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Influence of Rasayana on Fitness Parameters and Life span of *Caenorhabditis elegans* : Preliminary report

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ABSTRACT

We have used C.elegans for the first time to test Ayurvedic medicines. such as rasayana (herbal formulations). The objective of our study was to explore the potential of rasayana of Emblica officinalis drug on longevity, reproductive fitness of C.elegans. Increase in brood size, lifespan, and normal developmental time was observed in rasayana fed worms, compare to control one according to the one way ANOVA.

KEYWORDS : C.elegans, Emblica officinalis

Introduction

Ayurveda - Asia's traditional systems of medicine literally "Science of Life"-is based on the twin principles of wholeness and balance. Ayurveda is equally about maintaining or preserving good health as it is about treating disorders - "*Swaasthasya Rakshanam*" is one of the goals of Ayurveda (Charaka Samhita Sutrasthana, 2000a).[1]

The branch of *rasayana* or rejuvenation is one of the eight specialized branches of Ayurveda that primarily deals with the maintenance of health (Sushrutha Samhita Sutrasthana, 1972a)[2]. *Rasayana* is defined as any herb, food, or activity which confers youthfulness and cures diseases. If taken in a proper way the *rasayana* prevents early aging and keeps person young and active both physically and mentally[3] (Charaka Samhita Chikitsasthana,2000b). *Rasayana* is the method of treatment through which the *rasa* is maintained in the body. The purpose of *rasayana* is to give strength, immunity, *ojus,* vitality, will power and determination, and to strengthen the sense faculties[4], so that you are not exposed to sickness and disease as long as you live.

According to Sushrutha Samhita Sutrasthana (1972b) Sexuality and reproduction is so vital in Ayurveda that an entire discipline, known as *Vajikarana*, is dedicated to enhancing fertility and the rejuvenation of sexual and reproductive energy[5]. While some Vajikarana herbs work as aphrodisiacs, they also engender reproductive strength in order to increase the health of our offspring, or what vajikarana calls subahupraja[6] – children who are physically and mentally vital and have the fundamental qualities needed for a conscious life Charaka Samhita Chikitsasthana (2000c).

According to the literature there are few preliminary results where appropriately modified Ayurvedic *rasayanas* enhance the fertility and life span of *Drosophila melanogaster* (Pankaj *et al.,* 2010, Priyadarshini *et al.,* 2010,). After this there is no report on influence of rasayana on life span of other model organisms therefore, we have selected *Caenorhabditis elegans* (nematode) for the present study. The objective of the study is to evaluate the rasayana which is formulated as a new *rasayana* using organic herbs (*Emblica officinalis*) and maintaining traditional principles, precisely altered to reflect intrinsic differences between mammals and nematode.

Caenorhabditis elegans is a small free living, nematode naturally found in soil environments. *C. elegans* has a short life span (3 weeks at 22^0 C under optimal conditions), small size (1mm in length) easy to cultivate, and quick generation time, self fertilization, ability to frozon, measurable behavior genetic tractability and relevance to mammalian due to the high degree of conservation of gene sequence (Hope 1999)[9]. *C.elegans* occur in two sexes: self-fertlizing hermaphrodites and males.

. Most of the studies have focused on the effects of metals or agricultural chemicals. But little is known about the influence of the drugs, phyto-chemicals and man made chemicals on *C.elegans*. We used *C. elegans* to study effect of herbal rasayana of *emblica oficinalis* at endpoints of fitness parameters such as number of progeny (brood size) developmental time and also Longevity.

