

(72 h) to vitamin A solution. Hence, it is concluded that 48–72 h was the required duration of treatment for the development of ectopic limbs as has also been reported for *R. temporaria*<sup>17</sup> (3 days as the threshold level) and for *P. maculatus*<sup>14</sup> (48 h as the minimum exposure time). However, in *P. maculatus*<sup>12</sup>, *T. rolandae*<sup>15</sup> and *R. limnocharis*<sup>13</sup> ectopic limbs developed when the exposure time was for 24 h. Thus, it can be inferred that the induction of ectopic limbs is not time-dependent as otherwise proposed by Maden<sup>17</sup>.

It has been interpreted that hindlimb development is marked by the rising levels of thyroid hormone (TH)<sup>20</sup>. It is likely that during the regeneration process<sup>21</sup> at the amputated site, the TH combines with vitamin A palmitate and alters the tail hox code while repressing other genes resulting in the formation of limb blastema cells, particularly the ones that give rise to hindlimb fields<sup>17</sup>.

The effect of vitamin A palmitate at the tail amputated site of tadpoles causing homeotic transformation may be attributed to the conversion of the tail blastema cells to that of limbs. This changes the positional values at the tail tip. Subsequently, rearing in conditioned water after exposure to vitamin A, reverses the polarity along the rostral–caudal axis to generate ectopic limbs<sup>22</sup>. Some of the ectopic limbs develop from cellular mass at the site of amputation and mostly in a ventro-lateral direction similar to those observed in *P. maculatus*<sup>12,14</sup> and *T. rolandae*<sup>13,15</sup>. This is comparable to the finding in *B. andersonii*<sup>6</sup> in which continued exposure to vitamin A palmitate resulted in the folding of the fin to form a pouch at the tip of the regenerating tail. The cellular mass which is formed prior to the development of ectopic limbs may act as a reservoir for the transformed cells which needs to be investigated through histology. There was also regeneration of a complete tail that followed the growth of ESLs in *B. melanostictus* tadpoles as reported in *P. maculatus*<sup>12</sup>, *T. rolandae*<sup>15</sup> and *R. limnocharis*<sup>13</sup>. This is expected, in accordance with the interpretation schematically proposed by Bryant and Gardiner<sup>22</sup>. However, the interpretation of development of paired ectopic hindlimbs<sup>22</sup> is contradictory to the present finding as odd number (3) of ectopic limbs has been observed in *B. melanostictus* tadpoles as was also found in *T. rolandae*<sup>15</sup> and *R. limnocharis*<sup>13</sup>. Further, with the growth of an abnormally regenerated tail, there was also the development of extra-paired hindlimbs at the site from where only a single pair of hindlimb was supposed to develop. This observation was previously reported in *B. vulgaris*<sup>23</sup> and again in *U. systoma*<sup>9</sup>. These deformities could be related to the embryonal malformations caused by vitamin A<sup>24</sup>. In addition to the findings on *U. systoma*<sup>9</sup>, *R. temporaria*<sup>17</sup>, *T. rolandae*<sup>15</sup>, *R. limnocharis*<sup>13</sup> and *P. maculatus*<sup>10,12,14</sup>, the present study adds further to our knowledge on homeotic transformation in anuran tadpoles.

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## How does the nocturnal animal, *Mus booduga*, programme its activity in response to varying durations of light and darkness?

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In the temperate zone, animals adjust to various durations of light and dark by scaling the duration of activity with the durations of light (diurnal animals) or dark (nocturnal animals). They are also capable of advancing or delaying their activity depending on the durations of light or darkness. This study examines whether tropical animals which do not normally experience much variations in durations of light and



darkness can also do the same. Experiments with the field mouse *Mus booduga* show that their behaviour with respect to scaling the durations of activity matches that of temperate animals but not so with respect to anticipating the onset of darkness. It is speculated that scaling of durations of activity ( $\alpha$ ) may not require prior experience while anticipating the onset of light or darkness (i.e. showing the appropriate phase angle difference) being a more complicated adjustment cannot be achieved without prior experience.

