Increase in *Drosophila melanogaster* longevity due to *rasayana* diet: Preliminary results

S. Priyadarshini, J. S. Ashadevi[1], V. Nagarjun[2], K. S. Prasanna[3]

Government Ayurveda Medical College, Mysore, Karnataka, India, [1]Yuvaraja's College, University of Mysore, Mysore, India, [2]Uppsala University. Sweden, [3]Maharani's Science College for Women, Mysore, India

ABSTRACT

We report preliminary results from an ongoing series of experiments on lifespan extension by appropriately modified Ayurvedic *rasayanas* in animal models. Here data are presented indicating lifespan extensions of 51–55% (up to 70–95% in the pilot experiment) in a standard strain of *Drosophila melanogaster* (Oregon-K) using a standard *rasayana* (Ayurvedic herbal formulation for life-extension) suitably adapted for insects. In a first experiment, two groups of 20 unmated *D. melanogaster* strain Oregon-K kept at 22ºC received either *rasayana* or standard yeast diet; days of death were recorded. Another experiment investigated possible sex differences; equal sized (N = 30) groups of similar males, females, and controls were compared. Life lengths of all controls were in the strain's usual range: in Experiment 1, control life lengths were minimum 40 to maximum 53 days; experimental group figures were 81–91 days; groups were completely separated, experimental group minimum life length being 28 days more than control group maximum life length, i.e., about 2.5 full distribution widths – a sign test for the null hypothesis yields $p < 2^{-20}$, i.e., 10^{-6} as maximum p. Experiment 2 found no differences between life lengths of males and females; but the maximum life length of 30 controls (60 days in males and 66 days in females) was once again far shorter than the minimum life length of the 60 in the two experimental groups, strengthening the findings of Experiment 1. Despite group sizes being relatively small, results are conclusive: the *rasayanas* in question increase *D. melanogaster* strain Oregon-K life length. The complexity of the formulation suggests that multiple mechanisms are involved – worth further investigation.

Key words: Ayurveda, *rasayana*, *D. melanogaster*, longevity.

INTRODUCTION

Asia's traditional systems of medicine are person-centered, as much concerned with restoring high levels of health as with curing disease. South Asia's system of Ayurveda is no exception. Its name, meaning "knowledge of life and lifespan," implies an ability to prolong life by restoring health and reversing tendencies to aging. Its *rasayana* therapies – physical, mental, and spiritual – aim at achieving just that. *Rasayana* forms the seventh of eight subdivisions of Ayurveda's earliest extant text, *Charaka Samhita,*[1] but in fact are central to Ayurveda and its key concept of health promotion.

By the time of Charaka, *rasayana* health-promoting, herbal formulae were already well developed, indicating that detailed clinical observation and practice over centuries, if not millennia, had gone into their formulation. Ayurveda's second great text, the *Sushruta Samhita*, defines the term *rasayanapara*[2] as "one who continuously contemplates unique personalized management of senile / degenerative changes in the body, only achievable at the most refined levels attained during intense meditation. Qualities like taste and potency (*rasaveeryaoushada prabhavena*) and effect of the formulations are designed to retard ageing as much as possible, even to zero". Sushruta also describes *rasayanas* as "reversing naturally occurring senility" (*swabhava vyadhi nivarana*) and so "preventing death" (*marana nivarana*), further indicating that *rasayanas* are considered "special herbal formulations" (*vishishtaoushadhi chintaka*) conceived through prolonged consideration of their components' detailed properties.

Charaka[3] comments that those who specialize in changing aspects of the organism with age (*vayah prakriti satmyajnana*) can formulate *rasayana* according to need. His term "*Rasayana chintaka*"[3] meaning "one who contemplates the effect of *rasayana*," barely hints at the amount of research underlying the subject. Such people were said to have spent

Address for correspondence:
Dr. S. Priyadarshini, Assistant Professor, Government Ayurveda Medical College, Mysore, India.
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whole lifetimes contemplating all processes of aging and how to reverse them. The classic texts make no claims that *rasayana* as a subject is exhaustively understood. On the contrary, they state that research is an ongoing aspect of Ayurveda practice, implying that subjects such as *rasayana* are open to new possibilities even today.

