

Significance of style in cardamom corolla tubes for honey-bee pollinators

V. V. Belavadi, Venkateshalu and H. R. Vivek

Regional Research Station (UAS Bangalore), Mudigere 577 132, India

Cardamom flowers offer nectar in a corolla tube which is 23 (± 2.08) mm long and the style passes through the corolla tube. The honey-bee pollinators, *Apis cerana* and *A. dorsata*, despite their short tongue lengths (4.5 and 5.5 mm, respectively) drew nectar up to 11.45 (± 2.65) and 11.65 (± 1.85) mm. In controlled experiments, using capillary tubes of similar dimensions as cardamom corolla tubes, the depth of feeding by the two species of bees corresponded to their tongue lengths when there was no style. However, when a natural or an artificial style was introduced into the capillary tube the depth of feeding increased with increase in style thickness. We show here that the presence of style inside the corolla tube helps bees to draw more nectar from cardamom flowers. We also suggest that the plant facilitates pollinators to draw more nectar than it is possible with their short tongues, by keeping the style within, and that the thickness of style may have some significance in the evolution of the system.

The tubes of corolla of the common red and incarnate clover (*Trifolium pratense* and *incarnatum*) do not on a hasty glance appear to differ in length, yet the hive bee can easily suck the nectar out of incarnate clover but not out of the common red clover, which is visited by humble bees alone....

Charles Darwin, 1859

PLANTS that depend on insects for pollination offer nectar and/or pollen, and in some cases oils, as reward. Nectar is presented in the corolla tubes or in special nectaries in ways that make it available only to specific pollinator species. Several authors, including Darwin, have appreciated the differences in corolla lengths of flowers among plant species as regulators of pollinator visitation¹. Ever since Darwin, there have been several reports to corroborate this and it is now established that long-corolla flowers are visited by long-tongued insects like butterflies, bumble bees as well as birds, and short-corolla flowers by short-tongued bumble bees and honey-bees²⁻⁵.

In this paper we present a strategy used by the plant that facilitates pollinators to draw nectar from corolla tubes that are longer than the pollinators' harvesting organ. We argue that this strategy has implications for the evolution of the style feature.

Cardamom (*Elettaria cardamomum* Maton) grows in clumps of 20–25 pseudostems. New pseudostems bear panicles at the base on the second year, and flowers are borne on these panicles at the ground level; these

bloom between April and September with a peak during July–August. Anthesis occurs in the early morning hours (0430 to 0630 h) and anthers dehisce between 0730 and 0830 h (refs 6, 7). Stigma receptivity peaks at 12 noon⁶ and coincides with peak pollinator activity⁸. The flower lasts almost for a day, and withers off by 2000 h (refs 7, 8). Two species of honey-bees, *Apis cerana* and *A. dorsata*, constitute more than 98% of all the flower visitors, and are the true pollinators of cardamom^{8,9} (Figure 1). Cardamom flower has a corolla tube length of 23 mm (± 2.08). Nectar production is continuous and the corolla tube gets completely filled up by 0900 h (4.5 μ l, sugar concentration: 20%) if left unharvested¹⁰. In our earlier studies^{8,10} on cardamom pollination, we recognized a specific problem in the harvesting of nectar by certain insects: The pollinators (*A. cerana* and *A. dorsata*) have short proboscis (4.5 mm and 5.5 mm respectively), and hence can never exhaust the available nectar, but despite their short proboscis, they manage to draw nectar up to 11.5 mm depth, which is more than double the length of their proboscis. In the present paper, we attempt to provide a solution to this problem through field observations and controlled laboratory experiments.

All field and laboratory studies were conducted at the Regional Research Station, Mudigere, India (13°7'N and 75°37'E) during 1995–96.

We covered a set of 100 flowers with paper packets early morning (6 am) before the commencement of bee activity. At 11 am the mean nectar level in the corolla tube was 20 (± 1.26) mm. Fifty of these flowers were allowed a single visit by *A. dorsata* foragers and for the remaining 50, a single visit by *A. cerana* foragers. Immediately after the bee left, the flower was removed and the nectar level in the corolla tube was measured nearest to a mm, by holding the flower against light.

