EVALUATION OF *EMBLICA OFFICINALIS* RASAYANA ON FITNESS PARAMETERS AND LIFE SPAN OF NEMATODE: *CAENORHABDITIS ELEGANS*

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ABSTRACT: For the first time nematode *C. elegans* is exposed to test Ayurvedic medicine *Emblica officinalis* rasayana (herbal formulations). The objective of our study was to explore the potential of *Emblica officinalis* drug on reproductive fitness, longevity of *C. elegans*. Our results shows increase in brood size (number of progeny), lifespan was observed in rasayana fed worms, compare to control one according to the one way ANOVA.

KEY WORDS: *Caenorhabditis elegans*, Rasayana, *Emblica officinalis*.

Ayurveda is one of the ancient Indian medical sciences and oldest available classic of the world. which can be traced back to the Vedas, Vedas are the ancient books of knowledge or science from India. They contain practical and scientific information on various subjects beneficial to health, philosophy, engineering, astrology etc; Ayurveda combines physical, psyclogical and spiritual therapies in an approach to health that has addressed itself to the fundamental principles of good health and longevity. Ayurveda is precisely about maintaining or preserving good health as it about treating disorders–*"Swaasthasya Rakshanam*" is one of the goals of Ayurveda (Charaka Samhita Sutrasthana, 2000a).

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). *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 (Charaka Samhita Chikitsasthana, 2000b). The literal meaning of *rasayana* is "augmentation of *rasa*", the vital fluid produced by the digestion of food. Rasa provides nutrition, enhances the vigor, vitality and Longevity of life (Pankaj et al., 2010; Priyadarshini et al., 2010). *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, so that you are not exposed to sickness and disease as long as you live (Charaka Samhita Chikitsasthana, 2000b). *Emblica officinalis* is such herb used as rasayana in treatment of diseases. However effect of this drug on normal life activities has not been significantly validated. Therefore the present study was carried out with an *objective* to explore the potential of *Emblica officinalis* drug, on fitness of *C. elegans*. We have *C. elegans* as the test system which proved to be an excellent organism to test the effect of many drugs and other chemicals.

Valiathan has recognized the opportunity to create "Ayurvedic Biology" (Valiathan et al., 2006). 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 (2006) as saying that

rasayana should be tested on animal models. This rasayana is commonly used as rasayana therapy in Ayurveda medicine system but, possibly due to complexity and expense, only few trials have been conducted to test the rasayana formulations. There is urgent need to evaluate the influence of ancient herbal formulations of rasayana. According Pankaj et al. (2010); Priyadarshini et al. (2010); Guru Prasad et al. (2011) to literature there are few preliminary results where appropriately modified Ayurvedic *rasayana* enhance the fertility and life span of *Drosophila melanogaster*. After this there is no report on influence of rasayana on life span on any other model organisms, therefore we have selected *Caenorhabditis elegans* (nematode) for the present study as model. We have used *C. elegans* to study effect of herbal rasayana of *Emblica officinalis* fruits at endpoints of fitness parameters such as number of progeny (brood size), developmental time and also life span (Longevity).

Caenorhabditis elegans is a small free living, nematode naturally found in soil environments. It was chosen in 1963 by Sydney Brenner as a model organism to study the genetics of development and reproductive system. Today, this nematode *C. elegans* being simple, multicellular metazoan organism, transparent, free living nematode with a nervous digestive and reproductive system and specialized muscles (Riddle et al., 1997). It has been extensively used in biomedical research. *C. elegans* has a short life span (3 weeks at 22^{o}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).