The state of *Swastha* or perfect health,[4] is precisely described in Ayurveda, and is one of its central concepts, its achievement and maintenance being a common goal of all therapy. *Rasayana* thus holds a central position in Ayurveda.[3] *Rasayana*s are among its most commonly prescribed medicines, now recognized for their antioxidant power,[5] and ability to stimulate the immune system,[6] among other properties.

Valiathan has recognized the opportunity to create "Ayurvedic Biology."[7] His visionary perspective suggests that programs to create an evidence-base for Ayurveda should include clinical *rasayana* evaluations. He has since been quoted by Mashelkar[8] as saying that *rasayanas* should be tested on animal models. Mashelkar emphasized his personal dream to see *Drosophila melanogaster*, the well-known fruit fly, used for such tests. Its genome is sequenced, and humans and *Drosophila* are known to have 13,601 genes in common.[9] Gene sequences known to be associated with human diseases have been identified.[10] It constitutes a suitable system for testing myriad hypotheses.[9]

It is therefore with some satisfaction that we present a preliminary account of controlled experiments assessing the effects of a *rasayana* on life length of a particular strain of *D. melanogaster*. We report here two early trials of the viability of the method, including a study of possible sex dependence, using our first *rasayana* formulated for *Drosophila*. Later investigations of detailed mechanisms, long-term effects over many generations and so on, are still being completed, and will be published later.

AGING RESEARCH IN *D. MELANOGASTER* MODELS

D. melanogaster is one of a small number of organisms favored for human-related aging research (see Appendix). Few chromosomes in a small genome, with large numbers of spontaneous and induced mutations, make it well-suited to such work. Its genome is sequenced;[9] vertebrates have about four homologues for every gene found in *Drosophila*. The species shares large numbers of homologous genes with mammals, 13,601 with humans.[9] These have been analyzed to identify sequences related to those causing human diseases.[10] These facts make *D. melanogaster* particularly well suited for preliminary study of the genetics of disease, degeneration, and aging processes, since results should yield insights into what to seek in humans.[10] For example, the human *Xist* gene and the analogous *Drosophila Sxl* gene both control sex determination, and may both be involved in regulating lifespan.[11] This implies that any means of systematically influencing aging is worth studying for possible sex-dependence, as reported here.

Because of the foregoing, fruit fly models are popular for research on human-related topics. Partridge, in particular, has argued that research findings in *D. melanogaster* can throw light on probable happenings in human aging processes.[12] Dietary restriction (DR) is well recognized to extend *D. melanogaster* lifespan, a topic Partridge has discussed in detail,[13] noting that, because it reduces fecundity, a factor negatively affecting lifespan, DR's effects are greater in females. But DR can also produce conflicting results, different in different species,[14] and with complex interactions with pathology.[15]

D. melanogaster now forms a principal model for studying the biology of aging and longevity. Normal lifespan is about 40 days in males and females. *Drosophila* longevity genes with human homologues have been identified, as have their single gene mutations extending lifespan.[10] Selection of all such genes results in the "Methuselah" fly with a greatly extended lifespan.[16] Various inducers are known that can switch these genes on or off. Theoretically, it is therefore conceivable that an appropriate combination of such inducers could greatly extend Drosophila lifespan.

AGING AND ITS REVERSAL IN AYURVEDA

Maintaining the vitality of youth and preserving quality of life has long been a quest of civilized man. Ayurveda *Rasayana tantra* describes techniques providing multidimensional solutions to aging, premature aging, and their complications. Its *rasayana* division, also known as *jarachikitsa*, has been practiced as long as Ayurveda has been recorded.[1] The root of the word *jara*, meaning aging (*Jeerayate-iti jara*), is also that of the Greek word *gerios*, from which "geriatrics" is derived.

