Rasayana effect of *Guduchi Churna* on the life span of *Drosophila melanogaster*

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Abstract

Introduction: The *Drosophila melanogaster* is one of the most extensively studied animal models for understanding the process of aging and longevity. This fly is being used for genetic studies since almost a century. *Guduchi* (*Tinospora cordifolia* [Wild.] Miers.) is an Ayurvedic drug used as *Rasayana* (rejuvenation) due to its antiaging effect. **Aim:** To explore the potential of *Guduchi Churna* (powder of *T. cordifolia*) on the life span of *D. melanogaster* using adult feeding method. **Materials and Methods:** For experimental setups, the regular food media were mixed with *Guduchi Churna* in different concentrations (0.25 g/100 ml, 0.50 g/100 ml, and 0.70 g/100 ml). Flies were exposed to these concentrations for 30 days. **Results:** Increase in the lifespan was observed in both parent and F-1 generation. Applying of ANOVA revealed significant difference between control and drug groups. The longevity effect, in the drug group was seen even on F-1 generation. **Conclusion:** *Guduchi Churna* enhances the life span of *D. melanogaster* in both parent and F-1 generation.

Keywords: *Drosophila melanogaster*, *Guduchi Churna*, longevity, *Rasayana*, *Tinospora cordifolia*

Introduction

Rasayana (rejuvenation therapy), one of the most essential branches of Ayurveda,[1] practiced effectively and extensively since ages, is a group of medicinal preparations which are immunostimulants and capable of preventing the causation of many ailments such as untimely aging. The term *Rasayana* refers to nourishment or nutrition. *Rasayana* drugs act essentially on nutrition dynamics and rejuvenate the body and psyche. *Rasayana* drugs also promotes intellect and strength, prolongation of life and helps in the prevention of diseases.[2]

Aging is a process of unfavorable progressive changes associated with decline in vigor resulting in death. In humans, aging represents the accumulation of changes in a human being over time.[3] Aging is one among the largest known risk factors for most human diseases. However, every individual wants to stay healthy, look younger, and live longer. In recent years, scientists are interested to discover the scientific clues to the aging process and to determine if the process of aging is genetically or environmentally controlled, or by both. To this respect, different food components have been shown to increase life span of many organisms; however, no conclusive evidence in favor of any particular food component has yet been established.

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The vinegar fly, *Drosophila melanogaster*, is an attractive model organism for studying the mechanisms of aging due to its relatively short lifespan, convenient husbandry, and facile genetics.[4]

Guduchi (*Tinospora cordifolia* [Wild.] Miers.) is an Ayurvedic drug used for *Rasayana Chikitsa*.[5] The biological effect of this drug on life span has not been tested till now, but only being prescribed to human beings. Therefore, to revalidate the concept of *Rasayana Chikitsa* and in particular to assess the longevity effect of *Guduchi Churna*, the present study was carried out using *D. melanogaster* as the test system.

Materials and methods

Preparation of food media

Wheat cream agar medium was used as a food medium for *D. melanogaster*. This medium was prepared by boiling

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1000 ml of distilled water along with 100 g of jaggery. After complete dissolution of jaggery, 100 g of wheat flour (*Suji* or *Rava*) was added to the medium and then 10g of agar and 7.5 ml of propionic acid (antifungal) were added gently. The medium was distributed to glass vials of 8 cm × 2.5 cm size. The mouth of the vials was kept closed with sterilized cotton.[6] One day later, one or two drops of yeast solution were added to the food media. This medium was used after 24 h. At every step, heat vials were used for preparing medium, to prevent outbreak of pests and diseases. This culture was used as control culture. For experimental culture, different dose were calculated for *T. cordifolia.* There was no dietary restriction for the flies in both control and experimental cultures.

Preparation of cultures

To study the impact of *T. cordifolia* on longevity of *D. melanogaster* (Mysore strain), flies were obtained from *Drosophila* stock center, Department of Zoology, Mysore. The pure culture of these flies was maintained under standard food medium.[7] The effect of *T. cordifolia* was studied by adult feeding method. For this purpose, stocks of the flies were built up for five to six generation from isofemale line. The virgin females and bachelor males emerged from the normal media were isolated under ether anesthesia within 3 h of eclosion. Four groups were made and each group contain equal number of male (10) and female (10) flies and was marked as control, V group, S group, and T group. Test group flies were fed with 0.25g/100 ml, 0.50g/100 ml and 0.70g/100 ml of test drug with food media respectively. These flies were transferred to 8 cm × 2.5 cm autoclaved glass culture vials (to avoid contamination) containing equal quantity of normal food media used for *Drosophila* culture.

Experimental design

The stems of *Guduchi* was procured from its natural habitat, authenticated at Pharmacognostical Laboratory of IPGT and RA, Jamnagar, and made into fine powder (mesh size #72). For experimental setups, the regular food media were mixed with *Guduchi Churna* in different concentrations (0.25 g/100 ml, 0.50 g/100 ml, and 0.70 g/100 ml). For longevity (life span) study, twenty virgin females and bachelor males from all the four cultures were used. Daily, these flies were transferred to the fresh vials of all the three cultures and a controlled experiment (without any drug content) was also run simultaneously. Flies were exposed to experimental cultures of respective concentrations for 30 days. Later on, each vial was minutely monitored and the number of dead flies were recorded. For each experiment, life span of each fly was noted by simply noting the survivability of flies. All these experiments were conducted inside a BOD (Bio-Oxygen Demand) incubator with constant temperature (25°C) and other environmental conditions. The results were calculated in terms of number of days.

