ANALYSIS OF ANTI-OXIDANT AND ANTI-AGEING PROPERTIES OF ALOE VERA GEL ON LIFE HISTORY PARAMETERS OF DROSOPHILA MELANOGASTER

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ABSTRACT

If aging is due to or contributed by free radical reactions, as postulated by the free radical theory of aging, lifespan of organisms should be extended by administration of exogenous antioxidants In the present study, we tested the effect of larval diet supplementation with five different concentrations of Aloe vera gel on the adult longevity of short-lived D melanogaster populations. A vera gel supplementation of larval diet extended adult longevity in both the male and female flies without reducing fecundity but by efficient reactive oxygen species scavenging through increased antioxidant enzymes activity and better neuroprotection as indicated by increased locomotor activity in adult males.

Keywords: Anti-Oxidant, Drosophila Melanogaster, Fecundity, Longevity, SOD.

I. INTRODUCTION

Aging generally refers to the process of getting chronologically older and it is typically accompanied by senescence, the gradual loss of physiological functions. Both of these processes are to some degree, inevitable for all living organisms. Chronological aging is primarily predetermined by heredity, whereas senescence results from a complex interaction between environmental and genetic factors. Non-genetic factors such as nutrition, environmental quality, psychosocial factors, and lifestyle play an important role in healthy aging.

It is well known that Reactive Oxygen Species (ROS) have been implicated in more than 100 diseases, including malaria, acquired immune deficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes and cancer (Tanizawa *et al*., 1992; Alho and Leinonen, 1999). In *Drosophila*, a single locus, *Sod*, encodes the cytosolic enzyme Cu/Zn superoxide dismutase (CuZnSOD), which is an essential component in a major antioxidant defense pathway for scavenging reactive oxygen species (ROS) generated during aerobic respiration (Seto *et al.*, 1989; Noor *et al.*, 2002). Nevertheless, all aerobic organisms, including human beings, have antioxidant defenses that protect against oxidative damages. However, this natural antioxidant mechanism can be inefficient; hence, dietary intake of antioxidant compounds becomes important (Halliwell, 1994; Terao *et al*., 1994). Therefore, research for the determination of source of the natural antioxidants is important.

Therefore, research for the determination of source of the natural antioxidants is important. Recently there has been an upsurge of interest in the therapeutic potentials of plants, as antioxidants in reducing free radical

induced tissue injury. Medicinal plants are considered to be the best source for antioxidant compounds. As plants produce significant amount of antioxidants to prevent the oxidative stress caused by photons and oxygen, they are considered as potential source of antioxidant compounds. India is perhaps the largest producer of medicinal herbs and is rightly called the "Botanical garden of the World".

Aloe vera is the oldest medicinal plant ever known and the most applied medicinal plant worldwide. Aloe vera is a succulent plant species that is found only in cultivation, having no naturally occurring populations, although closely related aloes do occur in northern Africa (Akinyele 2007) The common names of Aloe vera are Aloe vera, Aloe, burn plant, lily of the desert, elephant's gall and Latin name is Aloe barbadensis The species is frequently cited as being used in herbal medicine since the beginning of the first century AD. Extracts from A. vera are widely used in the cosmetics and alternative medicine industries, being marketed as variously having rejuvenating, healing, or soothing properties. There is, however, little scientific evidence of the effectiveness or safety of Aloe vera extracts for either cosmetic or medicinal purposes, and what positive evidence is available is frequently contradicted by other studies (Ernst E 2000, Marshall JM 1990, Boudreau MD, Beland FA 2006, Vogler BK, Ernst E Oct 1999).

Fruit fly is one of the models to study aging and age-related diseases (Jafari, 2010). Humans actually share a huge amount of conserved biological pathways and diseases-causing genes with this tiny insect (Reiter et al., 2001; Bauer et al., 2004). Compared with other models, fruit fly is relatively easier to maintain in a large quantity due to their tiny body size and short lifespan. Previous reports have revealed that dietary modification, including calorie restriction and dietary supplementation, can extend lifespan and ameliorate certain age-related diseases (McCay et al., 1935; Lin et al., 2002; Partridge et al., 2005; Lee et al., 2006; Piper and Bartke, 2008).

In the present study, we analyzed the effect of ageing on the antioxidant enzyme system and compared with flies supplemented with Aloe vera gel. SOD activity was found to unregulated in *A vera* gel fed flies as compared to that of flies fed on standard *Drosophila food medium*. Protein content found increased in Aloe vera gel fed flies as compare to control flies. Our results suggest that supplementation with Aloe vera gel reduces oxidative stress and improves longevity.

II. MATERIAL AND METHODS

2.1 Fly Strains and Diet

Wild type strain of *Drosophila* was used in the present study. The flies were cultured on *Drosophila* food medium containing agar-agar, corn meal, sugar, yeast, anti-bacterial, anti-fungal agent at 21 ºC±1. The additional yeast suspensions were provided for healthy growth. Four experimental diets were prepared by adding Aloe vera gel at 3 ml, 5 ml, 7ml and 10ml in the control diet per liter.

2.2 Effect of Aloe vera gel on longevity and fecundity of Drosophila flies fed the basal diet

Two independent trials were conducted. For each trial, newly eclosed male flies were divided into 5 groups (n=200 each), and housed in 10 vials (20 flies per vial). The first group was maintained on the basal diet, while

the other experimental group was fed one of the Aloe vera gel diet. Dead flies were counted every 2–3 days and the remaining alive flies were transferred to a new vial containing the same diet. The maximum life spans in this study were calculated as the average life span of the 5% longest surviving flies. The same experiments described above were similarly repeated and the fruit flies were sacrificed in order to quantify the expression of SOD, and protein content.