A fascinating aspect of animal behaviour is the impressive synchronization (entrainment) of activity/rest cycles to natural photoperiods. Thus, diurnal animals confine their activity period to the light phase (L) and rest period to the dark phase (D), while nocturnal animals do the reverse<sup>1</sup>. This synchronization is maintained even under varying durations of light and dark in the laboratory, which may sometimes deviate considerably from the natural L:D ratios<sup>2</sup>. But how do varying durations of L and D affect the durations of activity (referred to as  $\alpha$ )? There is evidence<sup>3</sup>, for example, that chaffinches correspondingly increased their  $\alpha$  as the duration of L was increased from 8 to 20 h. Even when wider ranges of durations of L (or D for nocturnal animals) are used (or are present under natural conditions such as in some parts of the world, for example)  $\alpha$  shows an S-shaped curve. This means that  $\alpha$  remains almost the same up to certain durations of light, then increases linearly with increase in light and beyond a certain point reaches a plateau<sup>4</sup>.

A related question is how varying durations of L and D alter the time difference (positive or negative) between the onset of L (or D for nocturnal animals) and the onset of activity. Such time difference is referred to as the phase angle difference ( $\psi$ ) and may be measured as the difference between the onset of activity and onset of L (or D) ( $\psi_o$ ), midpoint of activity and midpoint of L (or D) ( $\psi_m$ ) or end of activity and end of L (or D) ( $\psi_e$ ). Observations under naturally varying photoperiods have shown that under long day length conditions for example, diurnal animals start their activity late (i.e. have a negative  $\psi_o$ ), reach the mid-point of their activity before the mid-point of L is reached (i.e. have a positive  $\psi_m$ ) and reach the end of their activity a little before the end of L (i.e. have a positive  $\psi_e$ ). Diurnal animals show exactly the opposite behaviour under short day conditions and nocturnal animals show similar behaviour with respect to long and short nights respectively<sup>4,5</sup>.

While these phenomena (both scaling of  $\alpha$  and phase angle difference with varying durations of L and D) have been investigated in the temperate zone, we know almost nothing about tropical animals, especially tropical mammals. Is it possible that since natural photoperiods are more or less constant in the tropics, these animals

are unable to respond in the manner described above when L:D ratios are altered drastically in the laboratory? This question motivated the present study.

Adult males of the nocturnal tropical field mouse *Mus booduga* ( $n=6$ ) were obtained from fields near Madurai University campus ( $9^{\circ}58'N$ ,  $78^{\circ}10'E$ ). They were maintained with *ad libitum* food and water in laboratory cages fitted with running wheels where an eccentrically placed magnet facilitated recording of locomotor activity. The rotations of the wheels were picked up by an Esterline Angus event recorder. The light intensity at the cage level during L phase was about 45 lx while absolute darkness prevailed during the D phase. The different LD duration ratios were achieved by altering the relative durations of L and D (by either lengthening a single light time (L) or a single dark time (D) as the case may be) while keeping the sum at 24 h. The various L/D ratios administered were: 22:2 h; 20:4 h;