C. elegans occurs in two sexes: self-fertilizing hermaphrodites and males. Adult hermaphrodites contain 959 somatic cells, while the males have 1031 cells (Hodgkin, 1988). Although there is limited number of cells in this novel animal, *C. elegans* has highly differentiated digestive muscular nervous system and reproductive system. *C. elegans* develops from a fertilized egg to a gravid adult in about 3 days at 20^{o}C by Hali & Altun (2008). A single hermaphrodite has ability to produce approximately 300 offspring. Offspring mature through four larval stages, L1-L4, growing in spurts between stages after molting old cuticles. The life cycle is short that its take 3 days eggs to L4 and next gravid (adult) will alive for 15 days in the bacterial diet (OP-50 strain) on agar or K media lawn (Byerly et al. 1976; Knight et al., 2002). At two points in the life cycle, if food is not available, nematode growth will arrest. Animals will survive for several hatchlings that arrest, as L1s or for several months starved L2s develop into dauer larvae (Lewis & Fleming, 1995). There is a wealth of knowledge available on *C. elegans* including its complete genomic sequence. There is a technology to quickly produce transgenic nematodes by Mello & Fire (1995) and the ability to observe all the cells in the living nematode by microscopy (Hali & Altun, 2008). The available microscopy techniques in *C. elegans* have led to the development of an exceptionally detailed in *C. elegans* reproductive and development biology. In addition, *C. elegans* is an ideal organism for assessing toxicity of heavy metals and detergents as well as organophosphate–induced mammalian neurotoxicity. A considerable amount of toxicity testing has been performed using *C. elegans* including lethality by Anderson and Boyd William (2008) and behavioral tests in aquatic media by Dhawan et al. (2008). 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*. The objective of the study is to evaluate the rasayana which is formulated as a new *rasayana* using (*Emblica officinalis* fruits) and maintaining traditional

principles, precisely altered to reflect intrinsic differences between mammals and nematode.

MATERIALS AND METHODS

Preparation of the *Emblica officinalis* Rasayana

The dried fruits of *Emblica officinalis* were grinded into powder and 5 gm of the powder was added to a bottle and extracted by reflux in 50 ml. 60% aqueous methnol for 2h. The mixture was filtered, and the filtrate was collected. The extract was then concentrated to dryness by rotary vapourization at 50°C under reduced pressure according to Quanbin et al. (2006) and a light brown powder (3.0 g) was obtained. This powder (300 mg) was directly dissolved in 25 ml distilled water.

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, Udayapura, Maharastra.

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°C. To obtain synchronized culture, gravid hermaphrodites were lysed in an alkaline hypochlorite solution (Sultson et al., 1980) and the eggs were transferred to fresh NGM plates. The culture was grown for 3 days at 20°C until the newly hatched worms reached adulthood. The L4 stage worms / gravid worms were washed with K-medium (53 mM NaCl, 32 mM KCl) according to Williams & Dusenbery (1990) and 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 10 µl of rasayana, this is designated as experiment I and control plates were also maintained without rasayana which is named as experiment II. All experiments were carried out at 22°C for 4 hours. The end points such as brood size (number of progeny), developmental time, and life span (number of survival rate) were evaluated.

Brood size (number of progeny) and Developmental time

To evaluate the effect of rasayana (*Emblica officinalis*) single worm was picked from pool exposed for 4h in above experiments and transfer to 500 µl of K-medium, adjusted to O.D 1.0 with OP-50 at 550 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 *Emblica officinalis* rasayana (experiment I) and control without rasayana (experiment II) for four hours at 22°C. After the exposure period, worms were washed thrice with K-medium and 50 L4 worms (designated as day 0 of life span estimated) were placed in a well of 15 mm flat bottomed 16–well plates, each well containing 500 µl of K-medium (OD at 550 nm adjusted to 1.0 with *E. coli* cells) and FudR (5-fluro-2-deoxyuridine) at a final concentration of 50 µm (Keaney et al., 2004). Worms were raised and maintained at 20°C. The survivability was scored every day by gentle touching with platinum 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 11.0.

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* (380.6±10.40) than the control one (313.6 ± 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= 124.10; $P < 0.001$). 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 (12.24 hours and 14.36 hours) where there is no such difference according to the One way ANOVA (F value= 11.92).

