ISSN: 2287-688X
Annals of Plant Sciences
2013, 02 (01), 49-54

# Wheatgrass (*Triticum aestivum L.*) Supplementation Promotes Longevity in *Drosophila melanogaster*

**Devesh C Pant, Mansi Dave, Anand K Tiwari***
School of Biological Sciences & Biotechnology, Indian Institute of Advanced Research, Koba Institutional Area, Gandhinagar-Gujarat-382007, India

**Received for publication:** December 22, 2012; **Accepted**: January 29, 2013.

**Abstract:** Wheatgrass is a rich source of vitamins, antioxidants and minerals in a bioavailable form. Traditionally, the juice of wheatgrass is used in the treatments of various diseases such as asthma, cancer, arthritis, diabetes, cardiovascular diseases etc. To promote longevity and life span, edibles containing high antioxidant capacities and other bio actives are considered to be ideal. We intended to test the activity of wheatgrass to counteract oxidative stress, which is one of the major causes of various diseases and ageing. In the present study, *Oregon* $R^+$ strain of *Drosophila melanogaster* was fed on standard diet (normal Drosophila diet), and diet supplemented with wheatgrass extract (10mg/mL). Our experiments suggest that supplementation of wheatgrass reduces oxidative stress and improves longevity in *Oregon* $R^+$ flies.

**Keywords:** Catalase, *Drosophila melanogaster*, Oxidative Stress, Superoxide Dismutase, Wheatgrass

## Introduction

Ageing, a biological process occurs in most of the animals leading to functional impairment and ultimately death of individuals (Kirkwood, 2005; Vijg and Campisi, 2008; Jacobson *et al*., 2010). Oxidative stress has been shown to play major role in ageing and it increases with the age. The main cause of oxidative stress induced damage is generation of reactive oxygen species (ROS), a highly reactive molecule that results an imbalance in aerobic metabolism and gives rise to disturbance in cellular homeostasis. Several studies have suggested that Plants and plants components (fruit, fruit juice/plant extract) contains high antioxidant compound and their consumption reduces oxidative stress, promotes health and delays ageing (Boyd *et al*., 2011; Harris, 2004; Liu 2004; Joseph *et al*., 2007; Pan *et al*., 2009). Thus, addition of antioxidants in diet maintains the balance between oxidant and antioxidant and increases the longevity. In recent year's use of alternate animal models have received great importance to study the effect of plants products on ageing.

Wheat (*Triticum aestivum L.*) belongs to Family Poaceae is the youngest stage of wheat plant which grows from the wheat grains (Porter, 1959). It can be also grown indoor in a tray as shown in figure 1. Wheatgrass is leafy and has a deep green colour and a strong sweet taste. It takes 6-10 days to germinate[10] and has maximum antioxidant property due to the presence of vitamins, minerals and phenolic compounds at the time of germination (Kulkarni *et al*., 2006; Hänninen *et al*., 1999). Since, this plant possesses superoxide scavenging activity (Peryt *et al*., 1992), antioxidant activity (Kulkarni *et al*., 2006, Aydos *et al*., 2011)′ anti-ulcer activity (Shah *et al*., 2011) and has ability to inhibit oxidative DNA damage (Falcioni *et al*., 2002)′ this plant is consumed as health food in various forms such as tablet, powder and juice. The beneficial effects of wheatgrass were associated with great antioxidant activity of wheatgrass plant (Kulkarni *et al*., 2006). Since there is no experimental evidence suggesting the potential activity of wheatgrass with longevity, the present study was designed using *Drosophila melanogaster* as an animal model.


**FIG. 1.** *Triticum aestivum* (Wheatgrass) plant grown in lab (Conditions : 23°C, 16h light, 8 h darkness, 50% humidity)

*Drosophila melanogaster* commonly known as fruit fly, is one of the well-studied model organism, has several advantages over other animal models, as it has shorter life cycle (10-12 days at 25°C), high fecundity (females lay ~800 eggs in a life time), easy to handle and fewer number of chromosomes (3 pairs of autosomes and 1 pair of sex chromosomes) with ~75% similarity with human genes (Sidow and Thomas, 1994; Rubin *et al*., 2000). Using fruit fly, many plant compounds/drugs can be easily mixed with fly food for administration to larvae/adult fly and its effect can be easily observed in next generation. Thus, due to above mentioned

