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 CCl_4. It is reported that galactosamine, a hepatotoxicant, causes reduction in liver RNA and protein synthesis[16]. In the present study no significant changes have been recorded in the liver DNA levels after CCl_4 treatment. Hepatic RNA levels, however, reduced significantly after CCl_4 administration. Observations of groups III and IV animals indicate that the slurry of *P. debilis* moderates significantly the CCl_4-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 CCl_4-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 CCl_4 treatment. It has been observed that removal of necrotic debris starts by 48 h after CCl_4 administration and is usually complete by one week[15].

Many compounds cited in the literature exhibit liver protection against CCl_4 either by decreasing the production of CCl_3 free radical[17] or by impairment of CCl_4-induced lipid peroxidation[18,19]. 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 CCl_4 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[20]. 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 hepatotonic 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*[1]. This protein, which is synthesized from the late

second instar stage onwards[2], 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[3–5]. The salivary gland secretion is said to be involved in cementing the puparium to a solid surface[6–10]. 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[11–14]. 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[15].

Preliminary investigations on the quantitative variation of this protein in *D. melanogaster*[4, 7, 16] and in a few other species of *Drosophila*[16] 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[17] were transferred to the culture vials (7.5 × 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[18] to isolate the glands. The glands were briefly fixed in 1 N HCl and later transferred to 2% lactoacetoorcein 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 uninucleate, 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*

Species	Size of glands[1]	No of cells[2]	Percentage glue[3]
***D. melanogaster* group**			
melanogaster subgroup			
D. melanogaster	0 425	129 9 ± 2 06	28 57 ± 1 66
D. simulans	0 375	129 0 ± 1 97	55 55 ± 1 88
D. mauritiana	0.450	129 9 ± 1 55	60 00 ± 1 67
D yakuba	0 375	146 0 ± 2 06	55 55 ± 1 44
ananassae subgroup			
D ananassae	0.300	128 9 ± 1 6	48 15 ± 2 24
suzuki subgroup			
D rajasekari	0 450	131 8 ± 1 03	—
***D. repleta* group**			
hydei subgroup			
D hydei	1 250	163 8 ± 1 89*	35 40 ± 1 41
***D. immigrans* group**			
immigrans subgroup			
D immigrans	1 800	130 1 ± 1 17	62 82 ± 1 70
hypocausta subgroup			
D rubida	2 100	130 0 ± 1 45	60 00 ± 0 65
D pararubida	1 960	130 0 ± 1 29	57.50 ± 0 91
nasuta subgroup			
D nasuta nasuta	0 726	129 4 ± 1 03	58 06 ± 1 21
D. n albomicans	0 840	130 7 ± 0 94	60 00 ± 1 45
D n kepulauana	0 720	128 1 ± 1 15	59 37 ± 1 08
D s. sulfurigaster	0 736	129 6 ± 1 17	56 25 ± 1 21
D s neonasuta	0 600	130 0 ± 1 33	55 17 ± 1 59

[1] The average size of a single lobe of a salivary gland in mm^2 ($n = 20$)
[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
*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[2]: (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[19] 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; therefrom 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[20]. Perusal of Table 1 reveals that among the species analysed, the size of the larval salivary glands ranges from a maximum of 2.1 mm^2 in *D. rubida* to a minimum of 0.3 mm^2 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[1]. Perusal of the literature reveals that the larval salivary glands in *D. melanogaster*[1] as well as in *D. hydei*, *D. subobscura* and *D. simulans*[21, 22] 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[21]. Further, all other specieswise 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[23, 24]. 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*[25].