Expert practice of *rasayana* therapy is said to offer the following benefits:[17] youthfulness, increased intelligence and creativity, improved functioning of all sense and motor organs, improved complexion, renewed vigor and vitality, better functioning of immune system, thereby bestowing resistance or faster recovery from illness, and freedom from fear of death and diseases. Its extreme form of *kutipraveshika* has also been described.[18,19]

Rasayana tantra[1] states time-tested properties of many *rasayanas*, herbs, minerals, special rituals, lifestyle

modifications, and their combinations, describing all in detail. A few are in use today, but, possibly due to complexity and expense, only sporadic trials have been conducted. There is thus a need to revalidate the ancient herbal formulations, as well as newer formulations appropriate to contemporary problems. In order to validate *rasayana* concepts more rigorously, we proposed using *D. melanogaster* models. We, therefore, formulated a new *rasayana* using organic herbs and maintaining traditional principles, precisely altered to reflect intrinsic differences between mammals and insects. We reasoned that if it could extend *D. melanogaster*'s lifespan, the same formula with insect changes reversed out would be of similar benefit to humans. In reality, the experiment constitutes an *in principle* test of the whole subject of *rasayana*, in particular the validity of applying it to animal models like *D. melanogaster*.

MATERIALS AND METHODS

Strain

D. melanogaster strain Oregon-K was selected for longevity experiments. Stocks were obtained from the Drosophila Stock Center, Department of Zoology, Manasagangothri, University of Mysore, and maintained at 22 ±1°C in 30 ml culture bottles containing wheat cream agar medium.

Stocks

Stocks were built up for three to four generations using standard propagation methods.

Procedure

Newly emerged unmated flies were released into 8 × 2.5 cm individual culture vials containing equal quantities of wheat cream agar medium and food supplement. Flies in each culture vial were transferred to fresh culture vials once every 4 days without being etherized.

Diet

Controls received 1 drop (10µl) of bacteriological grade yeast solution as food; experimental cultures received 1 drop *Drosophila rasayana* food supplement. No dietary restrictions were imposed. Flies could feed *ab libitum*.

Table 1: Lifespan ranges of *rasayana*-fed *D. melanogaster* and controls

Batch	N	Minimum (days)	Maximum (days)
Control	20	40	53
Rasayana	20	81	91

Experimental design

Experiment 1 consisted of two batches of 20 flies (experimental and control) [Table 1]; Experiment 2 [Table 2] comprised three batches of 30 flies for both males and females = 2 experimental batches (*rasayana* and *rasayana* + yeast supplement), and 1 control batch.

Observations

Daily count of number of surviving flies until all flies had died yielded numbers dying each day in each group.

Data analysis

Histogram distributions of Experiment 1 data are given, analyzed by sign test. For experiment 2, with larger numbers, additional analysis using Student's "*t*" test could be performed.

RESULTS

Table 1 presents results of a first, preliminary experiment. Control group life lengths ranged from 40 to 53 days, experimental group life lengths ranged from 81 to 91 days, roughly double the controls. Because of the small size of the groups in this first, preliminary pilot study, we do not give means or *t* values, the value of which would be doubtful in a population with undoubted residual genetic heterogeneity. Instead, the extremes of each distribution are given to show the large, 28-day difference between minimum experimental group, and maximum control group life lengths.

Table 1 compared lifespans of two batches of 20 *D. melanogaster* maintained on a normal wheat cream agar medium; batch 1 (control) diet was supplemented with yeast. Batch 2 (experimental) diet was supplemented with a special Ayurvedic *rasayana* designed for insects. Since

Table 2: Comparative study of male and female *D. melanogaster* Longevity on diets: Control, *rasayana*, and *rasayana* + yeast

		Mean ± SE	*t* value	Significance
Males	a) Control	52.63 ± 0.922	a/b 42.45	$P < 0.000$
	b) *Rasayana*	81.60 ± 0.294	a/c 88.91	$P < 0.000$
	c) *Rasayana* +Yeast	80.56 ± 0.901		
Females	a) Control	53.10 ± 1.36	a/b 23.71	$P < 0.000$
	b) *Rasayana*	80.23 ± 0.261	a/c 42.99	$P < 0.000$
	c) *Rasayana* + Yeast	80.36 ± 0.886		

df = 29 for each group

certain *D. melanogaster* aging processes occur differently for different sexes, one of our follow-up experiments investigated possible effects of both sexual differences and addition of the yeast supplement to the *rasayana* diet.