We first trained foragers from a colony of *A. cerana* to a feeding station in the laboratory. We used 20% sucrose solution (similar to cardamom nectar concentration) at the feeding station as well as in all subsequent experiments. Once there was a significant and steady recruitment of bees to the feeding station, we shifted the method of offering the sugar solution to a feeding platform, consisting of a petri plate (150 mm dia) covered with a thick yellow paper board. The yellow board had 25 holes (1.5 mm dia in a 5 \times 5 grid), into which we inserted capillary tubes (15 mm long \times 1.5 mm dia) open at either ends. The solution rose into the capillary tubes, offering sugar solution constantly. The purpose was to train bees to take sugar solution from the capillary tubes for our further experiments. The sucrose solution was changed on alternate days.

We conducted five experiments using 23 mm \times 1.5 mm capillary tubes, to simulate cardamom corolla tubes. One end of each capillary tube was closed with wax and the tubes were filled with 20% sugar solution. We fixed

these tubes on a yellow paper board covering a petri plate (150 mm dia) in 5×5 array of 25 tubes spaced equidistant from each other, similar to the feeding station. In the first experiment, we used only the capillary tubes. Since, in the cardamom flower, the style passes through the corolla tube, we hypothesized that the presence of style in the corolla tube might in some way help bees to draw more nectar. Hence, in our further four experiments, we used the cardamom style, the style from a tubular flower plant, *Ixora*, and two copper wires of thickness 0.1 and 0.4 mm. The thickness of cardamom style was 0.2 mm and that of *Ixora* 0.25 mm. Thus, we had four style or style-like structures with thickness of 0.1, 0.2, 0.25 and 0.4 mm, which we compared with no style. We repeated each experiment four times (Figure 2).

Similar experiments were conducted with *A. dorsata*. Since we failed in our attempts to train workers to come to a feeding station, we enclosed twenty foragers of this species in a cage $30 \times 30 \times 30$ cm and conducted



Figure 1. *Apis cerana* forager harvesting nectar from a cardamom flower.



Figure 2. Artificial flower used in laboratory experiments.

all our experiments after starving them for at least two hours.

A. cerana and *A. dorsata* did not differ significantly with reference to the depth to which they drew nectar from cardamom flowers ($z = 1.08$; $n = 50$ for each species), but they harvested nectar from much lower depths than their tongues could reach! (Figure 3).

In experiments with trained foragers of *A. cerana* and with caged foragers of *A. dorsata*, the depth of feeding was least when the sucrose solution was offered in capillary tubes without any style (Figure 4). However, the feeding depth differed significantly between the two species ($z = 2.54$; $P = 0.05$), but the levels corresponded almost to the lengths of their probosces. Thus, presence of style in the corolla tubes appears to help bees in drawing more nectar. In fact, when we inserted styles from cardamom flowers in capillary tubes, the depth of feeding rose to 10.94 ± 1.29 mm ($n = 97$) and 11.06 ± 1.34 mm ($n = 92$), respectively for *A. cerana* and *A. dorsata*. This indicated that the bees take advantage of the presence of style in the corolla tube of cardamom. The difference in depth of feeding between the two species was not significant ($z = 0.36$). The depth of feeding increased from 5.76 ± 0.87 mm ($n = 85$) and 6.46 ± 1.01 mm ($n = 89$)