***Corresponding Author:**
**Dr. Anand K. Tiwari,**
School of Biological Sciences & Biotechnology,
Indian Institute of Advanced Research,
Koba Institutional Area, Gandhinagar-Gujarat-382007, India

features, *Drosophila melanogaster* is an excellent animal model to study the longevity promoting properties of plant components and also to understand the mechanism of ageing. In recent years, flies have also been used as model organism for ageing, drug development and for testing of Ayurveda medicines, several drugs and environmental toxicant (Pathak *et al*., 2011; Tiwari *et al*., 2011; Dwivedi *et al*., 2012). There are several studies, suggesting the use of Drosophila as a model organism to study the effect of plants extract and their components on development of flies: Use of *Rosa damascena* plant extract decreases the mortality rate in male and female Drosophila (Jafari *et al*., 2008). Further, it has been shown that *S. Lavandulifolia,* Curcumin, Cocoa, Apple polyphenols, Blueberry, *Aloe vera* and Nectarine plant have a ameliorating effect on life span of Drosophila (Altun *et al*., 2010; Bahadorani and Hilliker, 2008; Lee *et al*., 2010; (Peng *et al*., 2011; Peng *et al*., 2012; Chandrashekara, *et al*., 2011; Boyd *et al*.,2011).

In this study, using *Drosophila melanogaster* as an animal model, we have shown that, supplementation of wheatgrass diet to fly results in reduced oxidative stress and improved longevity. To best of our knowledge, this is the first study showing the potential effects of wheatgrass on Drosophila longevity. However, the fundamental questions, which link ageing, stress, life span are still open.

## Materials and Methods

**Fly Strains used:**

*Oregon* $R^+$ was used as a wild type strain of *Drosophila melanogaster*. The flies were cultured on Drosophila food medium containing agar-agar, corn meal, sugar, yeast, anti-bacterial, anti-fungal agent at 22°C±1. The additional yeast suspensions were provided for healthy growth.

**Wheatgrass powder:**

Wheatgrass (*Triticum aestivum L*.) powder was obtained from Grime's Wheatgrass, Pune, India. The nutrient components present in per 100 gm of wheatgrass powder is given in Table.1 (Courtesy Grimes' Wheatgrass, Pune, India).

**Table.1:** Components of wheatgrass powder per 100 gm sample (adapted from Grime's Wheatgrass, Pune).

| COMPONENTS OF WHEAT GRASS | | | | | |
|---|---|---|---|---|---|
| NUTRIENTS | | VITAMINS | | AMINO ACIDS | |
| COMPONENT | CONTENT | COMPONENT | CONTENT | COMPONENT | CONTENT |
| Proteins | 34.10 g | A | 63.71 mg | Histidine | 17.1 mg |
| Carbohydrates | 87.27 g | $B_{12}$ | 0.041 µg | Isoleucine | 23.75 mg |
| Calories | 341.06 kcal | C | 6.23 µg | Leucine | 40.25 mg |
| Total dietary fiber | 9.62 g | E | 20 mg | Lycine | 34.23 mg |
| Chlorophyll (dry basis) | 5.99 g | $B_{17}$ | 2.79 µg | Threonine | 27.56 mg |
| Fat | 0.6 g | $B_9$ | 22 µg | Tryptophan | 5.02 mg |
| Calcium | 6.93 mg | | | Valine | 31.58 mg |
| Potassium | 31.50 mg | | | Methionine | 9.35 mg |
| Iron | 3.53 mg | | | Tyrosine | 28.32 mg |
| Sodium | 2.24 mg | | | Alanine | 57.21 mg |
| Zinc | 17.94 mg | | | Proline | 31.45 mg |
| Magnesium | 3.03 mg | | | Serine | 33.01 mg |
| Selenium | 6.12 mg | | | Phenylalanine | 34.08 mg |
| Phosphorous | 6.2 mg | | | | |
| Copper | 16.39 µg | | | | |
| Sulfur | 11.55 µg | | | | |
| Iodine | 1.98 µg | | | | |
| Manganese | 62 µg | | | | |

**Survivorship assay of adult flies:**

The effect of wheatgrass powder on the life span of adult flies was studied by feeding the *Oregon* $R^+$ flies (~150 flies from each group) to normal food and food mixed with wheatgrass powder from day 1 of their lifecycle (10 flies/vial and 15 vials per group). Flies were transferred to fresh vials every 2 days and the number of dead flies was recorded till the last fly was dead (Nazir *et al*., 2003).

