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EFFECT OF GANDHAKA AS RASAYANA IN THE LIFE SPAN OF DROSOPHILA MELANOGASTER

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ABSTRACT

Introduction: Gandhaka is an excellent rejuvenator that falls within the uparasa classification.. Drosophila is an ideal model organism for exploring ageing and lifespan mechanisms.

Aim: To study the effect of Shuddha Gandhaka in extending the life span of *Drosophila melanogaster* adopting adult feeding method.

Materials and Methods: Regular feeding media was combined with Shuddha Gandhaka at varying concentrations (12.5 mg/100 ml, 25 mg/100 ml, and 37.5 mg/100 ml) for the experimental setup. All the groups were given the same medium until it died.

Results: In the experimental media treated with Shuddha Gandhaka, there was an increase in lifespan. The effect of Shuddha Gandhaka on the longevity of *Drosophila melanogaster* were shown to be notable. The results clearly show that the G IV group had a 36.79 percent increase in life span, with a mean life span of 62.37.

It was discovered that there was a significant difference between the control and treatment groups. The findings appear to extend the lifespan of *Drosophila melanogaster* and to be consistent with their indicated human doses in Ayurvedic treatment.

Conclusion: In the context of the reported "Longevity" effects of Shuddha Gandhaka, the present in vivo study on a model organisms shows that Shuddha Gandhaka feeding significantly improves the life span of *Drosophila melanogaster.*

Keywords: *Drosophila melanogaster*, Shuddha Gandhaka, Longevity, Rasayana

INTRODUCTION:

Ayurveda is basically the science of life and longevity. The science has given much importance to longevity and is more evident in its definition.

Rasayana [Rejuvenation therapy] is one of the eight branches of Ayurveda which is most essentially practiced since ages extensively **[1]**. The Rasayana, can help to prevent early ageing and rejuvenate the body and mind. Rasayana drugs also promote immunity, strength and intellect, and strengthen the sensual organs, prolongation of life and helps in prevention of diseases **[2]**. Rasashastra being a main branch of Ayurveda which stands unique in the field of medical science by emphasizing the usage of processed minerals and metals in therapeutics. It is enriched with large number of potent medicines and formulations which are described as Rasayana. Gandhaka is one such wonderful drug which is a good rejuvenator enlisted under uparasa having 125 mgs to 1 gm as human dosage mentioned by many classical texts of Rasashastra **[3]**.

One of the most important fields of research, is one that is widely supported by various theories, is understanding the biology of ageing and life span extension. Due to its short life span, ease of raising and handling in the laboratory, low maintenance requirements, and rich genetic resource, *Drosophila* has been utilised extensively in ageing and longevity experiments. Most significantly, the *Drosophila* genome has been sequenced, with over 50% of fly genes having comparable human counterparts **[4, 5]**. *Drosophila* is an excellent model organism for exploring the mechanisms of ageing and longevity because of the above mentioned attributes. *Drosophila*, with its extensive genetic tools and well-studied developmental pathways, promises to be an excellent model for investigating the cellular and molecular mechanisms underlying the effects of various Ayurvedic formulations **[6]**.

The focus of this study is to evaluate Longevity effect of Shuddha Gandhaka in *Drosophila melanogaster*.

MATERIALS AND METHODS:

Strain

Drosophila melanogaster of Oregan K strain was selected for longevity experiments and was obtained from *Drosophila* stock center, University of Mysore.

Test Compound

Shodhana of Gandhaka was carried out in the Department of Rasashastra and Bhaishajya Kalpana, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan. It was carried out as per the reference of Rasaratna Samucchaya **[7]**.

Ashuddha Gandhaka was pounded in stone mortar. A vessel containing adequate milk to immerse all of the Gandhaka in it was used, and a white thin cloth was wrapped around its mouth and tied. Iron vessel containing ghee was heated on mild fire, when the ghee in the iron pot melted, the Gandhaka churna was added and the Gandhaka melted, it was transferred into a vessel containing milk via a cloth to filter the impurities in the Gandhaka. The Gandhaka, which solidifies in the milk, was washed with warm water and then pat dried each time. The dried Gandhaka was pounded into powder once again, and the process was continued six times with the same amount of components each time. Then finally obtained Shuddha Gandhaka is dried and stored in a container.