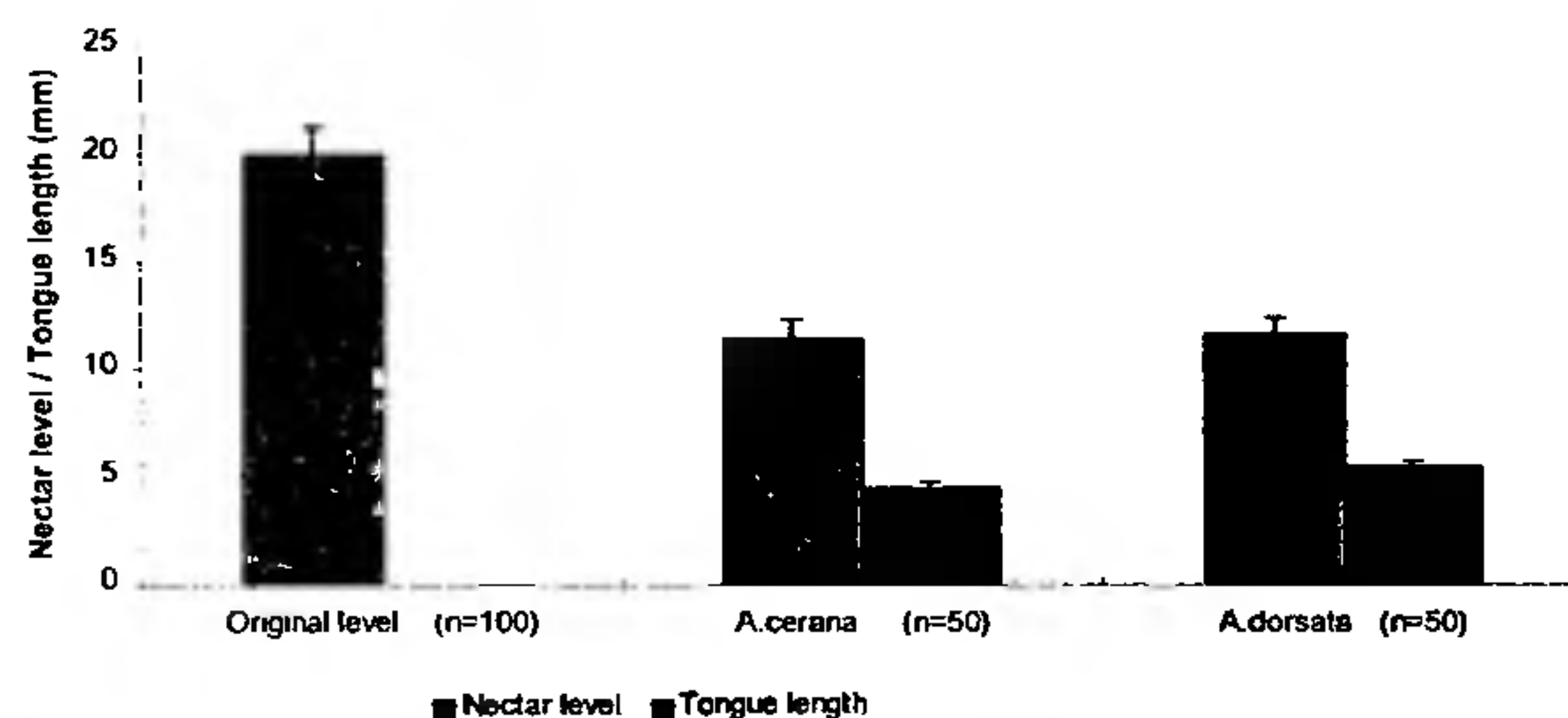


Figure 3. Depth of feeding by honey-bees from cardamom flowers. Median lines indicate standard deviations.