Perusal of the literature reveals that the increase in the size of the glands is associated with increased secretions of the respective hormones/proteins[15]. 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[26,27], 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 analysed, 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 made because 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. kepulauana*, *D. s. sulfurigaster*, *D. s. neonasuta*, *D. immigrans*, *D. rubida* and *D. pararubida* included in the present study belong to the *immigrans* group[28]. 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. neonasuta* (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*,

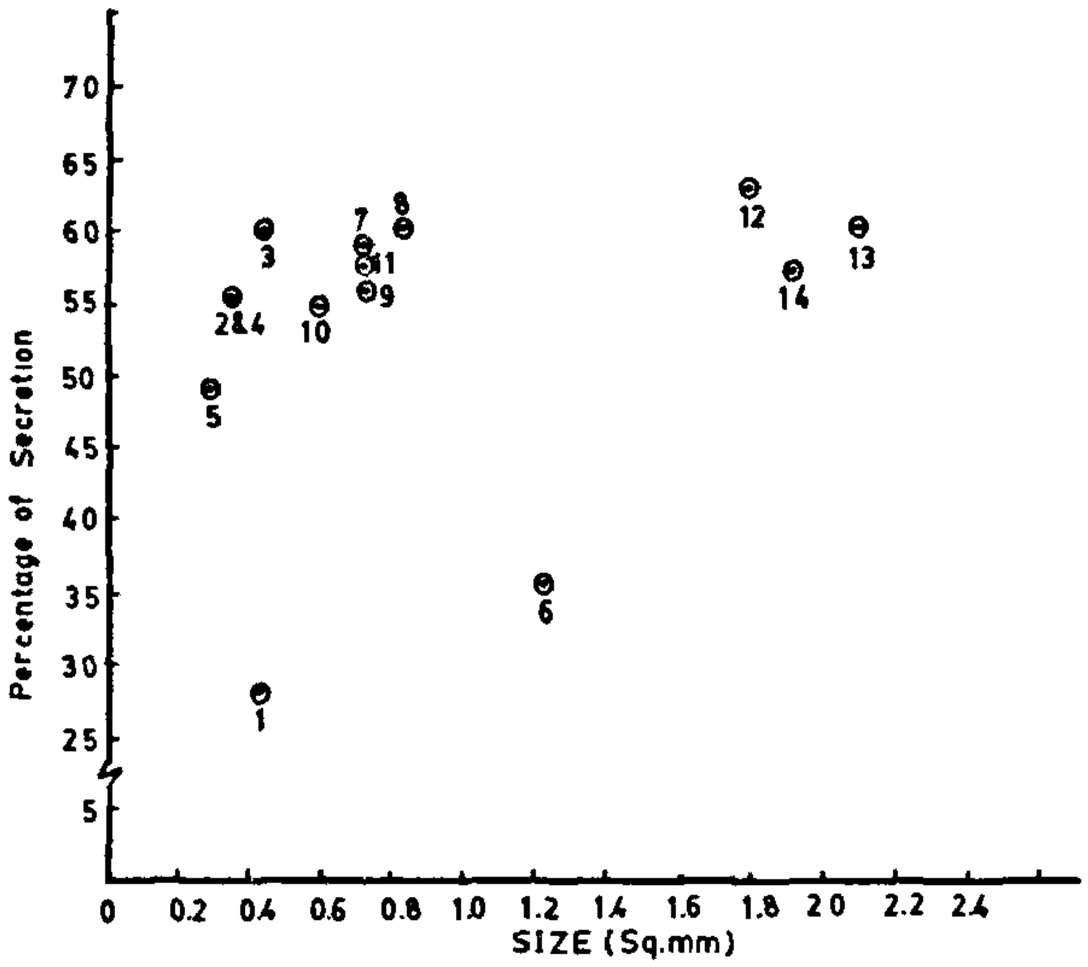

Figure 1. Percentage of glue protein in the late third instar larvae of different species of *Drosophila* in relation to the size ($L \times B$) of larval salivary glands 1, *D melanogaster*, 2, *D. simulans*, 3, *D. mauritiana*, 4, *D yakuba*, 5, *D ananassae*; 6, *D. hydei*, 7, *D. n nasuta*, 8, *D n albomicans*, 9, *D n kepulauana*, 10, *D s neonasuta*, 11, *D s. sulfurigaster*, 12, *D. immigrans*, 13, *D. rubida*, 14, *D. pararubida*

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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