Preparation of food media

The flies pure culture was cultivated in a standard food medium of wheat cream agar. This food medium was made by boiling 1000 ml of distilled water and 100 gm of jaggery together. After the jaggery was dissolved, 100gm of wheat powder (soji/ rava) was added to the medium, along with 10gm of agar and 7.5ml of propionic acid (an antifungal).

The culture bottles were filled with the medium. The mouths of the bottles were kept closed for a day by plugging them with sterilised cotton and leaving them undisturbed. Yeast granules were introduced to the food media a day later **[8]**. After 24 hours, this medium was used.

Heat vials were utilised for the prepared media at each step to avoid the spread of pests and pathogens. Control culture is based on the same culture. Different doses of Shuddha Gandhaka were estimated for experimental culture. The flies in both the control and experimental cultures had no dietary restrictions.

Preparation of Culture

Drosophila melanogaster flies were collected from Drosophila stock center, University of Mysore to evaluate the effect of Shuddha Gandhaka on longevity. Under the wheat agar medium, the files' pure culture was preserved **[9]**.

Adult feeding method was used to test the impact of Shuddha Gandhaka. Under ether anaesthesia, the virgin females and bachelor males who emerged from the wheat agar medium were segregated.

Four groups were made and each group contains 20 male and 20 female flies and was marked as G I, G II, G III, G IV Groups. Test group flies were fed with 12.5 mg, 25 mg, 37.5 mg of test drug with the wheat agar media respectively. The flies were aged 4 days and transferred to fresh culture vials containing 6 ml of wheat agar media.

Where G I - Group I i.e control group, G II- Group II treated with 12.5 mg, G III- Group III treated with 25 mg, G IV- Group IV treated with 37.5 mg of Shuddha Gandhaka.

Experimental design:

Table 1: Groups with dosage

Groups	Formulation	Dosage (mg/100ml)
G I	Control group	Wheat cream agar media
G II	Test group– Dose I	12.5mg + food media
G III	Test group– Dose II	25 mg + food media
G IV	Test group–Dose III	37.5mg + food media

(* G I - Group I, G II - Group II, G III - Group III, G IV - Group IV)

Wheat agar media was combined with Shuddha Gandhaka churna in different concentration for the experimental setups.

For longevity study, twenty virgin females and bachelor males were obtained from the culture and aged for 4 days were transferred to fresh culture bottles. The flies were maintained at 22°± 2°C with relative humidity of 70%, with artificial light in a sequence of 12 h light/12 h dark cycle.

Flies were maintained in culture bottles containing Wheat agar media and were maintained under aseptic condition throughout the experiment.

These flies were then transferred to new vials of all three cultures and a controlled experiment. Until its death, all of the groups were given the same medium. Longevity is defined as the period of time from birth to death. The survivability of flies was simply documented for each trial. The days were being used to calculate the results.

Statistical analysis:

SPSS version of one-way ANOVA test was used to statistically assess the data. Student- t test for comparison between control and test groups.

RESULTS

The effect of Shuddha Gandhaka on the longevity of *Drosophila melanogaster* were shown to be notable. The results clearly show that the G IV group had a 36.79 percent increase in life span, with a mean life span of 62.37.

Survival Mean ± SE of Bachelor Male in Control and treated *Drosophila melanogaster* **(Graph 1)**.

Illustrates the effect of control and varying concentrations of Shuddha Gandhaka on the lifespan of Female Virgin *Drosophila melanogaster* **Table 3**.

Survival Mean ± SE of Female Virgin in Control and treated *Drosophila melanogaster* (**Graph 2**).

In both sex, there were minimal differences in the rate of longevity of *D. melanogaster flies*. Female flies had a slightly longer life span than male flies in all of the groups **(Table 4)**.

Comparative effect of Shuddha Gandhaka Churna in Percentage of increase in life span of male and female flies (**Graph 3**).