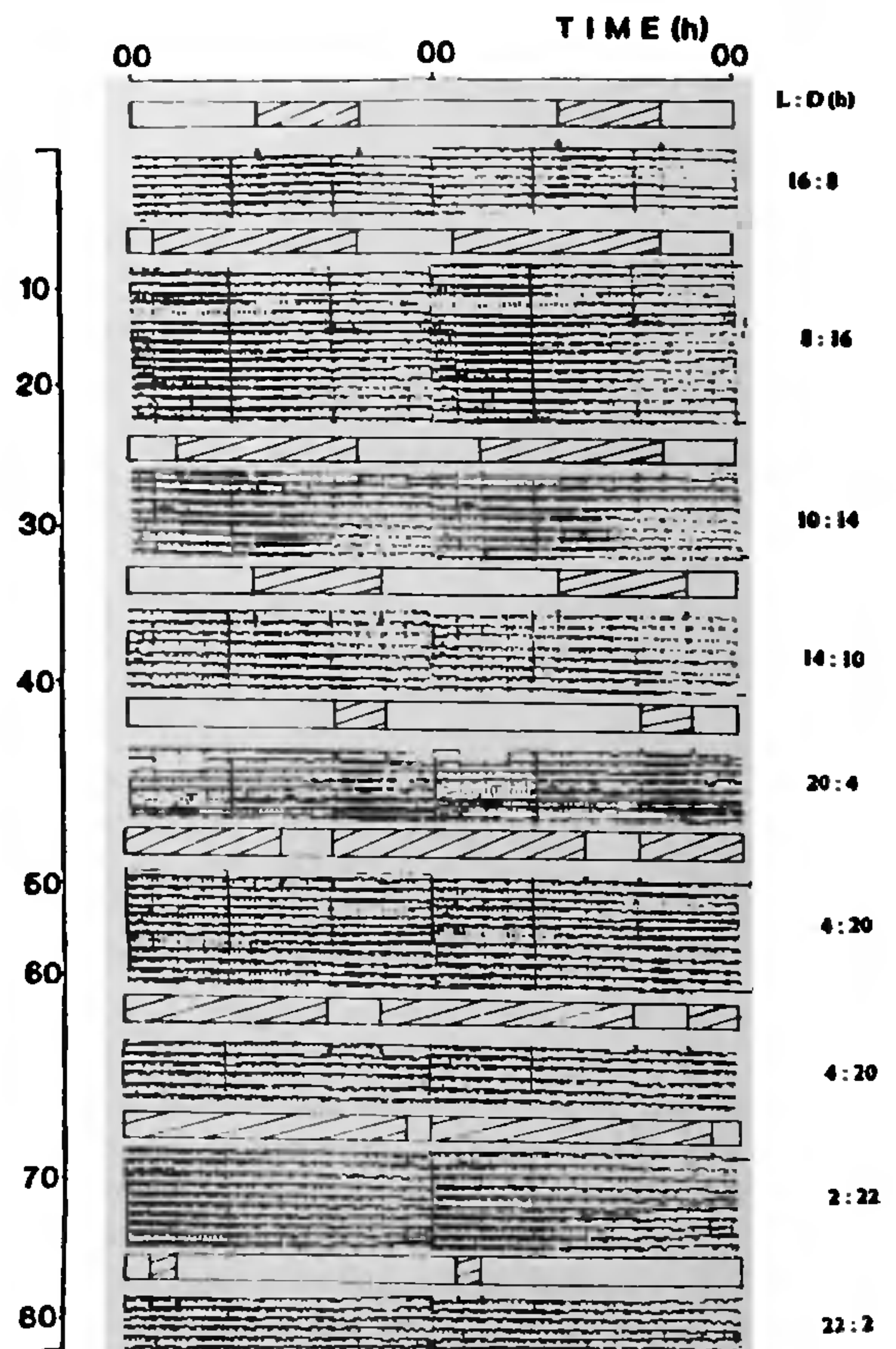


Figure 1. Actogram showing the activity pattern of an animal subjected to differing LD ratios. Open bars indicate durations of light time and filled bars indicate durations of dark time. The intensity of light during light phase was about 45 lx and absolute darkness prevailed during dark phase.



