plasma enzyme glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase
(GOT) were analysed according to the method of Reitman and Frankel\(^7\) and alkaline phosphatase (ALKP) was analysed according to the method of King and Armstrong\(^6\). The plasma cholesterol was estimated by the method of Zaltakis et al.\(^7\). The data from the groups were evaluated by ANOVA and Group I was compared with the rest of the groups using Student's t test.

The results in Table 1 show that CCl\(_4\) increases significantly the levels of GOT, GPT, ALKP and cholesterol in plasma. The results of group V indicate that the levels do not regain the control values after a recovery period of 6 days. There is a significant fall in liver RNA levels of the animals treated with CCl\(_4\) alone. The RNA levels regain near control values after a natural recovery period of 6 days. The effects of CCl\(_4\) have been effectively moderated by the administration of plant extracts, as is evident from the results of groups III and IV animals. The plasma parameters of the animals were less different from the control animals. Histopathological observations on the liver of CCl\(_4\)-treated animals revealed characteristic centrilobular necrosis indicated by significant number of swollen hepatocytes (Figure 1a). In the liver of groups III and IV animals, the extent of necrosis was significantly less compared to groups II and V animals. In animals treated with P. amarus slurry hepatocytes with dense cytoplasm were visible in periportal areas (Figure 1c) while in animals treated with P. debilis hepatocytes with dense cytoplasm were distributed uniformly (Figure 1d). This indicates localized regeneration induced by P. amarus slurry after CCl\(_4\) damage, whereas the regeneration is uniform after administration of P. debilis slurry. The liver histology of the animals administered this slurry shows less effect of CCl\(_4\) than that of animals which underwent a recovery period of 6 days after CCl\(_4\) exposure (Figure 1b).

Numerous studies have clearly shown that CCl\(_4\) causes hepatic injury. The structural, functional and compositional effects of CCl\(_4\) as a liver toxin are available in detail\(^8\). The metabolism of CCl\(_4\) releases CCl\(_3\) free radical, which initiates peroxidation and cleavage of fatty acids in the membranes\(^9,10\). In the present study CCl\(_4\) has been used as the model hepatic toxicant to investigate the efficacy of two plant slurries in the treatment of liver dysfunction. Alteration of serum enzyme levels can be monitored to evaluate the hepatocellular damage caused by various foreign compounds\(^11-13\). In this investigation plasma GOT, GPT and ALKP have been estimated to evaluate the hepatic damage. Liver RNA and DNA levels have also been estimated to assess the toxicant-induced changes in protein synthesis. Carbon tetrachloride causes a marked elevation in the transaminases\(^14\). The results of groups III and IV animals suggest that both plant species can control the increase in plasma GOT, GPT, ALKP and cholesterol levels to a considerable extent. Comparison of enzyme levels of groups III, IV and V indicated that in group V animals the plasma enzymes regained levels close to those of control animals, after 6 days of recovery, whereas the enzyme levels in groups III and IV reached close to control levels within 2 day after CCl\(_4\) administration. The earlier recovery of enzyme levels seen in animals administered plant slurries as compared to animals undergoing natural recovery clearly suggests significant hepatoprotective action of the plant slurries. It is reported that peak changes in plasma enzymes are noticed at 24 h after CCl\(_4\) administration but complete recovery is slow and takes about 14 days\(^15\). Observations on animals of groups III and IV provide a comparative account of the hepatoprotective action by both the plant species. Animals treated with P. debilis slurry

**Table 1. Plasma assays**

<table>
<thead>
<tr>
<th>Group parameters</th>
<th>Units</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPT</td>
<td>Units/l</td>
<td>39.20 ± 4.21*</td>
<td>85.20 ± 10.03***</td>
<td>56.20 ± 4.40***</td>
<td>40.00 ± 10.45*</td>
<td>57.60 ± 1.14***</td>
</tr>
<tr>
<td>GPT</td>
<td>Units/l</td>
<td>77.60 ± 24.04***</td>
<td>249.80 ± 24.71***</td>
<td>205.40 ± 21.92***</td>
<td>171.40 ± 26.54***</td>
<td>138.40 ± 10.64***</td>
</tr>
<tr>
<td>ALKP</td>
<td>K. units</td>
<td>22.20 ± 2.30</td>
<td>34.24 ± 4.32***</td>
<td>33.24 ± 2.065***</td>
<td>27.38 ± 2.94*</td>
<td>24.60 ± 2.79</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>mg/dl</td>
<td>33.60 ± 1.34*</td>
<td>55.54 ± 6.56***</td>
<td>38.50 ± 5.37</td>
<td>44.25 ± 4.34***</td>
<td>53.80 ± 5.93***</td>
</tr>
</tbody>
</table>

All values represent mean ± S.D. of 6 animals.
Differences which are significant are marked as follows: *P < 0.01, **P < 0.002, ***P < 0.001.

