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 CCI₄. 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 CCI₄ treatment. Hepatic RNA levels, however, reduced significantly after CCI₄ administration. Observations of groups III and IV animals indicate that the slurry of *P. debilis* moderates significantly the CCI₄-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 CCI₄-induced damage. The histoarchitecture of the liver of rats administered plant slurries is closer to that of control animals than of animals which underwent 6 days of recovery after CCI₄ treatment. It has been observed that removal of necrotic debris starts by 48 h after CCI₄ administration and is usually complete by one week.

Many compounds cited in the literature exhibit liver protection against CCI₄ either by decreasing the production of CCI₄ free radicals or by impairment of CCI₄-induced lipid peroxidation. The improved histology of liver as seen in histopathological observations on animals treated with plant slurries as compared to that seen in animals administered only CCI₄ indicates the possibility of both these plant slurries 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
second instar stage onwards, is stored in the form of secretory granules or vacuoles in the glandular cells and secreted into the gland lumen and later extruded to the exterior shortly before puparium formation. The salivary gland secretion is said to be involved in cementing the puparium to a solid surface. Electrophoretic analysis of glue proteins in different species of Drosophila has provided knowledge with regard to the extent of its ontogenetic, intraspecific and interspecific variations in nature and patterns. During our investigations on glue proteins, we found that the salivary glands in the third instar larvae of different species of Drosophila vary in size. Enlargement of the gland is associated with increased secretory activity.

Preliminary investigations on the quantitative variation of this protein in D. melanogaster and in a few other species of Drosophila have revealed that it is produced in varying amounts. In view of this, the present investigations were undertaken on 15 species of Drosophila belonging to three taxonomically different groups to determine whether there is any relationship between the size of the larval salivary glands and the quantity of glue protein synthesized and to analyse the extent of intragroup, intergroup and interspecific differences in the glue protein production, if any.

Table 1 gives the list of Drosophila species used in the present study. To maintain uniformity with regard to the density and age of the larvae, 50 eggs collected by modified Delcour technique were transferred to the culture vials (7.5 x 2.5 cm) containing equal quantities of wheat cream agar medium. After the larvae hatched out, extra yeast was added every alternate day (50 µl/vial) for feeding them and to maintain moisture in the cultures. The cultures were raised at a constant temperature of 22 ± 1°C.

To determine the number of cells present in the salivary glands, the third instar larvae were dissected in medium A to isolate the glands. The glands were briefly fixed in 1 N HCl and later transferred to 2% lactic-acetoceorcin stain. After 5 min, the glands were placed on a clean slide and gentle pressure was applied on them through the cover glass placed on the glands to facilitate the spreading of cells. Since the larval salivary gland cells of Drosophila are uninnucleate, the number of nuclei in the glandular region were counted under low magnification using a binocular research microscope to determine the number of cells constituting the salivary glands. Ocular and stage micrometers were used to measure the length and breadth of the salivary glands of the late third instar larvae. The glands in which the secretions had not yet poured into the lumen of the gland were taken for making the measurements. The ‘length’ includes the measurement from the end of the glands up to the glandular duct and the ‘breadth’ includes the measurement at the centre of the lobe.

Table 1. Relationship between the size, no. of cells and the quantity of secretion proteins (glue proteins) of salivary glands in different species of Drosophila

