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Efficacy of manjistha and ginger powder in extending the lifespan of *Drosophila melanogaster*

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Ageing is a condition of deterioration of the physiological functions necessary for the survival and fertility over time. Amongst the various factors that accelerate the ageing process, reactive oxygen species produced during normal metabolism is one of the major contributors. In this study, we have evaluated the effect of manjistha and ginger on the lifespan of *Drosophila melanogaster*. It was found that feeding manjistha and ginger can extend the lifespan of flies significantly, unlike those fed on control media. Further, we demonstrated that manjistha was effective against paraquat-induced oxidative stress. Upon paraquat treatment, mortality rate of 12% and 8% was observed in male and female flies respectively that were reared on manjistha supplemented media whereas mortality of 100% and 92% were observed in male and female flies, respectively when reared on control media. The present study suggests potential benefits on the longevity of the flies by the dietary supplementation with ginger and manjistha, especially in the oxidative stress condition. These findings should be further examined to identify active components present in these plant products that show its manifestation on the well-being of the fly.

Keywords: Antioxidants, *Drosophila,* Ginger, Lifespan, Manjistha, Paraquat

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Ageing, a physiological deterioration process, is a complex process resulting due to several inter-related factors. One of the most popular and investigated reason for ageing is the presence of free radicals. The free radicals produced during normal metabolism have been attributed to the age- associated damage at the cellular and tissue levels[1]. Free radicals like superoxide anion, hydroxyl radical and hydrogen peroxide, etc. produced in the body are called the reactive oxygen species (ROS) which react with various biological molecules namely lipids, proteins and deoxyribonucleic acids resulting in the imbalance between oxidants and antioxidants. Such imbalance over time disturbs the homeostasis of the organism resulting in ageing and death[2]. Antioxidants are reported to counter the effect of damage produced by these ROS by its ability to trap free radicals[3]. The body produces many antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase, which neutralize many types of free radicals[4,5]. Besides these the antioxidants present in food play an important role in protecting health. Studies have proved that the antioxidant property of the plants is capable of exerting protective effects against oxidative stress in biological systems[6].

Dietary modification is assumed to be able to extend the lifespan and slow down age-related diseases. Further, calorie restriction has been shown to prolong lifespan in various animal models; while, supplementation of different chemicals into the daily diet has been also acknowledged as an impending way to slow the ageing process[7]. Plants possess a plethora of chemical compounds exhibiting antioxidant properties. These antioxidants occurring in plants are largely; ascorbic acid, carotenoids and phenolic compounds[8,9]. *Rubia cordifolia* (L.) commonly known as Indian Madder or Manjistha is a well-known medicinal plant. The key chemical components found in this plant are anthraquinones, glycosides, flavonoids, steroids, phenols and saponins[10,11]. *Zingiber officinale* Roscoe commonly known as ginger, is another such plant whose extracts

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are reported to contain polyphenol compounds (6-gingerol and its derivatives), which is reported to have high antioxidant activity[12].

Drosophila melanogaster commonly known as the fruit fly is one of the commonly used models in the ageing research because it shares many similarities with humans in nutrient sensing pathways and similarity in more than 70% of known diseases-related genes[13].

In the present study, the effect of the two plants-manjistha and ginger on the longevity of *D. melanogaster* was compared by supplementing the commonly used wheat cream agar media (control) with manjistha and ginger of known concentration. Also the longevity of the adults reared on the different diets was assessed following treatment with paraquat, a potent superoxide radical generator.

Materials and Methods

Control media

All the experiments were performed on *D. melanogaster* (Oregon K) flies procured from the *Drosophila* Stock Centre, University of Mysore, Karnataka, India. The stocks (control and experimental cultures) were maintained on standard wheat cream agar media seeded with yeast and propionic acid at 22°C ± 1°C and 70-80% relative humidity in BOD. This media was used as control food wherever indicated.

Experimental media

Powdered manjistha roots and fresh ginger rhizomes were procured from the local market. Ginger rhizomes were air dried in shade and powdered in an electric mixer to obtain fine powder. 100 mg of manjistha and 10 mg of ginger were added separately to 100 mL of normal standard media each, immediately after the preparation of the media. The concentration of manjistha and ginger in the test media were 1 mg/1 mL and 0.1 mg/mL respectively. Around 4 mL of test media were dispensed into the experimental vials while still warm.