**Table 2. Tissue biochemistry**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>mg/g</td>
<td>5.29 ± 0.81*</td>
<td>5.576 ± 0.99</td>
<td>5.135 ± 0.59</td>
<td>5.389 ± 0.25</td>
<td>5.456 ± 0.86</td>
</tr>
<tr>
<td>RNA</td>
<td>mg/g</td>
<td>117.74 ± 10.56*</td>
<td>72.522 ± 0.7282*</td>
<td>77.878 ± 4.67*</td>
<td>85.302 ± 6.23*</td>
<td>103.59 ± 17.60*</td>
</tr>
</tbody>
</table>

All values represent mean ± S.D. of 6 animals.
*P < 0.001

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show enzyme levels closer to the control levels than animals treated with *P. amarus* slurry. This indicates a better regulatory effect of *P. debilis* slurry over *P. amarus* slurry in the moderation of hepatic damage due to CCl₄. It is reported that galactosamine, a hepatotoxicant, causes reduction in liver RNA and protein synthesis. In the present study no significant changes have been recorded in the liver DNA levels after CCl₄ treatment. Hepatic RNA levels, however, reduced significantly after CCl₄ administration. Observations of groups III and IV animals indicate that the slurry of *P. debilis* moderates significantly the CCl₄-induced fall in liver RNA levels. The recovery of RNA level is, however, more in group V animals. In histopathological observations cellular regeneration was evident in animals treated with *P. debilis* slurry and also in animals treated with *P. amarus* slurry. The liver of animals administered with *P. debilis* slurry showed regenerating hepatocytes both in centrilobular and periportal areas, whereas in animals treated with *P. amarus* slurry the regeneration was localized to periportal areas only, where the cells showed dense cytoplasm. This further suggests that the liver of rats treated with *P. debilis* slurry showed more areas with regenerating hepatocytes compared to rats treated with *P. amarus* after CCl₄-induced damage. The histarchitectural of the liver of rats administered plant slurry is closer to that of control animals than in animals which underwent 6 days of recovery after CCl₄ treatment. It has been observed that removal of necrotic debris starts by 48 h after CCl₄ administration and is usually complete by one week.

Many compounds cited in the literature exhibit liver protection against CCl₄ either by decreasing the production of CCl₄ free radical or by impairment of CCl₄-induced lipid peroxidation. The improved histology of liver as seen in histopathological observations on animals treated with plant slurry as compared to that seen in animals administered only CCl₄ indicates the possibility of both these plant slurry being able to induce accelerated regeneration of liver cells, reducing the leakage of GPT, GOT and AlkP into the blood. Serum transaminase returns to normal with the healing of liver parenchyma and regeneration of liver cells. Though both plant species are being used in traditional medicines for the treatment of liver disorders, the present investigation provides adequate evidence to the view that *P. debilis* is a better hepatoprotective than *P. amarus*. Further toxicological and pharmacokinetic studies are needed to substantiate this distinction in the action of the two species so as to suggest the dosage for a treatment regimen. Some of these studies have already been initiated.


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Increase in size of the gland is not always associated with increased secretion: An evidence from the larval salivary glands of *Drosophila*

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The quantity of the larval salivary gland secretions (glue proteins) in relation to the gland size was analysed in 15 species of *Drosophila*. Such an analysis revealed that in most of the species, the gland size variation was due to hypertrophy and not hyperplasia and the quantity of glue synthesized is double compared to that in *D. melanogaster*. Further, it is evident that the quantity of secretions synthesized is independent of the size of the salivary glands.

A tissue-specific protein called the glue protein is synthesized by the larval salivary gland cells of *Drosophila*. This protein, which is synthesized from the late