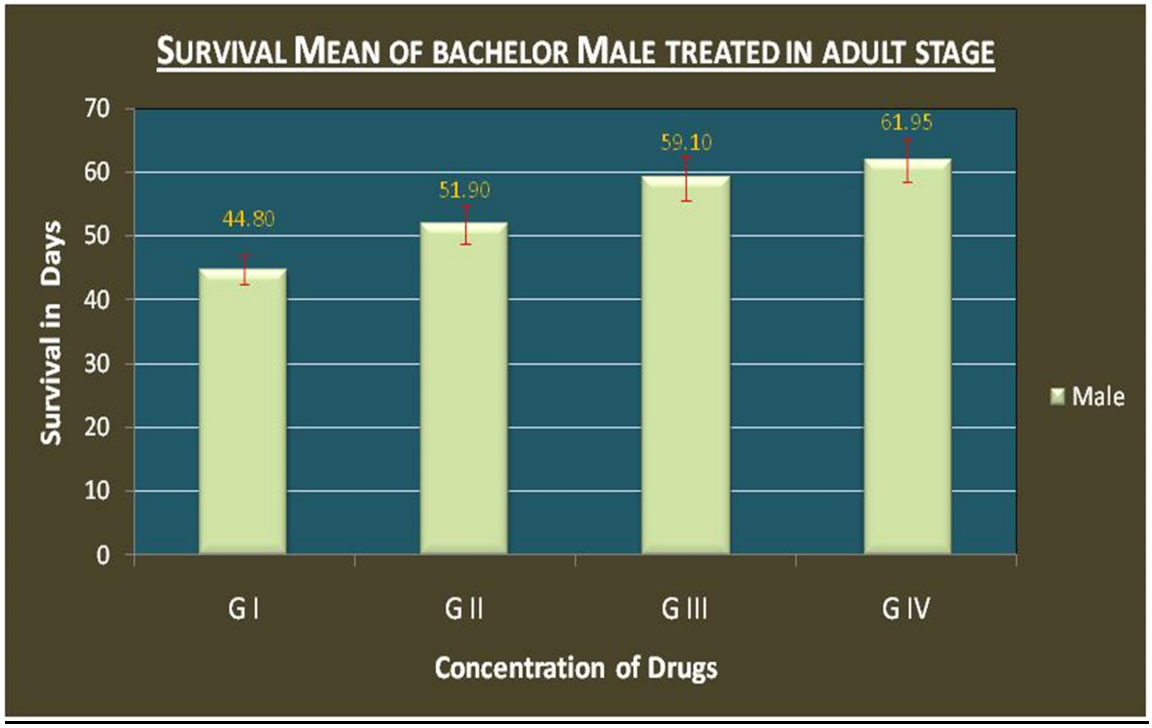
Comparison of survivability percentages across all groups (**Graph 4**).

Table 2: Illustrates the effect of control and varying concentrations of Shuddha Gandhaka on the lifespan of Bachelor Male *Drosophila melanogaster*:

Groups	Dosage (mg/100ml)	Mean± SE	Percentage of increase in life span	F Ratio	Sig
G I	-	44.80±2.30			
G II	12.5	51.90±3.10	15.85	27.29	0.000
G III	25	59.10±3.43	31.92	8.64	0.004
G IV	37.5	61.95±3.43	38.28	10.63	0.002

*** The mean difference is significant at 0.5 level (P < 0.05)**

G I (Control), G II (Shuddha Gandhaka 12.5mg), G III (Shuddha Gandhaka 25mg), G IV (Shuddha Gandhaka 37.5mg)



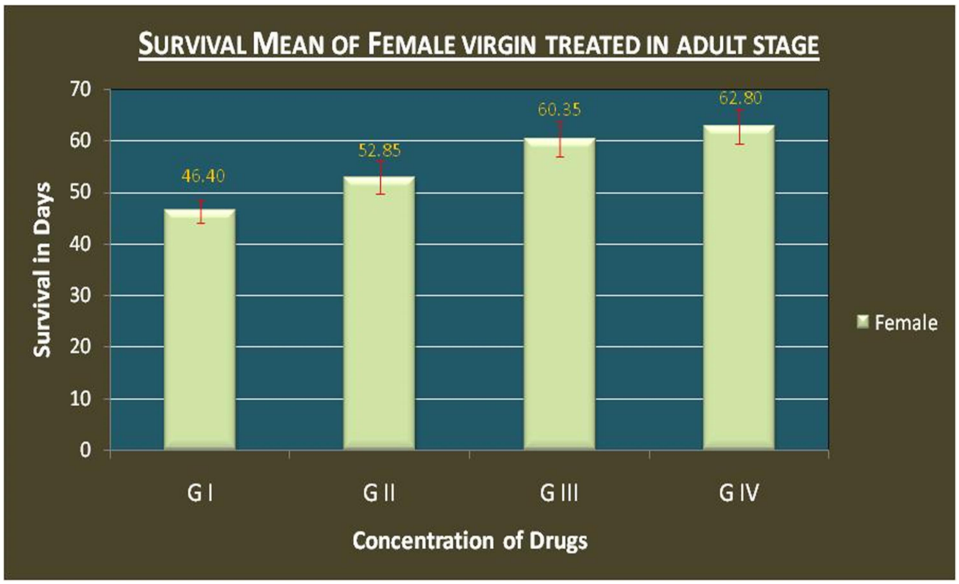
Graph 1:

Table 3:

Groups	Dosage (mg/100ml)	Mean± SE	Percentage of increase in life span	F Ratio	Sig
G I	-	46.40±2.22			
G II	12.5	52.85±3.21	13.90	14.34	0.010
G III	25	60.35±3.54	30.06	10.72	0.017
G IV	37.5	62.80±3.48	35.34	15.84	0.008

*** The mean difference is significant at 0.5 level (P < 0.05)**

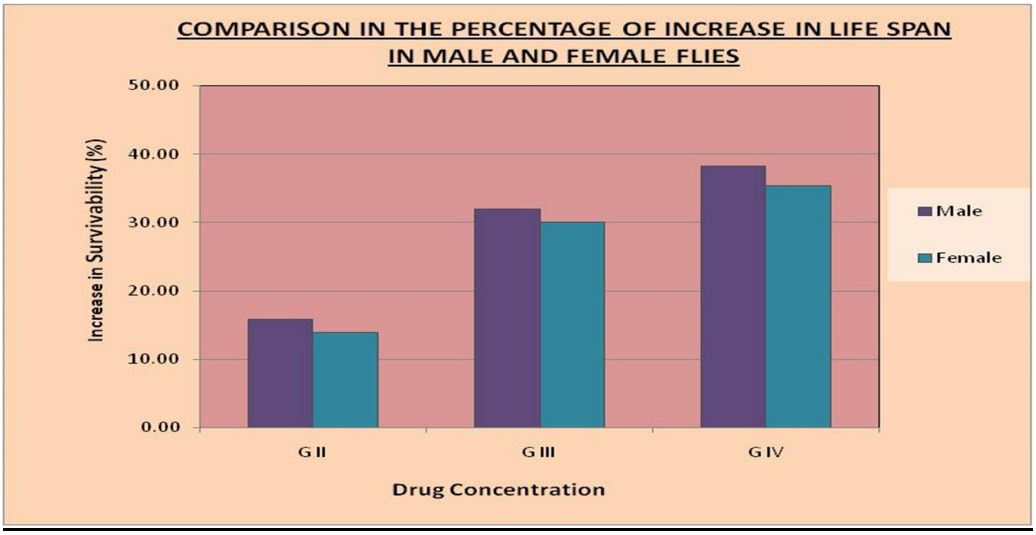
G I (Control), G II (Shuddha Gandhaka 12.5mg), G III (Shuddha Gandhaka 25mg), G IV (Shuddha Gandhaka 37.5mg)



Graph 2

Table 4: Comparative effect of various concentration of Shuddha Gandhaka on the survival of male and female Drosophila flies.

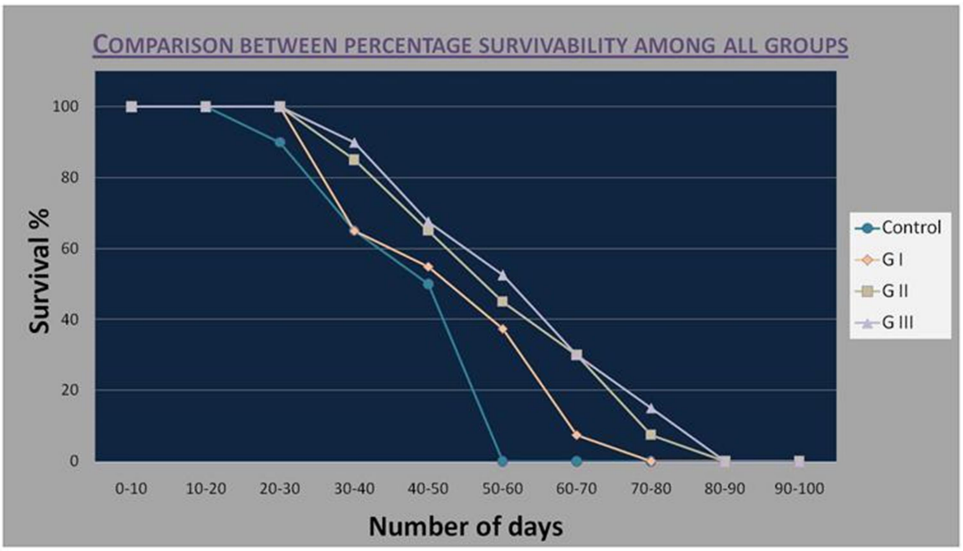
Groups	Dosage (mg/100ml)	Male (Mean±SE)	Percentage of increase in life span	Female (Mean±SE)	Percentage of increase in life span
G I	-	44.80±2.30	-	46.40±2.22	-
G II	12.5	51.90±3.10	15.85	52.85±3.21	13.90
G III	25	59.10±3.43	31.92	60.35±3.54	30.06
G IV	37.5	61.95±3.43	38.28	62.80±3.48	35.34