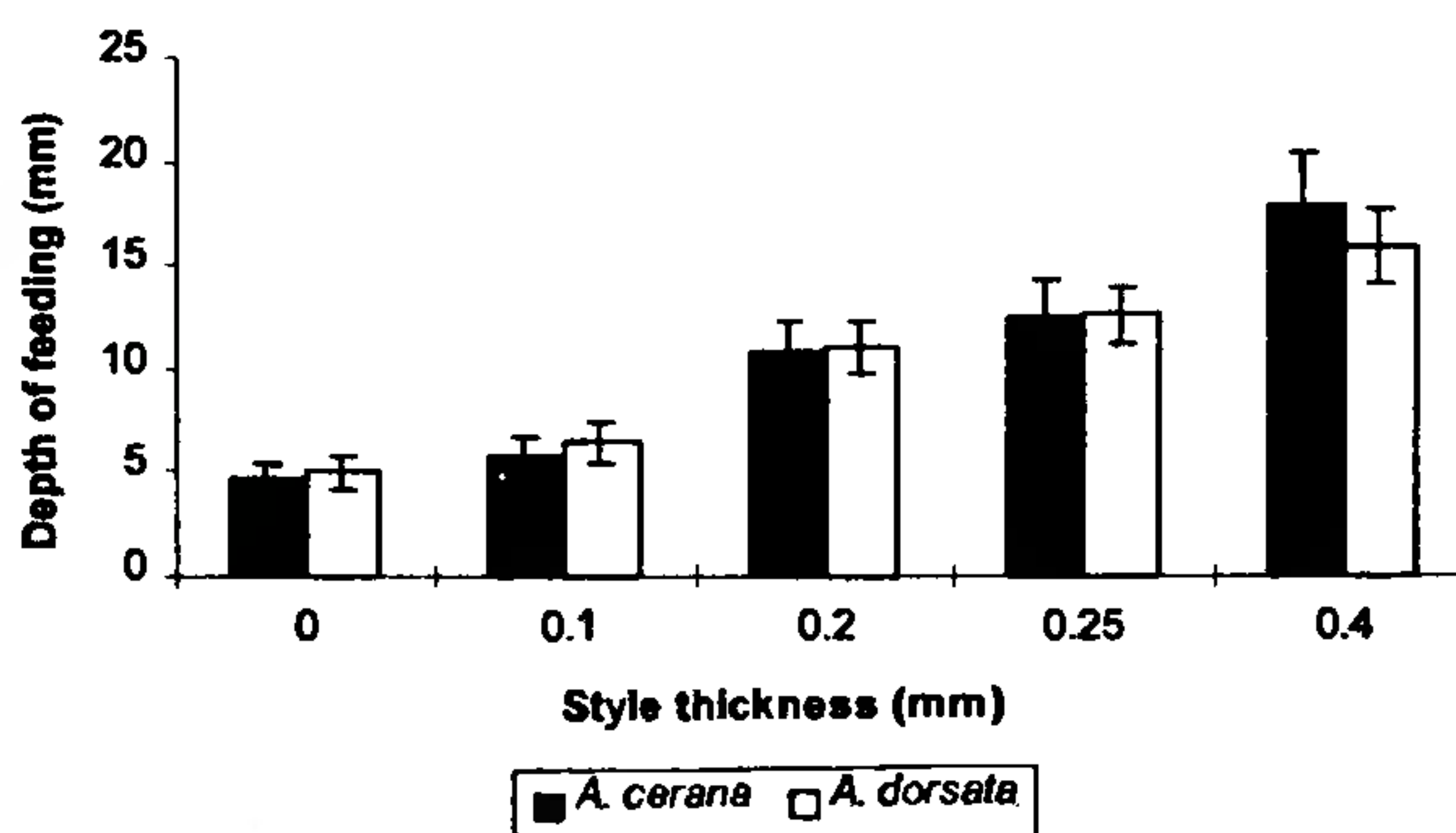


Figure 4. Depth of feeding from capillary tubes by honey-bees in relation to thickness of style. Median lines represent standard deviations of feeding depths.

with 0.1 mm style, to 17.86 ± 2.64 mm ($n=72$) and 15.98 ± 1.82 mm ($n=69$), for 0.4 mm style for the two species (Figure 4). The depth of feeding differed significantly between the two species of bees with 0.1 mm ($z=2.91$, $P=0.05$) and 0.4 mm ($z=5.37$, $P=0.05$) thick styles, while it was not significant for 0.2 and 0.25 mm thick styles. Thus, the depth to which bees could harvest nectar appears to be a function of size of style in relation to the corolla tube diameter.

We attribute this to surface tension forces acting upon the liquid and to a certain extent to cohesive and adhesive forces. The extent to which a liquid rises in a capillary tube is inversely related to the radius of the tube. If r is the radius of the capillary/corolla tube, h the height of the liquid, τ the density of the liquid and g the acceleration due to gravity, the surface tension (T) which is responsible for the rise of a liquid to a height (h) follows the equation,

$$T = \tau g r / 2 \cos \theta (h + r/3).$$

Since, for a liquid that wets the tube, $\cos \theta = 1$

$$\begin{aligned} T &= 1/2 \tau g r (h + r/3) \\ &= 1/4 \tau g d (h + d/6), \end{aligned}$$

where d is the diameter of the capillary/corolla tube. When a style passes through the corolla tube, the effective diameter of the tube is reduced correspondingly. If the thickness of the style is x then the effective diameter of the tube becomes $(d-x)$ or d' .

Then, $T = 1/4 \tau g d' (h + d'/6)$,

$$\begin{aligned} \text{or } h &= 4T/\tau g d' - d'/6 \\ &= 4T/d' (\tau g - 1/6) \\ &= K_1/d' (K_2), \end{aligned}$$

where K_1 and K_2 are constants.

In short, the rise in the level of the liquid (h) becomes inversely related to the effective diameter of the tube as shown in Figure 5.

