Validation of Ayurvedic formulations in animal models requires stringent scientific rigor

Sir,

The paper by Priyadarshini, et al. entitled “Increase in Drosophila melanogaster longevity due to rasayana diet: Preliminary results,” published in April 2010 issue of Journal of Ayurveda and Integrative Medicine concludes that dietary supplement of a specially formulated “rasayana” to Drosophila melanogaster flies significantly enhances their lifespan and that this observation seemingly agrees with what is stated about rasayana in Ayurvedic literature. This can be an important finding. Unfortunately, however, the present paper falls significantly short of a scientific analysis and reporting.

Although strong claims are made in the rather verbose “Introduction” and “Discussion” sections, the real description of the methods is very limited. The major limitations are as follows:

- Neither the nature of the rasayana nor its method of preparation is described in the paper. Authors use terms like “Drosophila rasayana” or “inset rasayana,” which certainly do not exist in Ayurvedic texts! For a scientific study, one would like to know how it differs from or resembles the typical rasayana preparations.
- How much rasayana was actually available to the flies and how was it mixed with food? With respect to the diet, authors state “Controls received 1 drop (10 µl) of bacteriological grade yeast solution as food; experimental cultures received 1 drop Drosophila rasayana food supplement. No dietary restrictions were imposed. Flies could feed ad libitum.” This does not provide any information about the concentration or the nature of rasayana given. Nor is any mention made of the concentration of yeast solution nor the quantity of food to which the “1 drop” of yeast solution or the rasayana supplement was added. What quantity of the standard fly food was provided in each vial?

With such limited information about the methods of study, no one can reproduce the results for verification! A basic requirement of a scientific analysis, whether “preliminary” or more definitive, is that the materials and method used in the study should be explicit enough so that those interested can use the same for verification or for more advanced studies. In the present case, this is not possible because such essential information has not been provided.

It is surprising that on the basis of “a preliminary study,” as title of the paper states, authors claim their “finding” that the rasayana-fed flies displayed significantly increased longevity is as definitive “as a law of physics or chemistry”! It is likely that the life span is indeed enhanced by the ”rasayana” feeding. However, establishing it, especially from the viewpoint of implications for human society, requires more in depth data and analysis together with all the relevant details. Enough data must be provided to establish that the modified rasayana used by authors in the present study is essentially similar in its actions and composition to the traditional rasayana. Hiding the essential details is not justifiable in a scientific study.

In addition to the above serious limitations of experimental part of the study, there are factual errors relating to some scientific facts. For example, the mammalian Xist and Drosophila Sxl genes are not at all analogous since unlike the protein coding Sxl gene of Drosophila, the Xist gene is a noncoding one and is not involved in sex determination! Although both have roles in dosage compensation, their mechanisms of action are entirely different. Thus they are not even functional analogues. It is also not true to say that for every Drosophila gene, there are about four homologues in vertebrates. The methuselah mutant of Drosophila is a single-gene mutation rather than it being the result of selection of all genes involved in ageing in flies as seems to be implied in the statement of authors on p. 115. Such erroneous claims in a scientific paper can be very misleading indeed. Another seemingly trivial but important point is that the P value cannot be less than “zero” (or <0.000 as given in Table 2).

In recent years, there has been a welcome flurry of research activity to justify the principles and practice of Ayurveda and other traditional medical systems. There is no question that Drosophila is a very good model for studies directed to understand molecular basis of the Ayurvedic formulations. However, unless such studies are rationally planned and conducted with the required experimental rigor, the apparently “positive” results may actually be more damaging to the system which the authors would want to strengthen. The published study by Priyadarshini et al. under reference does not seem to meet the basic rigor
Sir,

This is in response to the letter from Lakhotia S related to our work published as a preliminary report. Professor Lakhotia is right in pointing out the need for more studies on rasayana effects in animal models. We also appreciate his concerns and questions related to our article.

It is important to understand that rasayana relates to rasa, which not only has organism-specificity but it is also dependent on other significant factors. According to Sushruta Samhita (sarvabhuta chinta sharera Su.Sm. Sh chapter 1/1–3), all living organisms have certain features in common, and others that distinguish them.

Our earliest research started in June 2007, when we began a series of tests on four commercial formulations of human-optimized rasayanas on small numbers of flies. Some of these showed marginal changes in longevity, but none were striking, nor what we expected for a "rasayana-effect." On the contrary, we observed significant adverse effects including lethality. This suggested that the paste-like dosage form of rasayana formulations prescribed for humans was adversely affecting the feeding process. This led us to consider developing "Drosophila-friendly" rasayana formulations. Basic principles of Ayurveda especially of dravyaguna shastra provide the way for developing such preparations. Rasayana in liquid dosage forms would provide an easy way to alter the concentration in the feed and to arrive at precisions stated in our paper as "1 drop of Drosophila rasayana food supplement."

A considerable amount of innovation went into these developments and we wanted to protect the intellectual property. We thank J-AIM editors for agreeing to a black box approach in our reporting the experiments, and to our withholding details of its formulation. However, we confirm that the details of the formulation including chemical standardization data will be made available to J-AIM once patent formalities are completed.

The extensive introduction was included to communicate fundamental understanding of basic principles to suit the diverse backgrounds of the multidisciplinary readership of J-AIM. Both reported longevity experiments were made on D. melanogaster Oregon-K strain obtained from the Drosophila Stock Center, Department of Zoology, Manasagangothri, University of Mysore. Our research team included both experienced Drosophila scientists and Ayurveda experts. We followed standard experimental protocols and do not understand the grounds on which Lakhotia claims that the study was poorly executed. However, we do appreciate his comments on the way it has been reported and discussed. As regards the reporting of P values, SPSS-10 and 16 both generate this form, and we have seen such practice in many reputed journals when highly significant P values are achieved. Of course there could be different viewpoints. The errors in the discussion section especially those relating to ideas promoted by John Tower [3] are regretted but in no way affect the confidence that we place in our data and its significance.

We continue to stand by our results using rasayanas modified to suit Drosophila and confirm that they are consistent, significant, and reproducible. However, in the best interest of scientific enquiry and to dispel doubts about our results, we would be happy to work with Drosophila experts like Lakhotia. Under suitable nondisclosure agreement, we are also open to sharing required information and data as well as test material for possible repetition of our experiments in other laboratories.

Deeper understanding and appreciation of Ayurveda is essential for the way ahead. It will bring rapid success in these kinds of trans-disciplinary research activity and in collaborations searching for answers to questions concerning concepts from ancient knowledge. We, therefore, feel that it is essential to involve experienced Ayurveda experts on a basis founded on mutual trust and respect for the two seemingly diverse but mutually consistent and compatible disciplines.

S. Priyadarshini, J. S. Ashadevi, V. Nagarjun, K. S. Prasanna

of a scientific study. Publication of such poorly executed study, which is presented more in “popular” article style rather than a professional scientific report, is also not good for the journal.

Letters to the Editor

Prof. S. C. Lakhotia
Department of Zoology, Cytogenetics Laboratory, Banaras Hindu University, Varanasi - 221 005, India.
E-mail: lakhotia@bhu.ac.in

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