2.3. Climbing Assay

Climbing ability of fruit flies was assessed using the climbing assay. In this assay 10 male flies were placed in a plastic vial, given 10 s to climb up. At the end of each trial, the number of flies that climbed up to a vertical distance of 8 cm or above was recorded. Each trial was performed three times.

2.4 Statistical Analyses

In all cases except survival function analysis, the population means were used as the units of analysis. The significance of the difference between means was assessed using one-way analysis of variance. The differences among treatments were compared by Tukey–Kramer Minimum Significant Difference. The significance of the difference between adult survival curves was analyzed using Kaplan–Meier log-rank test.

III. RESULTS

3.1 Longevity and Fecundity

Supplementation of diet with *A vera* significantly changed the average longevity of female flies ($F7,16 = 5.4159$, $p = .005$) but not of male flies ($F7,16 = 2.984$, $p = .10$). The median life span of both female and male flies was not significantly altered by diet supplementation. However, the maximum life span was significantly altered in both the sexes (Table 1). The increase in longevity of female flies was not linked to loss of fertility as there was no significant effect of diet supplementation with *A vera* on lifetime fecundity ($F7,16 = 2.0296$, $p = .114$). The survival rates of both female and male flies were significantly affected by *A vera* supplementation.

Table 1: Data on mean values of Fecundity, Percent viability and longevity of male and female individuals of Drosophila melanogaster fed on Control and Aloe vera gel supplemented food medium.

Treatment groups	Fecundity	Percent Viability	Longevity (Days)	
			Male	Female
Control	171	91.13	50	56
A vera (3ml/lt)	180	91.2	57	70
A vera (5ml/lt)	187	93.8	61	75
A vera (7ml/lt)	195	96	63	78
A vera (10ml/lt)	197	97.3	65	80

3.2 Superoxide Dismutase Activity

Superoxide dismutase activity was significantly upregulated in male ($F7,16 = 66.4002$, $p = .000$) and female ($F7,16 = 18.9459$, $p = .000$) flies by *A vera* supplementation.

3.3 Climbing Activity

Male climbing activity was significantly influenced by diet supplementation with *A vera* ($F7,16 = 138.7598$, $p = .000$). Flies reared on 10ml/litre of A vera gel were the most active, whereas the standard control flies were the least active (Table 2).

Table 2: Data on mean values of chill coma recovery and climbing assyay of Drosophila melanogaster fed on Control and Aloe vera gel supplemented food medium.

Treatment groups	Climbing Time	
	Male	Female
Control	11.3	8.4
A vera (3ml/lt)	9.5	7.5
A vera (5ml/lt)	7.2	6.3
A vera (7ml/lt)	6	5.4
A vera (10ml/lt)	5	4.3

IV. DISCUSSION

In the present study, anti-oxidant and anti-ageing effects of Aloe vera gel were analyzed on the life history parameters of D. melanogaster. Aloe vera gel could prolong the mean lifespan of fruit flies by >10% compared with the control. The present study also demonstrated that supplementation of aloe vera gel was associated with elevated mRNA level of SOD at Drosophila. The increased locomotor activity of flies reared on media supplemented with resveratrol could have been due to its neuroprotective activity (Mokni M, Elkahoui S, Limam F, et al. 2007; Araki T, Sasaki Y, Milbrandt J. 2004; Parker JA, Arango M, Abderrahmane S, et al. 2005; Wang Q, Xu J, Rottinghaus GE, et al. 2002; Wang Q, Yu S, Simonyi A, et al. 2004; Han YS, Zheng WH, Bastianetto S, et al. 2004). In another study, feeding fish with resveratrol-supplemented diet prevented age-dependent neurodegeneration (Valenzano DR, Terzibasi E, Genade T, et al. 2006). In addition to neuroprotection, resveratrol could have stimulated the growth and regeneration of nerve fibers that could have resulted in increased preadult viability, because the larval growing media was modified in this study, unlike all other studies that altered the adult diet. The lipid content of the flies raised as larvae on *A vera* extract–supplemented diet suggests that *A vera* extends longevity through mechanisms other than calorie restriction, as increased longevity in dietary restriction studies were associated with increased lipid content and reduced dry weight and fecundity (Simmons FH, Bradley TJ. 1997). Longevity extension by *A vera* is probably mediated through prevention of neurodegeneration and/or regeneration of nerve fibers as indicated by increased locomotor activity. Increased activity of detoxifying enzymes SOD and catalase seems to be able to clear the system of the toxic elements that could have otherwise caused damage and lead to no improvement in longevity. *Aloe vera* is known to contain a plethora of phytochemicals, such as 1,8-dihydroxyanthraquinone derivatives and their glycosides, proteins, lipids, amino acids, vitamins, enzymes, inorganic compounds, and small organic compounds (Hamman JH. 2008), some, if not most of them could have contributed to improved health through

correction of many metabolic processes—anthraquinone is a starting material for production of antioxidants to cite one example. *Aloe vera* extract seems to mimic the longevity extension effects of resveratrol as well as morphine (Dubiley TA, Rushkevich YE, Koshel NM, et al. 2011) through regeneration of nerve fibers, neuroprotection as indicated by increased locomotor activity, and upregulation of detoxifying enzymes.

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