With reference to the cardamom corolla tube and all our experiments with capillary tubes, it should be noted that the lower end of the capillary tube was closed. When a style is present, the bee invariably inserts its proboscis in the gap between the style and the wall of the capillary or corolla tube to draw the liquid. The feeding mechanism in honey-bees involves a stomodeal pump located in the head connected to the pharynx¹¹. For the pump to be effective, the proboscis should be in contact with the liquid. It is reasonable to assume that this is achieved by the reduction in radius of the tube by the presence of the style. Further, a liquid that is rising in a column establishes a cohesive force between the molecules of the liquid, resulting in a continuous column. In the presence of a style, the area of adherence increases, and thicker the style, smaller will be the effective radius of the corolla tube, and therefore, greater are the surface tension and cohesive forces, resulting in a higher nectar level which can be pumped up by the bee.

Our study suggests that relative diameters of nectar tube and style are important in regulating the rewards available for the pollinators. A relatively thick-styled system favours pollinators because they could harvest more nectar from each flower, need to visit fewer flowers in each bout and fewer bouts for meeting their daily energy needs. This would obviously be a loss to the plant, because it reduces its pollen transfer efficiency.

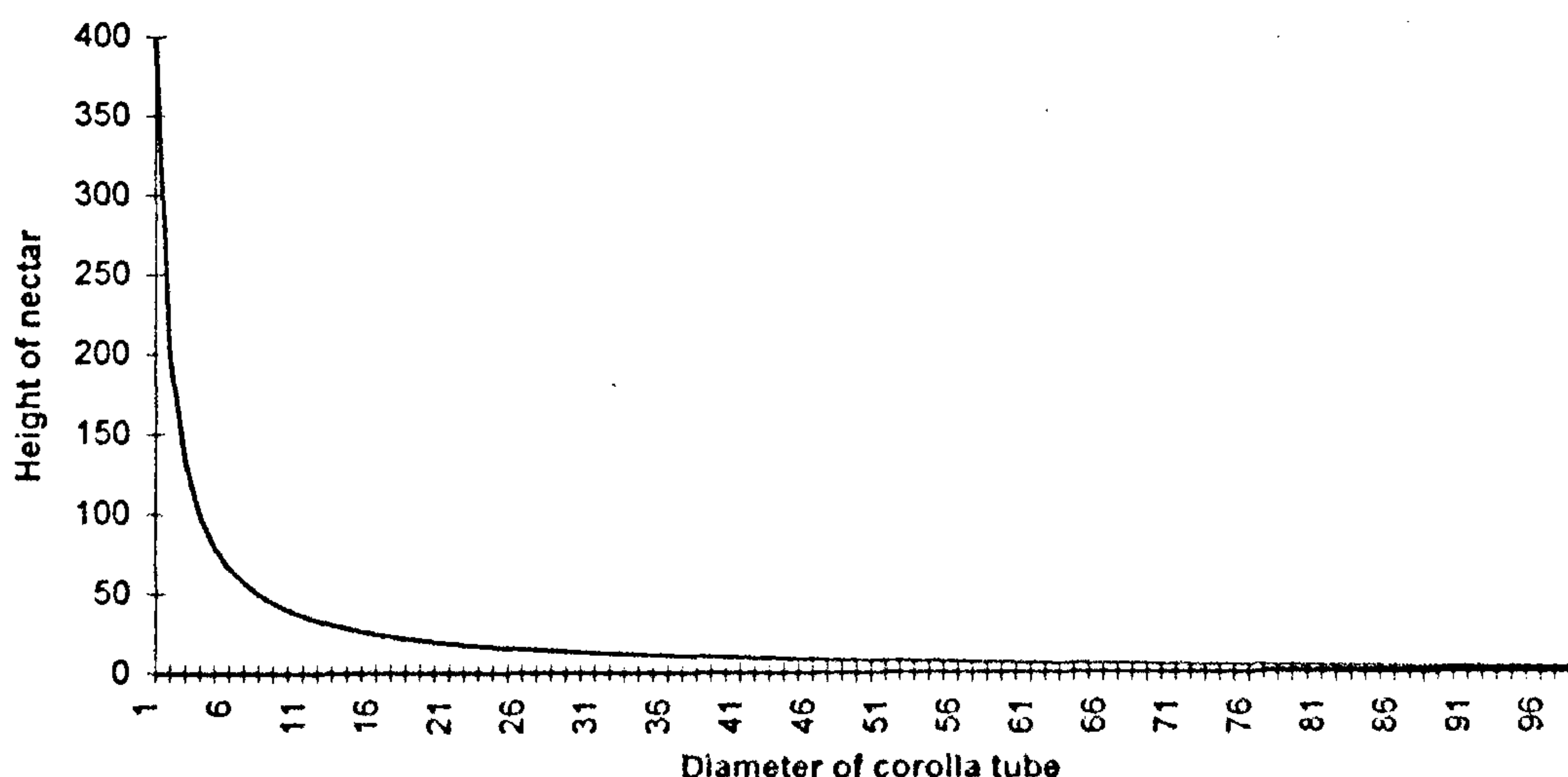


Figure 5. Expected inverse relation between height to which nectar rises and the diameter of nectar tube. A style passing through the corolla tube reduces the inner diameter effectively.

On the other hand, a relatively thin style ensures repeated visits of pollinators to the flower though too thin a style might also discourage pollinator visitation. Hence, the style diameter can be expected to be a tradeoff between these risks and benefits. Thus, our study offers an hitherto unknown parameter that might have shaped the evolution of styles and nectar tubes.

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Nicotinamide and α -tocopherol combination partially protects *t*-butyl hydroperoxide-induced neurotoxicity: Implication for neurodegenerative disease

M. S. Parihar*, Y. Manjula**, Saira Bano*, Taruna Hemnani*, Tarangini Javeri* and Prem Prakash*

*Biochemistry Division, School of Studies in Zoology, Vikram University, Ujjain 456 010, India