Materials and Methods

Strains

Ceanorhabditis elegans, wild-type strain (N2), was obtained from the *Caenorhabditis* Genetics Center (CGC, Minniepolis, MN, USA), which is funded by the NIH National Center for Research Resources (NCRR). This is borrowed from the east west college,

Preparation of nematode cultures

The worms were cultivated on NGM plates (3 g l^{-1} NaCl, 2.5 g l^{-1} proteose peptone, 5 mg l^{-1} cholesterol, 1 mmol $l^{-1}CaCl_2$, 1 mmol l^{-1} $MgSo_4$, 25 mmol l^{-1}Potassium phosphate, p^H 6.0,17g l^{-1} agar) on an established lawn of *Escherichia coli* strain OP50 (Brenner,1974) and maintained at 20^{0}C. To obtain synchronized culture, gravid hermaphrodites were lysed in an alkaline hypochlorite solution (Sultson et al., 1988) and the eggs were transferred to fresh NGM plates. The culture was grown for 3 days at 20^{0}C until the newly hatched worms reached adulthood. The L4 stage worms/gravid worms were washed with K-medium (53mM NaCl, 32mM KCl) (Williams and Dusenbery, 1990), peleted by centrifugation (3000 rpm, 5 min), washed again thrice with cold K-medium and finally suspended in K-medium to obtain 30-50 nematodes per 10 µl.

Procedure

Experiments were performed in 12–well sterile tissue culture plates. Approximately 100 early gravid animals which is considered as animal pool were transferred in 500µl of K-medium, adjusted to O.D 1.0 with OP-50 *E. coli* bacteria strain (diet of *C.elegans*) at 55O nm containing one drop of rasayana, this is designated as experiment I and control plates were also maintained without rasayana which is named as experiment II (procedure of Priyadharshini *et al.,* 2010). All experiments were carried out at 22^{0}C for 4 hours. End points such as brood size (number of progeny), developmental time, and life span were evaluated.

Brood size and Developmental time

To evaluate the effect of rasayana single worm was picked from pool exposed for 4h in above experiments and transfer to 500µl of K-medium, adjusted to O.D 1.0 with OP-50 at 55O nm 72 hours after incubation. The number of progeny produced by single worm was counted by staining with 0.1% Methylene blue under stereomicroscope (35x). This was screened for both experiment I and II. The developmental time refers to the time taken for development from eggs to L1. Developmental time is observed in the terms of hours, at least 3 replicates for each experiment and five times were performed for statistical purposes.

Lifespan assay

Age synchronized adult worm (L4 stage of *C.elegans*) were exposed to rasayana (experiment I) and control without rasayana (experiment II) for four hours at 22^{0}C. After the exposure period, worms were washed thrice with K-medium and 20±1 L4 worms (designated as day 0 of life span estimated) were placed in a well of 15mm flat bottomed 16–well plates, each well containing 500µl of K-medium (OD at 550nm adjusted to 1.0 with *E.coli* cells) and FudR at a final concentration of 50µm (Keaney et al., 2004). Worms were raised and maintained at 20^{0}C. The survivability was scored every day by gentle touching with platinium wire. The worms which failed to move in response to touch were considered as dead. Mean, standard deviation and One way ANOVA was applied to all parameters using SPSS 10.0. Developmetal time is expressed in hours

Results

The data (Table1) on the brood size and developmental time is represented in mean and standard deviation. The brood size of *C.elegans* was very high in case of rasayana fed *c.elegans* (340.6±10.40) than the control one (315.6 ± 4.50) in the experiment II (table 1). According to One way ANOVA there is a significant difference between the brood size between the experiment I and II (F value=126.10; P<0.001). But in case the developmental time of the *c.elegans* from egg to L1 stage does not have such difference between both experiments I and II rasayana fed worms took 13.24 hours and 13.36 in control hours where there is no such difference according to the One way ANOVA (F value= 14.92).

The life span of *C.elegans* data is depicted in the form of the figure1 where its shows the percentage of survivability of the *c.elegans* which is fed in rasayana and control one. According to the figure life span of the rasayana fed animals was high and it went more than 30th of day the percentage of the *C.elegans* of the control group was nil at almost on the 25th day.