Statistical analysis

The data were statistically analyzed by one-way ANOVA test.

Results

Results revealed significant effect of *Guduchi Churna* on lifespan of *D. melanogaster*. Results clearly indicates that there was 85.91% increase in life span of T group and the mean life span was 53.08 ± 0.14 [Table 1 and Figure 1].

The linear plot [Figure 1] shows that in control group, there was no survivability of flies in 30–40 days. In V group, the rate of survivability was 62% for 80–90 days. In S group, the rate of survivability was 47% for 70–80 days. In T group, the rate of survivability was 39% for 60–70 days. Slight differences in the rate of survivability of *D. melanogaster* flies were found in both the sexes [Table 2]. The life span of female flies was slightly higher when compared to male flies in all the groups [Figure 2].

The difference in mean life span of control group and V group flies was 55.50 days which is statistically significant ($P > 0.001$). The difference of mean between control group and S group was 44.40 days, which again reflecting toward the positive effect of drug on the longevity of flies ($P > 0.01$). However, comparison between control and T group shows statistically insignificant result ($P < 0.05$) though the difference of mean was 23.80 days [Table 3].

In F-1 (first generation) of control group, the life span of male and female flies was 24 days. The highest life span of flies (F-1 generation) was observed in 0.25 g/100 ml concentration, i.e., 46 days. In 0.50 g/100 ml concentration (F-1 generation), the life span of flies was 33 days. In the highest concentration, i.e., 0.70 g/100 ml (F-1 generation), the rate of survivability is less compared to other drug groups

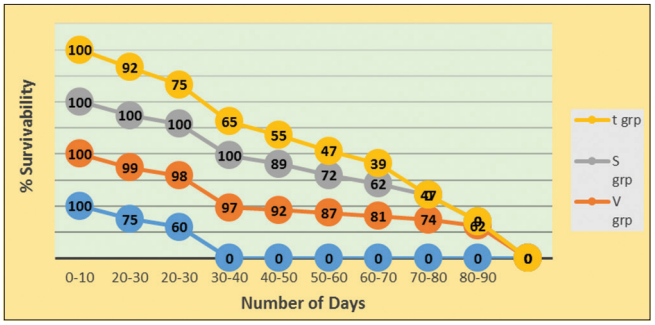

Figure 1: Comparison between percentage survivability among all the groups

Table 1: Effect of *Guduchi Churna* in different concentration on life span of male and female Drosophila flies (n=100)

Groups	Dosage (g/100 ml)	Survivability	
		Survival days	Percentage of increase in life span
Control	-	28.55±0.14	-
V group	0.25	83.89±0.24	193.83
S group	0.50	72.99±0.25	155.65
T group	0.70	53.08±0.14	85.91

Table 2: Comparative effect of *Guduchi Churna* in different concentration on life span of male and female Drosophila flies

Groups	Dosage (g/100 ml)	Survivability (in number of days)				
		Male *Drosophila* (mean±SE)	SD	Percentage of increase in life span	Female *Drosophila* (mean±SE)	Percentage of increase in life span
Control	-	29.05±0.29	39.019	-	28.08±0.94	-
V group	0.25	83.76±0.34	30.401	188.33	84.02±0.33	199.4
S group	0.50	71.71±0.30	38.203	146.85	74.26±0.30	164.45
T group	0.70	51.48±0.30	37.559	77.21	54.68±0.70	94.72

SD: Standard deviation, SE: Standard error

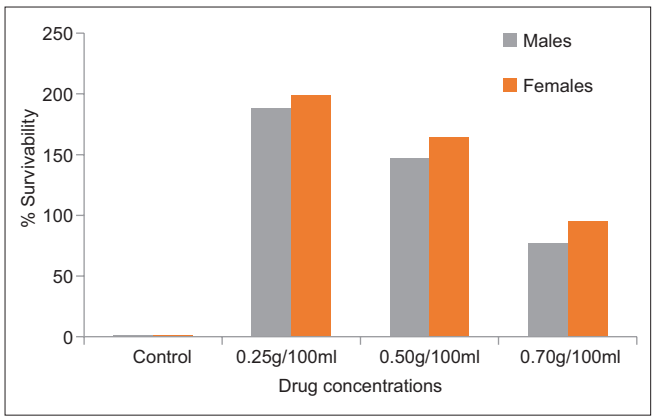

Figure 2: Comparative effect of *Guduchi Churna* in different concentration on life span of male and female flies

as the dose of *Guduchi Churna* increased, the life of flies was decreasing [Table 4].

The differences in longevity period between F-1 generation of V group and control group was 22 days which is statistically significant ($P < 0.001$). The difference of mean between control group and S group was only 9 days which is statistically insignificant ($P > 0.05$). However, comparison between control and T group shows statistically insignificant result ($P > 0.05$) though the difference of mean was only 5.00 [Table 5].

