

Authors' reply

Sir,
This is in response to the letter from Lakhotia S related to our work published as a preliminary report.[1,2] 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, Manasagangotri, 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.

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Letters to the Editor
Sir,

Ayurveda states that *jara* (aging) is a "swabhavika" (natural) property of all organisms – unicellular and multicellular. Interventions ranging from environmental, lifestyle, genetic, and sometimes even chemical to delay *kalaja jara* (predictable and unavoidable aging) and prevent *akalaja jara* (untimely or avoidable aging) should be and are possible. Of the examples now available, some use as little as a single, simple chemical. In 2002, for instance, the late Seymour Benzer and colleagues reported life extension in *Drosophila* [1] by feeding a “drug” 4-phenylbutyrate (PBA).

The studies also provided early evidence of the mode of action... “a global increase in histone acetylation as well as a dramatically altered pattern of gene expression, including induction or repression of numerous genes.” Aging and longevity are clearly very complex and real traits. Such studies seem to support Ayurveda’s "Rasayana Tantra," which claims innumerable rejuvenating and life-extending practices and formulations (see Prof. Ajay Kumar Sharma’s "Elements of Rasayana Therapy in Ayurveda," ISBN 81-7030-831-3, for a brief but detailed account of Rasayanas). The paper by Priyadarshini et al. [J-AIM 1(2):114–119] shows that similar studies can be performed using rasayanas, but raises questions about this important but underexplored and underappreciated area. The most significant aspects of the experiments and their results as reported in the paper are their obvious weakness! By not providing details of either the formulation or the way in which it was arrived at, and by doing so in the manner done... the paper should initiate an important debate. Such debates hold latent benefits – I illustrate this using an example from a similar set of circumstances, with which I was closely involved in the early days of plant DNA research. In the late 1970s and the early 1980s, the big challenge was being able to prepare high-quality plant DNA from a tissue rich in phenolics and other secondary compounds, which readily complexed with the DNA making it unsuitable for any kind of enzymatic investigation. Such was the order of magnitude of this problem that several meetings were held around Europe with the sole aim of bringing together researchers to discuss methods to improve results. Later, in the mid-1980s, several informal and very short contributions helped shape and speed the generation of plant DNA libraries in bacteriophage lambda cloning vectors purely by appropriate selection of bacterial hosts. These important and nontrivial successes were due to researcher skills based on deep appreciation of bacterial genetics.

The single example below from the Maize Genetics Cooperation Newsletter (http://www.agron.missouri.edu) was particularly significant and is worth citing as its insights and choice of bacterial strain helped very rapidly move the entire field of construction of plant genomic DNA libraries in bacteriophage lambda cloning vectors, and enabled the isolation and characterization of genes from several plant species – all to be carried out single-handedly by a PhD student like me! The background to the challenges faced in choice of bacterial host strains like *Escherichia coli* Q358 and K803 and their genotypes and compatibility with Enterobacteria phage λ (lambda phage)-based cloning vectors available are all in this brief contribution by Nina Federoff. I quote below (in italics) from:

Notes on cloning maize DNA by Nina Fedoroff

Maize Genetics Cooperation Newsletter; Volume 57, 1983
[http://www.agron.missouri.edu/mnl/57/125fedoroff.html]

To determine whether the inability to form plaques on Q358 is an inherent property of recombinant phage containing maize DNA, recombinant phage were propagated on K803 and tested for their ability to grow on the various strains. The results are shown in Table 2.

REFERENCES
1. Lakhotia S. Validation of Ayurvedic formulations in animal models requires stringent scientific rigor. J Ayurveda Integr Med 2010;1:171-2.
2. Priyadarshini S, Ashadevi JS, Nagarjun V, Prasanna KS. Increase in *Drosophila melanogaster* longevity due to rasayana diet: Preliminary results. J Ayurveda Integr Med 2010;1:114-9.
3. Tower J. Sex-specific regulation of aging and apoptosis. Mech Ageing Dev 2006;127:705-18.