<table>
<thead>
<tr>
<th>Species</th>
<th>Size of glands</th>
<th>No. of cells</th>
<th>Percentage glue</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. melanogaster group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>melanogaster subgroup</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. melanogaster</td>
<td>0.425</td>
<td>129.9 ± 2.06</td>
<td>28.57 ± 1.66</td>
</tr>
<tr>
<td>D. simulans</td>
<td>0.375</td>
<td>129.0 ± 1.97</td>
<td>55.55 ± 1.88</td>
</tr>
<tr>
<td>D. mauritana</td>
<td>0.450</td>
<td>129.9 ± 1.55</td>
<td>60.00 ± 1.67</td>
</tr>
<tr>
<td>D. yakuba</td>
<td>0.375</td>
<td>146.0 ± 2.06</td>
<td>55.55 ± 1.44</td>
</tr>
<tr>
<td>ananassae subgroup</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. ananassae</td>
<td>0.300</td>
<td>128.9 ± 1.6</td>
<td>48.15 ± 2.24</td>
</tr>
<tr>
<td>suzuki subgroup</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. repleta group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hydei subgroup</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. hydei</td>
<td>1.250</td>
<td>163.8 ± 1.89*</td>
<td>35.40 ± 1.41</td>
</tr>
<tr>
<td>D. immigrans group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>immigrans subgroup</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>D. immigrans</td>
<td>1.800</td>
<td>130.1 ± 1.17</td>
<td>62.82 ± 1.70</td>
</tr>
<tr>
<td>hypocausta subgroup</td>
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<td></td>
</tr>
<tr>
<td>D. rubida</td>
<td>2.100</td>
<td>130.0 ± 1.45</td>
<td>60.00 ± 0.65</td>
</tr>
<tr>
<td>D. pararubida</td>
<td>1.960</td>
<td>130.0 ± 1.29</td>
<td>57.50 ± 0.91</td>
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<tr>
<td>nasuta subgroup</td>
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<tr>
<td>D. nasuta nasuta</td>
<td>0.726</td>
<td>129.4 ± 1.03</td>
<td>58.06 ± 1.21</td>
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<tr>
<td>D. n. albicans</td>
<td>0.840</td>
<td>130.7 ± 0.94</td>
<td>60.00 ± 1.45</td>
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<tr>
<td>D. n. kephiliana</td>
<td>0.720</td>
<td>128.1 ± 1.15</td>
<td>59.37 ± 1.08</td>
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<tr>
<td>D. s. sulfurigaster</td>
<td>0.736</td>
<td>129.6 ± 1.17</td>
<td>56.25 ± 1.21</td>
</tr>
<tr>
<td>D. m. nasuta</td>
<td>0.600</td>
<td>130.0 ± 1.33</td>
<td>55.17 ± 1.59</td>
</tr>
</tbody>
</table>

1. The average size of a single lobe of a salivary gland in mm² (n = 20).
2. The average number of cells in a single lobe of a salivary gland (n = 20).
3. Relationship between the total gland proteins (including the glue plugs) and only the secretory proteins.
4. Statistically significant at 5% level.
For the determination of the quantity of glue proteins, the larvae which were about to pupate were selected and two kinds of samples were prepared following the procedure described by Ramesh and Kalisch: (i) the secretion along with the gland tissue and (ii) only the secreted glue, which was isolated by dissecting the glandular cells from 95% ethanol-fixed bloated salivary glands of the late third instar larvae using a pair of extra-fine needles. The quantity of protein was determined by micromethods with bovine serum albumin (BSA) as the standard. The difference in the protein quantity between the two kinds of samples provides the amount of glue produced; therefore, the percentage of protein constituting the secretion was calculated.

Table 1 shows the species of Drosophila used in the present study, the data on the size of the larval salivary glands (length × breadth), the number of cells present in the glands and the percentage of glue proteins synthesized.

The increase in the size of an organ may occur either due to hyperplasia and/or hypertrophy. Perusal of Table 1 reveals that among the species analyzed, the size of the larval salivary glands ranges from a maximum of 2.1 mm² in D. rubida to a minimum of 0.3 mm² in the case of D. ananassae. The larval salivary glands of Drosophila, which develop from the lateral discs of the developing embryo, consist of a limited number of cells and the growth of these glands occurs due to an increase in the cell volumes of both duct and glandular cells. Perusal of the literature reveals that the larval salivary glands in D. melanogaster as well as in D. hydei, D. subobscura and D. simulans consist of an average of 128 cells/lobe. From the present analysis (Table 1) it is evident that, though there is a lot of variation in the size of the glands, the number of cells constituting the glandular part of the salivary glands in different species of Drosophila do not vary significantly except in the case of D. hydei. The number of cells were found to be highest in D. hydei, being 163 ± 1.89/lobe. While in all other species the number of cells/lobe ranges from 128 ± 1.5 to 130.7 ± 0.94, the glands of D. yakuba have 146 ± 2.06 cells/lobe.