Discussion

The fruit fly *D. melanogaster* has been used as a model in biological research on aging for exploring the longevity phenotypes, artificial and natural selection responses.[8] *T. cordifolia* whole plant extract had shown neuroprotective property *in vivo* in *Drosophila*.[9] In view of the health-promoting potential of *Guduchi Churna*, impact on longevity in *Drosophila* was planned.

In control group, the life span of male and female flies was 29 and 28 days, respectively. The highest life span of male and female flies was observed in 0.25 g/100 ml concentration, i.e., 83.2 and 84.8 days, respectively. In 0.50 g/100 ml concentration, the life span of both male and female flies was 33 days. This is suggestive of *Guduchi Churna* exhibits *Rasayana* effects irrespective of sex. It was also observed that increase in the concentration of drug led to decrease in the survivability rate. At lower concentration of *Guduchi Churna*

Table 3: Mean difference of longevity in different groups, multiple comparisons versus control group (Holm-Sidak method)

Comparison	Difference of mean	*t*	*P*
Control and V group	55.50	3.40	< 0.001
Control and S group	44.40	2.72	< 0.01
Control and T group	23.80	1.46	> 0.05

Table 4: Comparative effect of *Guduchi Churna* in different concentration on life span of Drosophila flies in F-1 generation

Groups	Dosage (g/100 ml)	Survivability (in number of days)	
		Mean±SE	Percentage of increase in life span
Control	-	24.05±12.5	-
V group	0.25	46.00±10.9	91.26
S group	0.50	33.00±12.5	37.21
T group	0.70	29.00±12.6	20.58

SE: Standard error, F-1: First generation

Table 5: Multiple comparisons versus control group (Holm-Sidak method) in F-1 generation

Comparison	Difference of mean	*t*	*P*
Control and V group	22.00	4.26	< 0.001
Control and S group	9.00	1.74	> 0.05
Control and T group	5.00	0.97	> 0.05

F-1: First generation

there was a significant increase in lifespan of the flies. Higher concentrations progressively reduced the life span of flies significantly. Increasing the concentrations further did not show more increase in lifespan. This indicates that there is a particular concentration of *Guduchi Churna* which causes maximum lifespan extension (around 0.25 g/100 ml of food). Concentrations beyond that limit do not further increase the lifespan, which may be due to the absorption threshold of *Guduchi Churna*. The increase in lifespan of the flies due to *Guduchi Churna* can be attributed due its high antioxidant properties. Researches suggest that, the alcoholic and aqueous extracts of *T. cordifolia* are reported to have beneficial effects on the immune system[10] and have been tested successfully for their immunomodulatory activity.[11-14] Although the exact

mechanisms of the action of *Guduchi Churna* by which this plant's products are able to enhance the survivability of *D. melanogaster* could not be ascertained from this study, but has validated the concept of *Vayasthapana* and *Rasayana* effect of *Guduchi Churna*.

Conclusion

Guduchi as *Rasayana*, enhances the life span of *D. melanogaster* which validates the concept of *Rasayana*. Increase in the life span of F-1 generation of *Drosophila* is suggestive of *Rasayana* effect of *Guduchi Churna* even prevails to next generation.

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Conflicts of interest

There are no conflicts of interest.

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हिन्दी सारांश

ड्रोसोफिला मेलनोगेस्टर के जीवनकाल पर गुडूची चूर्ण का रसायन प्रभाव

पंकज पाठक, महेश कुमार व्यास, हितेश ए व्यास, मुकेश बी नारिया

जरा और दीर्घायु प्रभाव की प्रक्रिया को समझने के लिए ड्रोसोफिला मेलनोगेस्टर एक मुख्य एनिमल मॉडल है। यह मक्खी एक शतक से आनुवांशिक अध्ययनों के लिए प्रयोग में ली जाती रही है। गुडूची (टिनोस्पोरा कॉर्डिफोलिया विल्ड.) एक आयुर्वेदिक औषधि है जो रसायन प्रभाव के कारण आयुवर्धन के लिए उपयोग की जाती है। इसी उद्देश्य से गुडूची चूर्ण की आयुवर्धन गुणवत्ता को ज्ञात करने के लिए एडल्ट फीडींग तकनीक से ड्रोसोफिला मेलनोगेस्टर को खाद्य के रूप में दिया गया। प्रयोगिक स्वरूप के आधार पर प्रतिदिन गुडूची चूर्ण को अलग अलग मात्रा (0.25g/100ml, 0.50 g/100ml और 0.70g/100ml) में खाद्य के साथ ३० दिनों तक दिया गया। परिणाम स्वरूप F-1 वंश और दोनों अभिभावकों में आयु की वृद्धि पाई गई। प्रयोग किए जा रहे कंट्रोल और ड्रग समूह के बीच परिणाम में अंतर पाया गया। प्रस्तुत अध्ययन से यह सिद्ध होता है कि गुडूची चूर्ण ड्रोसोफिला मेलनोगेस्टर के जीवन काल को विवर्धन करने में सार्थक है।