Data in Table 2 show no discernible difference between male and female *D. melanogaster* fed on any of the three diets. The yeast supplement made no difference to life lengths of those fed on the insect *rasayana*. The effect of the *rasayana* supplement is to increase the life length of both males and females apparently equally by about 51–55%. Males and females can, therefore, be regarded as a single group. These 120 *rasayana*-fed *D. melanogaster* versus consistently lived far longer than any of the 60 controls, yielding $P < 2^{-120}$ value for the statistical significance with which the null hypothesis was broken, according to a simple sign test.

This experiment obtained similar lifetimes as the first experiment, confirming its results: no significant differences were observed between male and female lifetimes, whether on normal, *rasayana*, or *rasayana* and yeast diets; nor were lifetimes of pure *rasayana* diet significantly different from those on a *rasayana* and yeast diet. As in Experiment 1, experimental groups (all four) lived so much longer than ordinary diet control groups that, as in Table 1, their distributions had no overlap and were completely separated. The robustness of the overall experimental results was established: an Ayurvedic *rasayana* designed for insects can increase life length by at least 50%.

DISCUSSION

For small groups with possible residual genetic heterogeneity, statistical values such as mean and SD have less meaning than raw data distributions. Data presentation of the first experiment is thus limited to range of lifetimes [Table 1], and a histogram of days of death [Figure 1]. Similarly, a sign test rather than parametric statistical tests of significance is more appropriate, and sufficient to yield a highly significant *P* value for the null hypothesis: $P < 2^{-20} = 0.95 \times 10^{-6}$ [Table 1].

For the second experiment given in Table 2, data strongly indicate that the *rasayana* benefits both sexes equally: zero sexual differences were indicated. Similarly, adding a yeast supplement had no discernible effect on life length. This means that the four *rasayana*-fed groups of 30 (2 male + 2 female) *D. melanogaster* can be combined into a single group of 120, for which parametric statistics are valid. Again the complete separation of groups yields sign test statistical significance of $P < 2^{-120}$, but this value pales into insignificance compared to parametric statistics applied to the difference of 27.84 between the 60 strong control

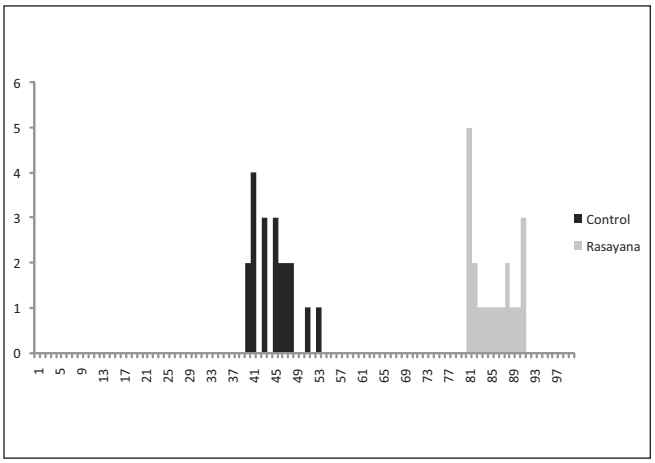

Figure 1: Histogram of days of death for experiment 1

group with df = 59, mean ± SE = 52.85 ± 0.99, and the 120 strong experimental group with df = 119, mean ± SE = 80.69 ± 0.29, an increase of 52.7%. The two means are separated by no less than *21.74* times the sum of reduced SE values (1.28).