Graph 3

Table 5: Illustrates the effect of control and varying concentrations of Shuddha Gandhaka on the lifespan of both Male and Female Drosophila melanogaster:

Groups	Dosage (mg/100ml)	Mean± SE	Percentage of increase in life span	F Ratio	Sig
G I	-	45.60±1.58			
G II	12.5	52.37±2.20	14.86	34.96	0.000
G III	25	59.72±2.43	30.98	15.32	0.000
G IV	37.5	62.37±2.41	36.79	17.46	0.000
* The mean difference is significant at 0.5 level (P < 0.05)					



Graph 4

Table 6: Mean difference of longevity in different group with control group G I (Student-t test)

Comparison	Difference of mean	t	sig
G I (Control) & G II	6.775	7.827	0.000
G I (Control) & G III	14.125	10.391	0.000
G I (Control) & G IV	16.775	12.353	0.000

DISCUSSION

In biological research on longevity, the *Drosophila melanogaster* has been utilized as a model to evaluate lifespan phenotypes, artificial and natural selection responses **[10]**. *Drosophila* and vertebrates share a huge number of genes and similar metabolic activities that keep sugar, lipid, and amino acid homeostasis in check **[11, 12]**.

They also share the highly conserved PI3K/Akt/mTOR signaling pathways, which play key roles in oxidative metabolism and ageing regulation in both groups **[13]**. Because of these factors, as well as its exceptional genetic tractability, the fly model is increasingly being employed to study human health issues **[14]**.

The impact on longevity in Drosophila was planned due to rasayana property of Shuddha Gandhaka. Flies which were fed on Shuddha Gandhaka churna had longer life span than

the flies which were fed on normal food media that is in control group.

Male and female flies in the control group lived 44.8 and 46.4 days, respectively. The highest life span of male and female flies was observed in G IV - 37.5mg/100 ml concentration, i.e., 61.9 and 62.8 days, respectively. Male and female flies had a life span of 52.3 days in G II -12.5mg/100 ml concentration, and 59.7 days in G III - 25mg/100 ml concentration. This suggests that Shuddha Gandhaka has Rasayana effect regardless of gender.

Antioxidant activity of Shuddha Gandhaka might have contributed to the increased longevity in treated adult flies. Antioxidants play important role in checking the free radicals which are main cause for DNA damage. DNA damage is one of the issues in decreasing longevity **[15]**.

As there is increase in longevity in adult treated *Drosophila melanogaster*, it can be said that it might be due to the effect of drug because, control group which were fed only on wheat agar media failed to live longer and there was no involvement of environmental factors as all the experimental groups were maintained in homologous condition.

In *Drosophila melanogaster* longevity is under genetic control so it can be assumed that Shuddha Gandhaka have influenced the genes of longevity in adult treated to make them live longer.

Rasayana are said to enhance immunity, impart vigour, act as aphrodisiac, and slow down the ageing process in Ayurveda.

Drosophila, with its extensive genetic tools and well-studied developmental pathways, promises to be an excellent model for investigating the cellular and molecular mechanisms underlying the effects of various Ayurvedic formulations.

CONCLUSION

The physiological state following Shuddha Gandhaka in flies suggests that Shuddha Gandhaka reduces ageing conditions and hence contributes to "healthy ageing" in humans, so the findings in the fly model have therapeutic implications. Shuddha Gandhaka as Rasayana extends the life span of *Drosophila melanogaster*, validating the Rasayana property of Shuddha Gandhaka.

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