Experimental setup

Longevity assay

Longevity of both virgin and mated flies was studied. Three independent set of wild type flies were cultured on control, manjistha- and ginger-supplemented media. After 3 days, all the adult flies were removed from these culture bottles to generate flies of similar age which were left in the bottle. Longevity assessment was carried out as described by Harini and Ramachandra[14]. Thirty virgin females and unmated males were collected from the synchronous culture. The flies from each of the media (control as well as test media) were then transferred to fresh media of respective type every third day (at a density of n = 1 fly/vial) and were subsequently monitored for their longevity. To record the life span, the flies were observed daily and dead flies were counted until the death of the last fly in each of the food vial.

For longevity assessment in mated flies, synchronous culture of flies on three different food media was generated as earlier. 30 virgin females and unmated males were collected from these culture bottles and aged for about 5 days in separate vials. Subsequently, single mating pair was made by transferring a male and a female on their designated food media on the 6th day post-eclosion. Mating was allowed for next two days, following which the mated males and females were withdrawn and aged separately. Longevity of mated pairs was compared only between flies reared on control media to the flies raised on manjistha supplemented media.

In both the experimental setups for longevity assay, the flies were transferred to fresh vials on every third day to prevent sticking of flies to the food surface. Each experimental setup was made in the replicates of three. The lifespan of each fly was recorded till their natural death.

Paraquat treatment

As described above, virgin female and unmated male flies were collected from control and manjistha media and were aged separately for five days. Paraquat (Paraquat dichloride hydrate, PESTANALTM, Analytical Standard; Cat No. 36541, Sigma-Aldrich, India) exposure of the flies was carried out as previously described[15]. They were then starved for four hours and transferred to vials (density n = 10 flies each sex/vial) containing circular Whatmann filter paper #1 discs soaked with freshly prepared 20 mM of paraquat prepared in 5% sucrose solution. Two such set ups were made for each media and sex.

Experiment was replicated thrice for each sex and for each media. Numbers of dead flies were counted every 12 h for 3 days (72 h).

Statistical analysis

The significance of difference between means was assessed using Student's *t*-test. Data for more than

two treatments were subjected to one-way ANOVA and the mean values were separated using Tukey's test[16]. Differences between two groups were considered to be significant at $p<0.05$.

Results

Differences in the longevity of the flies reared on the three different media were observed in the present study. In our experimental setup, we recorded that wild type unmated flies raised on normal media survived up to an average of ~34 days and ~30 days in males and females, respectively. It was found that both the plant supplements (ginger and manjistha) significantly extended the longevity of flies in a sex-independent manner as compared to control media. We observed that manjistha restricted early ageing and death in both males and females by prolonging the average lifespan of flies up to ~56 days and ~54 days, respectively. A notable increase in lifespan of 64.7% in males and 80% in female was observed in comparison to flies cultured on normal media. Similar survival trend was observed in unmated males and virgin females raised on ginger-supplemented media. An average lifespan of ~46 days and ~43 days was recorded, which was a substantial increase of about 35.29% and 43.33% in males and females, respectively as compared to the control flies. Based on these two data sets, it was evident that both the plant ingredients were potent dietary supplements for slowing down the ageing process and restricting early death. Lifespan of flies of both the sexes reared on the three media differed significantly ($p<0.05$) with each other (Fig. 1). However, amongst the two test media, manjistha appeared to elevate lifespan more effectively than ginger so further experiments were conducted only with manjistha as the test media.

A significant difference in the longevity of mated flies was also observed when reared on control media and manjistha media. The average longevity of mated males was 20.66 days on control media and 44 days on manjistha media showing an increase in lifespan of 112.97%. In the case of mated females, the life span on control media was 19.30 days and 40.4 days on manjistha media which was an increase of 109.32 % (Fig. 2).

After paraquat exposure, the flies were tracked for next four days. Flies that were raised on normal media displayed higher and early incidences of death post-paraquat treatment. In the case of males grown on normal media, mortality was observed by the 12th h post-paraquat exposure that gradually increased reaching a 100% mortality by the 72 h (Table 1). On the contrary, males fed on manjistha-supplemented media, showed delayed mortality and attained a maximum mortality of ~12% by 72 h post-paraquat treatment and ~8% mortality was observed in the case of females. About 92% of females from control group were found to be dead by the 72 h. The increase in mortality was observed with time in the control media and almost all flies were dead by the end of 3rd day but in manjistha media, flies mortality occurred only in the initial few hours and flies survived after that at least till 3rd day (72 h).