Such a magnitude is clearly within the range of possibility, since the sum of all genetically favorable modifications also produces increases of this order. Nevertheless, the potential advance to present medical knowledge represented by the magnitude of this increase in lifespan *from a single formulation* needs to be set in context: no pharmaceutical comes close to it.

Jafari[20] emphasizes that failure to find pharmaceuticals significantly helping to slow or reverse human ageing processes reflects the number of genes and biochemical pathways involved; aging is an inherently *complex* process: no single *chemical drug* targeting a single enzyme is going to be effective against it. He suggests "mass screening of pharmaceuticals and botanicals to produce effective therapeutics for human aging," as a means to develop protocols for complexity-based therapy.

His group's subsequent research investigated the effects of single plant extracts, clear examples of complexity, on *D. melanogaster* lifespan. Extracts of three Chinese mushrooms yielded no effects, while *Rhodiola* (Hong Jing Tian) extended lifespan,[21] the hypothesized mechanism involving increased resistance to stress, i.e., adaptogenicity. A potent antioxidant, *Rosa damascene*, also produced decreases in *D. melanogaster* mortality rate.[22]

Results reported here confirm and extend Jafari's approach. As *complex* herbal formulations, Ayurvedic *rasayanas* inherently amplify complexity levels of single plant extracts.[6,,23] The greatly increased lifespans reported here

are a reflection on the essential complexity of traditional formulations, their particular adaptation for insecta, and the inherent advantages such an approach offers to *complex* biological phenomena like ageing. In this context, the considerable surpassing of the 35% increase in lifespan ascribed to the Methuselah fly[16] by our observed 51–55% mean increase in lifespan in Experiment 2 has considerable significance. It suggests that Ayurveda's methods may go beyond genetic effects, and possible achievements within the context of the genomic paradigm. The traditional approach, validated by millennia of practice, overcomes a major deficiency in modern drug programs.

These experiments have profound implications for the broad scope of applications of fruit-fly models to studies of human developmental and degenerative processes, and pathologies, e.g., birth defects; development of the respiratory and circulatory systems; cardiovascular development and disease; eye development, and blood cell development; circadian rhythms, smell, taste, sight, touch, and hearing; learning and memory; sleep; drug abuse; brain disease, diabetes, malaria, Parkinson's disease; and cancer to name a few. The results suggest that *applications of Ayurveda herbal products to all such areas, including investigation of mechanisms of action, could advantageously be carried out in fruit-fly models*.

CONCLUSIONS

Unlike the *P* values of most drug experiments, $P < 0.05$ or even 0.01, the *P* values obtained in Tables 1 and 2 are of the same order of significance as *P* values obtained from graphical representations of algebraic laws of physical science regarded as correlations. For these, definite statements are made. Thus, *extension of life length of D. melanogaster by the special "insect rasayana" is as definite as a law of chemistry or physics*.

We can legitimately omit cautionary words like "suggests" or "indicates," universally used as qualifiers in biomedical experiments. In contrast to such reservations, we can maintain that *inclusion of the insect rasayana as a dietary supplement definitely increases the lifetime of D. melanogaster*; roughly equally for males and females, and by at least 50% in both experiments.

Reiter *et al*, conclude, "The utility of *Drosophila* as a model organism for the study of human genetic disease is now well documented."[10] *D. melanogaster* models are especially valuable for elucidating physiological mechanisms. Combining these preliminary results of our life-extending *rasayana* therapy for *D. melanogaster* with statements concerning *rasayanas*' value from the ancient texts,[1-3] suggests that even for complex mixtures, the two organisms' closeness holds valid and *D. melanogaster* models maintain their utility. This should be tested in detail.

Almost any question concerning Ayurvedic formulations should, therefore, be testable on *D. melanogaster* systems. Various mutants will form suitable models to investigate advanced questions such as synergy between components, and overall mechanisms of complex formulations. In particular, as Valiathan and Mashelkar have suggested,[7,8] any program to establish *Ayurveda's* overall evidence base should include its system of *rasayana*, which aims to restore health[4] and increase longevity.

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