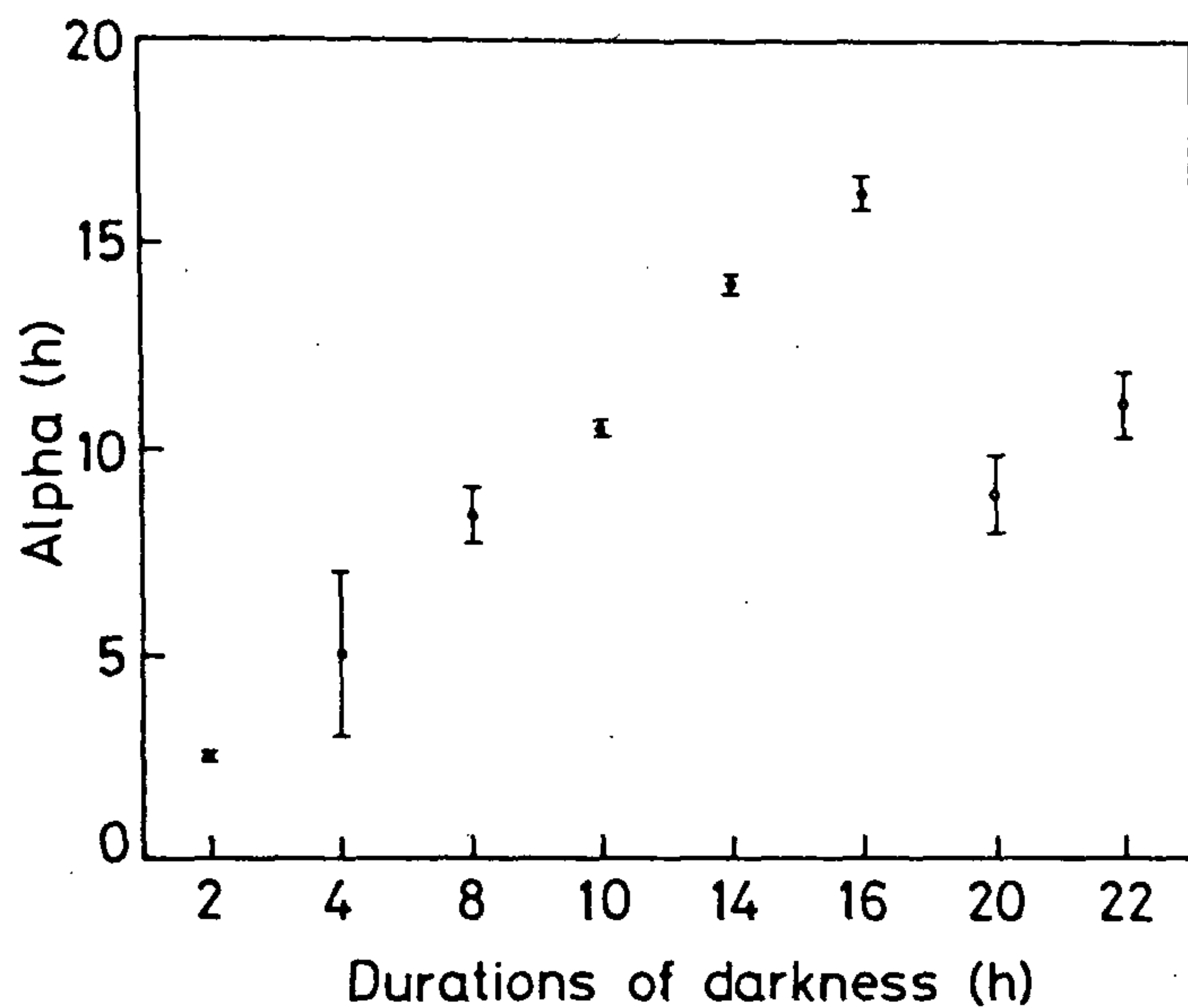


Figure 2. Durations of activity ( $\alpha$ ) as a function of durations of darkness. Mean  $\pm$  SD values are shown.

16 : 8 h; 14 : 10 h; 10 : 14 h; 8 : 16 h; 4 : 20 h and 2 : 22 h. Only the duration of light or dark was altered and the intensity of light during L phase was kept constant. Actograms were double plotted using conventional methods (Figure 1).

This study yielded two major results. One was that  $\alpha$  did vary with durations of D (as expected in nocturnal animal) all the way from 2 to 16 h, although the positive correlation between  $\alpha$  and D broke down after 16 h (Figure 2). This means that these tropical nocturnal animals were able to scale their  $\alpha$  with varying durations of D much as temperate animals are capable of doing so, although they would not be expected to have adaptations to such drastic variations in LD ratios. It is true however, that the scaling of  $\alpha$  with durations of D broke down when the duration of D was 20 or 22 h. Here  $\alpha$  was  $8.92 \pm 0.96$  and  $11.13 \pm 0.78$  respectively. This breakdown of the scaling of  $\alpha$  at extreme D durations suggests that the scaling observed when D was varied from 2 to 16 h is in fact an intrinsic property of the entrainment mechanism rather than a simple masking effect of light. In other words, the activity of this nocturnal animal was not merely suppressed by the presence of light. If that were so, there is no reason why  $\alpha$  should not have kept pace with D even beyond 16 h. It can also be seen from Figure 1 that the scaling of  $\alpha$  with durations of D from 2 to 16 h was quite independent of the phase in which the animal was when the LD ratios were varied. This again argues that the observed scaling of  $\alpha$  with durations of D was not a masking effect as masking has shown to be phase dependent<sup>6,7</sup>.