Discussion

Plant extracts and their isolated constituents have always been a significant part of various therapeutic systems[17,18]. The active phytochemicals present in

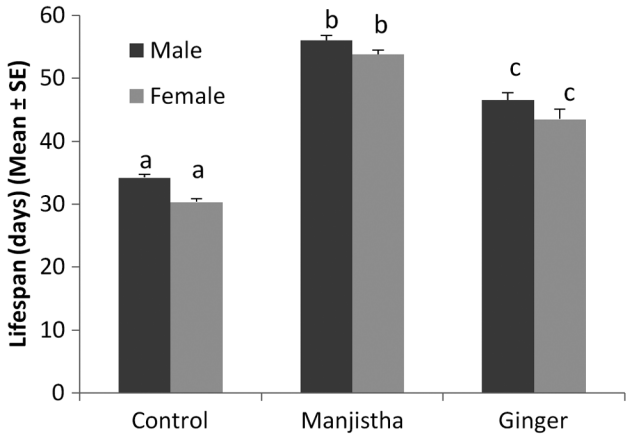

Fig. 1 — Lifespan of *D. melanogaster* (unmated flies) on different food media. Bars of a particular sex with different superscripts differ significantly ($p<0.05$)

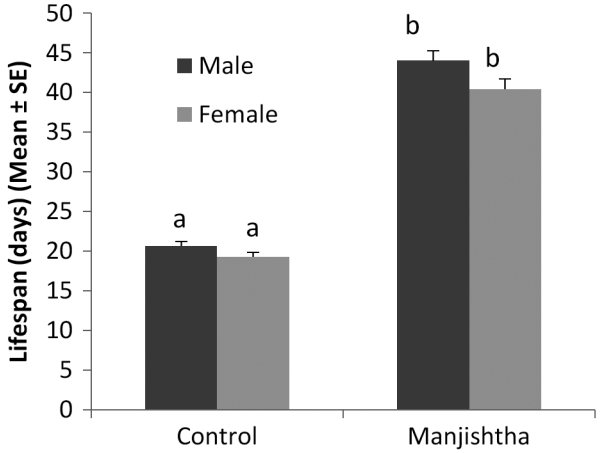

Fig. 2 — Lifespan of *D. melanogaster* (mated flies) on different food media. Bars of a particular sex with different superscripts differ significantly ($p<0.05$)

Table 1 — Post paraquat treatment mortality in the adult flies of *D. melanogaster* reared on normal and manjistha media

Hours	% Mortality			
	Control		Manjishtha media	
	Male	Female	Male	Female
12	8.33	11.67	0.00	8.33
24	31.67	25.00	11.67	8.33
36	40	51.67	11.67	8.33
48	48.33	51.67	11.67	8.33
60	65	68.33	11.67	8.33
72	100	91.67	11.67	8.33

R. cordifolia and the root extracts of *Z. officinale* have been used for several pharmaceutical purposes[18-20]. It is for the first time that manjistha is tested for their effect on longevity in *Drosophila* although there are few reports of ginger on the same property either tested *in vitro* or *in vivo* on *Drosophila* and other model organisms such as *Caenorhabditis elegans*[21-25].

The mechanism of action for these two plant extracts on *D. melanogster* is uncertain but the potency to increase the lifespan was established. The longer longevity of the flies on manjistha and ginger supplemented media as compared to those flies reared on the normal fly culture media (control) could be due to the antioxidant property of the active components present in these plants which may have reduced the cell damage caused due to free radicals[26]. Antioxidative activity of alcoholic extract of roots of manjistha was observed in male mice against lead. Oral administration of manjistha led to marked improvement in both hematological and serum biochemical changes in the mice[27].

Increased lifespan in *Drosophila* has also been observed when supplemented with plant extracts like curcumin, apple polyphenols, ginger, pomegranate, cocoa etc.[28-34]. Under the condition of oxidative stress by different chemicals and physical conditions, ginger powder supplemented media proved to be efficient in increasing the life span of fruit flies[24].

Paraquat causes lethality by inducing oxidative stress in flies. The oxidative stress due to paraquat treatment also was well resisted by the flies reared on manjistha supplemented media as their survival time was increased when compared to those flies reared in the normal fly media. This is indicative of the antioxidative property of manjistha as paraquat is a known stress inducer which results in the release of superoxide radicals[33].

Conclusions

Molecular and physiological analysis needs to be done to understand the exact mechanisms of the action of the active components of these two plant extracts but the present study provides a preliminary basis for the study of the antioxidant properties and efficacy of manjistha and ginger on increasing the life span of *D. melanogaster.* Such dietary supplement which is easy to procure might prove as an effective way to dampen the effect of ROS in our body. More research is needed in this field to identify the active components in these plant powders which might be able to counter the effect of oxidative stress in the body.

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Conflict of Interest

Authors have no conflict of interest.

Authors' Contributions

Concept was given by BT; experiment design by ASN & BT; experiment and data collection by PA, GG, AR and AY; data analysis and graph by ASN and BT; draft of the manuscript by PA and GG; ASN and BT reviewed and finalized the manuscript.

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