**Department of Chemical Engineering, Indian Institute of Technology, Mumbai 400 076, India

In light of evidences of impaired energy metabolism and oxidative damage in neurodegenerative disorders, we have investigated the potential role of compounds which may improve the mitochondrial metabolism and can ameliorate the toxic effects of free radicals, and thus compensate for disease-related defects. Treatments with three doses of *t*-butyl hydroperoxide (*t*-BuOOH) resulted in increased lipid peroxidation in corpus striatum. The superoxide dismutase (SOD, EC 1.15.1.1) activity increased while reduced glutathione content declined. Supplementation with α -tocopherol and nicotinamide and their combination resulted in decline in LPO and showed the tendency towards normalization of SOD activity and GSH content. The results showed that supplementation of a combination of α -tocopherol and nicotinamide provides protection against *t*-BuOOH-induced neurotoxicity.

THERE are considerable experimental evidences to support

age-related changes in general^{1,2} and aging of central nervous system (CNS) in particular^{3,4} which are inflicted by oxygen-free radicals such as superoxide anion radical ($O_2^{\cdot-}$), hydroxyl radical ($\cdot OH$), peroxy radical (RO_2^{\cdot}), alkoxyl radical (RO^{\cdot}) and its non radical derivatives such as hydrogen peroxide (H_2O_2), singlet oxygen (1O_2) and hypochlorous acid ($HOCl$)⁵. The brain is highly vulnerable to attack by oxygen-free radicals during aging owing to high lipid contents and low level of antioxidants to neutralize these molecular renegades along with utilization of large amounts of O_2 (refs 4, 6). Further, strong evidence to indicate that defect in mitochondrial energy metabolism plays an intrinsic role in several neurodegenerative disorders⁷⁻⁹, is emerging. Reduced complex I activity has been reported in Parkinson's disease patients¹⁰. In Huntington's disease, multiple enzyme defects have been found^{7,11,12}. Reduction in complex IV activity was reported in Alzheimer's disease patients¹³⁻¹⁵. One potential mechanism whereby mitochondrial dysfunction could occur is due to increased generation of free radicals and oxidants^{12,16}. Impairments of mitochondrial energy metabolism will result in interruption of oxidative phosphorylation, which results in decreased ATP production¹⁷. Under circumstances of electron transport chain inhibition or molecular defects, the levels of free radicals production increases^{9,10,18,19}. Mitochondrial complex I appears to be the most sensitive. The ability of α -tocopherol (T), a hydrophobic antioxidant to stop the chain reaction initiated by free radicals, has been demonstrated in a wide variety of paradigms^{3,20,21}.