Discussion

In the present study an effort is made to study the effect rasayana of *Emblica officinalis* on fitness parameters and life span. Our results confirms there is a influence of *Emblica officinalis* which is mixed in experimental culture experiment I. These results were similar to some workers Charaka Samhita Sutrasthana.2000a. Sexually reproducing animals are endowed with special features, first to produce fertile offspring and second to adapt to a particular environment. From our studies, rasayana of *Emblica officinalis* shows high number of progeny (table 1) This finding agrees with the observation of Gruwes *et al.,* (1971) where they noticed oviposition rhythm in *Drosophila melanogaster* and Vogel (1972) has demonstrated that certain aziridine analogous have discernible effect on fecundity in *Drosophila*. Table 1 also incorporates mean fertility per female in adult experimental diet with *Emblica officinalis* was seriously increase compare to control, and they were more fertile than control, (P<0.001 by ANOVA). Several workers have made studies on the effect of different chemicals on fertility in *Drosophila melanogaster* (Vasudeva and Krishnamurthy 1983). The present study of the author agrees with them that influence of the chemicals will alter the fertility in *Drosophila. More over* Pankaj 2010, demonstrated the influence of the rasayana on the fertility of *Drosophila.*

The estimation of fitness is the first step in understanding the adaptive evolution of a population. Rate of development is another parameter, which is used to analyze the some chemicals clinically. In the present investigations the genetic constitution, amount of the food, temperature, and space were kept constant. Obviously the differences in the developmental must have been determined by the chemical used or not by the other factors. This type of effect on the developmental time by different chemicals in *Drosophila melanogaster* has been shown by Luning (1966). Contrast to the above studies our results suggest that there is no such influence of rasayana on developmental time of *C.elegans* according to the mean value of both experiment I and II (table1).

emphasizes the importance of the pytochemicals on aging is an inherently *complex* process: no single *chemical drug* targeting a single enzyme is going to be effective against it. According to the Pankaj 2010 and Priyadharshini 2010 there is a extension of life span in Drosophila which is fed by the rasayana compare to non fed flies. These results are similar to our results where worms in the experiment I which is treated with rasayana show more long life span compare to the experiment II (control one). We authors confirms the developmental time is not early in case of the rasayana fed worms.This suggests the time taken to the completion of the life cycle is normal and there is relationship between the prolong life span and normal developmental time.

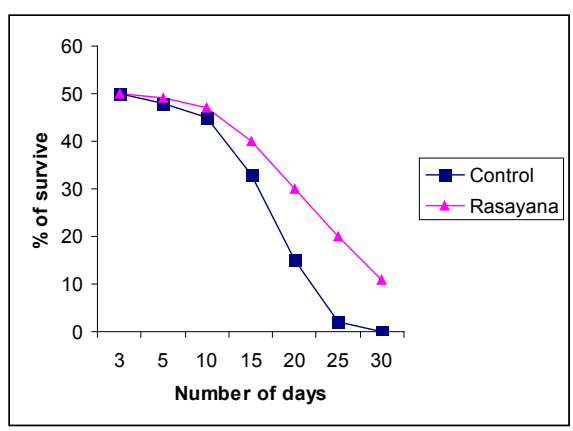
Conclusion:

Like *Drosophila, C.elegans* is also one of the important model organisms in field of life science. One can use *c.elegans* in screeening the pytochemicals and any drugs. Our data shows there is influence of rasayana on brood size and lifespan of *C.elegans* worms. This is contrast to the developmental time where there is no early development of the egg into L1 stage and extension of lifespan of nematode worms.

Table 1: fittness paramaters of *Rasayana*-fed and Control one of *C.elegans*

Batch	N	Number of progeny	Developmental time (in hours)
Rasayana (Experiment I)	25	340.6 ± 10.40	13.24 ±1.09
Control (Experiment II)	25	315.6 ± 4.50	13.36 ± 0.99
F value		126.10	14.92
P value		0.000**	0.686

Figure 1 Lifespan of *C.elegans* in rasayana (experiment I) and control plates (experiment II)



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