Interspecific comparisons of the variation in the number of cells in the salivary glands were made. Such an analysis revealed that except for the comparison made between D. hydei and D. yakuba, the extent of variation in D. hydei when compared to all the other species is statistically significant at 5% level (Student’s t test). The present investigations reveal that the number of cells per lobe in the case of D. hydei is higher than the one reported earlier. Further, all other species wise comparisons made by application of Student’s t-test revealed that the variation in cell number is statistically insignificant. Differences in culture conditions and genotype may influence the final cell number in the salivary glands. In the present study though the larvae of different species of Drosophila are raised under uniform conditions of temperature, humidity, food and larval density, their salivary glands attain different sizes before they pupate. This variation in the size of the salivary glands in the case of D. hydei could partly be due to increase in cell number. In the other species under study, it is evident that increase in the size of the salivary glands is due to increase in cell volume and not due to increase in cell number, since the variation in cell number is statistically insignificant (see Table 1). A similar situation wherein the growth of the salivary glands is found to be the result of hypertrophy and not hyperplasia has been reported in Chironomus.

Perusal of the literature reveals that the increase in the size of the glands is associated with increased secretions of the respective hormones/proteins. Among the species which belong to melanogaster group (Table 1), it is observed that the quantity of glue protein synthesized is minimum in D. melanogaster, constituting 28.57% of the total protein content of the salivary gland. The amount of secretions synthesized by the larval salivary glands of its closely related species, namely D. simulans, D. mauritiana and D. yakuba, is double of that in D. melanogaster, though the gland size in D. simulans and D. yakuba is smaller and in D. mauritiana slightly larger. While D. ananassae, which also belongs to the melanogaster species group, possesses salivary glands which are the smallest among all the other species analyzed, it synthesizes glue proteins that constitute 48.15% of the total salivary gland proteins. Such quantitative estimations in the case of D. rajasekari could not be predicted even with a variety of protein precipitating agents the secretions of salivary glands could not be obtained as plugs.

The species D. n. nasuta, D. n. albomicans, D. n. kexpulaana, D. s. sulfurigaster, D. s. neonasa, D. immigrans, D. rubida and D. pararubida included in the present study belong to the immigrans group. Among these, the maximum quantity of glue (62.82%) was seen to be synthesized in the case of D. immigrans and minimum in the case of D. s. neonasa (55.17%). Though the salivary glands of D. immigrans, D. rubida and D. pararubida are 2–3 times larger than those of the nasuta subgroup species and 4–5 times larger than those of D. simulans, D. mauritiana and D. yakuba of the melanogaster group, the proportion of glue produced is almost the same. The amount of glue synthesized by the larval salivary glands of species belonging to the immigrans group is double compared to that in D. melanogaster. In contrast, the secretions constitute only 35.4% in the case of D. hydei (repleta group) in spite of the fact that the number of cells constituting the glandular part of the salivary glands is significantly higher and is more than one-and-a-half times larger than that of the nasuta subgroup species, and three times larger in gland size than melanogaster species group. Further, our results on the quantity of glue proteins in D. melanogaster, D. hydei,
D. n. nasuta and D. n. albomicans are similar to the ones reported earlier.4,16

From the results of the present investigations it is clear that the quantity of glue protein synthesized in different species varies. Except in the case of D. hydei and D. ananassae, in all the other species analysed the proportion of glue protein produced is double or more compared to that in D. melanogaster. Though the salivary glands of D. hydei have more number of cells and larger size, it produces around half the quantity of glue compared to the other species. D. yakuba produces the same proportion of glue proteins as in most of the species though the number of cells in the glands is slightly higher.

The coefficient of correlation analysis (r = 0.3) revealed that the increased size of the glands has no correspondence with the quantity of glue protein production, which means that the increase in size of the salivary glands is not always associated with increase in secretions. The correlation plots made between the percentage of glue produced and the size of the larval salivary glands fall into two clusters. D. melanogaster and D. hydei do not belong to any one of the clusters (Figure 1). The regulatory elements/factors with different specificities could account for the differential synthesis of glue proteins in various species of Drosophila. The importance of the increased quantities of glue protein synthesis in species other than D. melanogaster and D. hydei is being investigated.

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