The second result of this study was that the phase angle differences did not correspond to the general

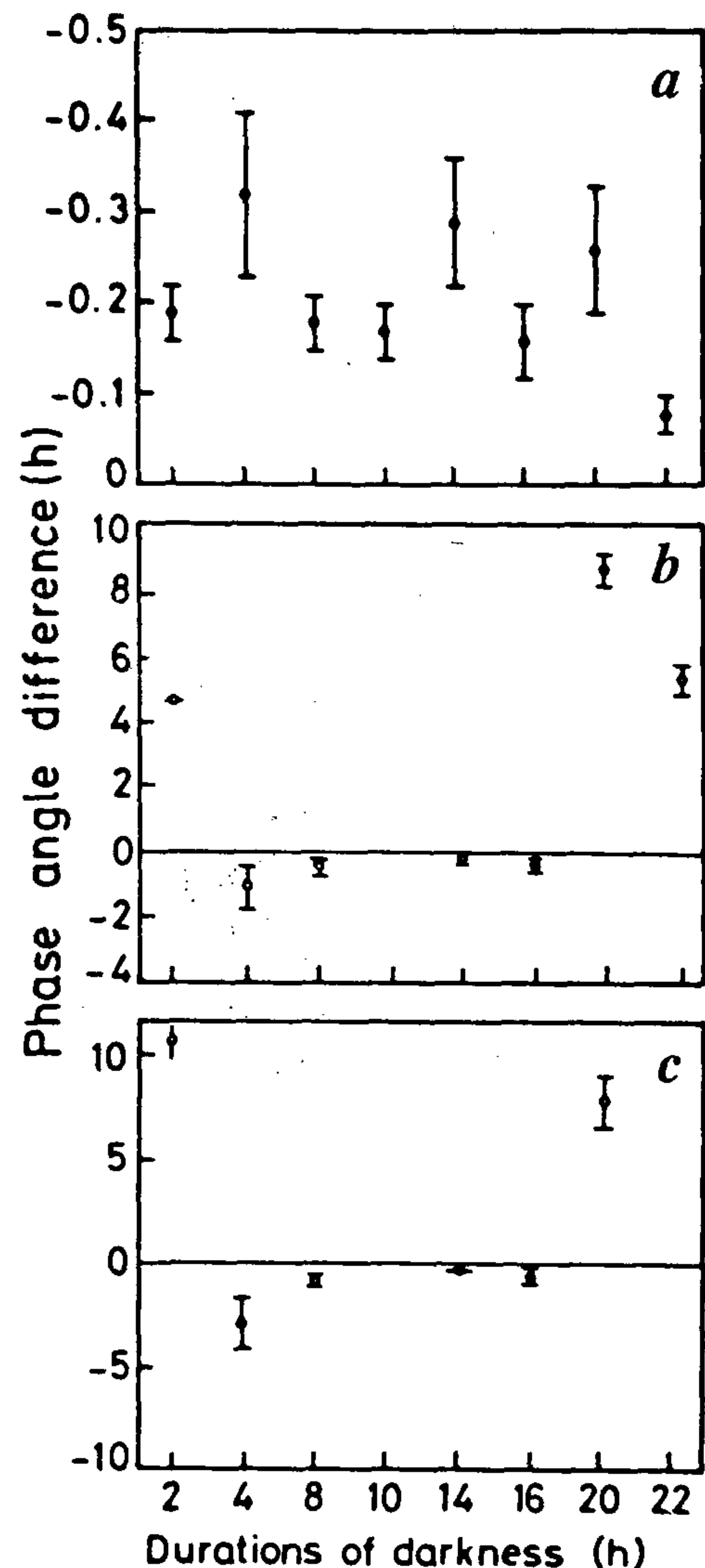


Figure 3. Phase angle differences as a function of durations of darkness. *a*, onset ( $\psi_0$ ); *b*, mid point ( $\psi_m$ ); *c*, end ( $\psi_e$ ). Mean  $\pm$  SD values are shown. Data not available for 10 h of durations of darkness.

pattern observed in temperate zone animals described earlier. Indeed, there was no discernible pattern in the phase angle difference whether measured as  $\psi_0$ ,  $\psi_m$  or  $\psi_e$ , with increasing durations of darkness (Figure 3). The absence of differences in phase angle in response to varying LD ratios as expected in a temperate animal is not really surprising for *Mus booduga*. Tropical animals are not expected to be exposed to much drastic variations in LD ratios and are thus not expected to have the required adaptations. The longest night in Madurai for example is 12.5 h while the shortest night is 11.4 h. Studies on the tropical bat *Hipposideros speoris* have shown that these bats do seem to anticipate the onset of darkness<sup>8</sup>. However, since only the natural seasonal variations in LD ratios were used in this case, the bats may be expected to be able to be adapted to such variations.