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 (*Emblica officinalis)* 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* of rasayana on fitness parameters and life span. Our results confirm there is an influence of *Emblica officinalis* rasayana 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 shows high numbers of progeny (Table 1). Our finding agrees with the observation of Grawes 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*. Several workers have made studies on the effect of different chemicals on fertility in *Drosophila melanogaster* (Vasudeva & Krishnamurthy, 1983). The present study of the author agrees with them that influence of the chemicals will alter the fertility in *C. elegans*. Moreover (Pankaj et al., 2010; Guru Prasad et al., 2011) showed the influence of the rasayana of *Emblica officinalis* on the fertility of *Drosophila*.

The estimation of fitness is the first step in understanding the adaptive evolution of a population. The 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 Jafari et al. (2007). 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).

Jafari et al. (2008) emphasizes the importance of the phyto-chemicals on aging is an inherently *complex* process: no single *chemical drug* targeting a single enzyme is going to be effective against it. Along with this his group is also suggested that failure to find pharmaceuticals significantly helping to slow or reverse human ageing processes reflects the number of genes and biochemical pathways involved; He suggests "mass screening of pharmaceuticals and botanicals to produce effective therapeutics for human aging is essential in increase in life span. 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 (Jafari et al., 2008). According to (Priyadarshini et al., 2010; Guru Prasad et al., 2011) 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 confirm the developmental time is not early in case of the *Emblica officinalis* rasayana fed worms. This suggests the time taken to the completion of the life cycle is normal and there is effect of *Emblica officinalis* rasayana on prolong life span and normal developmental time.

CONCLUSION

Like *Drosophila, C. elegans* is also one of the important model organisms in field of testing some of the plant products. Our data shows there is influence of rasayana on brood size number of the progeny and extension of lifespan of nematode worms. This is contrast to the developmental time where there is no early development of the egg into L1 stage according to the statistics.

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Table 1. Fitness parameters of *Emblica officinalis rasayana*-fed and Control one of *C. elegans*.

Batch	N	Number of progeny	Developmental time (in hours)
Rasayana (Experiment I)	25	380.6 ± 10.40	12.24 ±1.09
Control (Experiment II)	25	313.6 ± 4.50	14.36 ± 0.99
F value		124.10	11.92
P value		0.000**	0.686

Values are in M ± SE, **P<0.001.

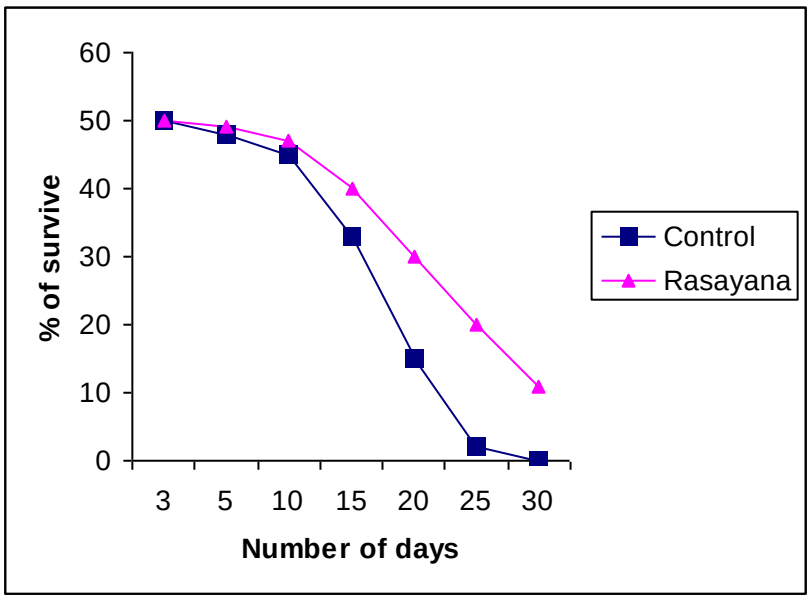

Figure 1. Lifespan of *C. elegans* in *Emblica officinalis* rasayana (experiment I) and control plates (experiment II).