The contrasting results with respect to the observed scaling of  $\alpha$  with durations of D and the absence of

the expected patterns of differences in phase angles with varying LD ratios in the present study, raise an interesting question. How is it that a tropical animal, not used to much variations in LD ratios (in its recent evolutionary past) can still show the same behaviour as temperate animals with respect to scaling of  $\alpha$  but cannot do so with respect to varying the phase angles. It is interesting to speculate that scaling of  $\alpha$  with durations of D is a simpler phenomenon not really requiring prior experience or adaptation while varying the phase angles as temperate animals do is a more complicated phenomenon which involves anticipating the onset of light or darkness. This may be dependent on prior experience or adaptation. However, this speculation is based on this single result with a tropical animal. Given the extreme paucity of information on the behaviour of tropical animals under varying LD ratios and the unusual results obtained in this study, more work along these lines on other tropical animals, diurnal and nocturnal, is sorely needed.

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## Potent amoebicides from plant extracts – An *in vitro* assessment with the gum-oleo-resin of *Commiphora wightii*

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The medicinal value of *Commiphora wightii* has been believed by tribals to be mainly due to its yield of guggulipid, which has been scientifically shown to have hypochloesteromic, anti-septic, anti-pathogenic, anti-parasitic properties. It is also used for non-specific diarrhoea and dysentery. Amoebic dysentery is a common disorder of a large number of people

in the tropics. In our studies we have reviewed the active principles of most known anti-amoebic plants. Further, we have tested the crude extracts of oleo-gum-resin obtained from *C. wightii* against *E. histolytica* NIH 200 using microdilution technique. They were found to be comparable with quassinoids; Ailanthinone and Bruceantin. The need for linkages between chemical characterization with established *in vitro* techniques is demonstrated.

AMOEBIASIS by *Entamoeba histolytica* is an important cause of dysentery. Recent global estimates indicate the increasing trend from 480 million people (excluding China) annually suffering from amoebiasis<sup>1</sup>. The search for herbs and medicines for this scourge, from all possible sources is an ongoing exercise. Many natural products of plant origin are an important source of biologically active compounds and have potential for the development of novel antiprotozoal drugs as studied by *in vivo* and *in vitro* experimentations<sup>2</sup>. Table 1 shows known anti-amoebic plants studied since the last decade for their active fractions and principles.

*Commiphora wightii* (Arnott) Bhand, (Burseraceae) is a small tree or large-sized shrub which produces a gum 'guggal' believed to have high medicinal value. It is commonly known as 'Indian bdellium' or 'guggal' in India<sup>3</sup>. It is found in the arid rocky tracts of Rajasthan, Gujarat, Karnataka and Maharashtra states of India; Sindh and Baluchistan states of Pakistan; Bangladesh and Arabia<sup>4</sup>. The trunk is knotty, outer bark comes off in rough flakes leaving an inner layer which is bright, shining and peels off in thin rolls like paper. The latex oozes out through wounds or cuts as a yellow fluid which hardens to form a golden brown, yellow or reddish brown oleo-gum-resin. Guggal gum is a mixture of 61% resin, 29.3% water, 0.6% volatile oil and 3.2% foreign matter<sup>3</sup>. Guggal gum is known for its therapeutic properties in various ailments, particularly arthritis, many vascular and neurological complications, hypercholesteremic conditions<sup>5</sup>, rheumatism and possesses anti-inflammatory activity<sup>6</sup>, in cure of ischaemic heart disease, obesity, neurological disorders, ills of syphylitic nature<sup>7</sup>, scrofulous infections, urinary disorders and a few skin diseases<sup>4</sup>. Its essential oil also possess antibacterial, antifungal and antihelminthic activity.

As one of the ingredients in 'Arogya Vardhini Bati', an Ayurvedic drug, *Commiphora* is used for the treatments of diarrhoea and dysentery in man and its efficacy has been tested both *in vitro* and *in vivo*<sup>8</sup>. Alcohol extract of its oleo-gum-resin was tested *in vitro* against axenic cultures of *E. histolytica* NIH 200 but proved less effective than *Curcuma zedoaria*<sup>9</sup>. The most optimal comparisons of other such plants which were similarly studied for their active principles are *Brucea javanica*, *B. antidysenterica* and *Simarouba amara* with quassinoids; Bruceantin and Ailanthinone as their active compounds.