**Gustatory Assay:**

This was done according to method described by Babhadorani *et al*., (2008). In brief, 30 newly eclosed males and females flies (15 flies per vial) were collected and cultured on a fresh standard medium for 4 days. Afterwards, flies were starved for 20 h on water-soaked Whatman filter paper No. 1 and subsequently transferred to fresh vials containing wheatgrass supplemented medium mixed with 0.8% orange red (Food colour dye, ESSCO products) for 2 h. For control flies, culture medium was supplied only with 0.8% food dye.

The degree of abdomen redness for each fly was blind scored using a subjective grading scale ranging from grade 0 (colorless abdomen) to grade 5 (fully red abdomen). The level of abdomen redness was used as an index of the amount of food taken by the fly.

***Preparation of homogenate:***

The tissues of control and treated flies were homogenized in cold 0.1M phosphate buffer (pH 7.4) containing 0.15 M KCL to obtain 10% homogenate. After centrifugation at 8000 rpm for 10 minutes, 25 *µL* of supernatant was used for antioxidant enzyme assays and 1*µL* for protein estimation.

**Antioxidant enzyme assay:**

**Superoxide Dismutase (SOD) assay:** The method for estimating cytosolic Cu-Zn SOD described previously (Nishikimi *et al*., 1972) was followed with minor modification (Gupta *et al*., 2005). The experiment was carried out in 15 mL falcon tube. The assay mixture consisted of 1.2 mL of 0.052 M sodium pyrophosphate buffer (pH 8.3), 100 *µL* of 186 *µ*M phenazine methosulphate, 300 *µL* of 300 *µ*M nitroblue tetrazolium, 200 *µL* of 780 *µ*M reduced nicotinamide adenine dinucleotide and 25 *µL* of 10 % homogenate. Tubes were vortexed and kept for 90 sec in dark followed by addition of 1 mL of Glacial acetic acid and 4 mL of n-butanol. After addition of butanol violet ring appears. The tubes were incubated for 10 min followed by centrifugation at 3000 rpm for 10 min. One unit of enzyme activity is defined as enzyme concentration required for inhibiting chromogen production (optical density at 560 nm, using Spectronic Helios Gamma UV-Vis Spectrophotometer, Thermo Fisher scientific, USA) by 50% in 1 min under assay conditions and expressed as units/mg/protein.

**Catalase (CAT) assay:** CAT activity in the control and wheatgrass fed flies was measured by following the ability of the enzyme to split $H_2O_2$ within 1 min of incubation time. The experiment was carried out in 15 mL falcon tube. The assay mixture consisted of 1 mL of 0.01 M sodium phosphate buffer (pH 7.0), 25 *µL* of sample 10% homogenate. The final volume was made 1 mL by adding distilled water and the tubes were vortexed. 500 *µL* of 0.2 M Hydrogen peroxide (1:3 by volume) was added followed by 2 mL dichromate acetic acid (5% solution of $K_2Cr_2O_7$). Tubes were vortexed again and kept for boiling for 15 min and were cooled under tap water before measuring the optical density (OD). OD was measured at 570 nm (Spectronic Helios Gamma UV-Vis Spectrophotometer, Thermo Fisher scientific, USA) (Sinha, 1972).

**Protein estimation:** Protein concentration was determined by standared Bradford method (Bradford, 1976). OD was measured at 595 nm using a Spectronic Helios Gamma UV-Vis Spectrophotometer (Thermo Fisher scientific, USA).

**RNA isolation and PCR analysis:** Total RNA was isolated from control and wheatgrass fed flies using TRIzol reagent (Ambion, USA) following manufacturer's instructions. This was used for cDNA synthesis using Revert Aid™ H Minus first strand cDNA synthesis kit (Fermentas MD, USA) following the manufacturers' protocol. Each reaction mixture consisted of total RNA (3 *µ*g), 0.5 *µ*g/*µL* oligo (dT) 18 primer (1 *µL*), 5X reaction buffer (4 *µL*), 20 U Ribolock™ ribonuclease inhibitor, 10 mM dNTP mixture, 200 U Revert Aid™ H Moloney Murine Leukemia Virus reverse transcriptase (M-MuLV RT) and DEPC water to make a final volume of 20 *µL*. The synthesized cDNA (~1 *µL*) was used for PCR amplification in a thermocycler (S1000TM Thermal cycler, Bio-Rad, CA, USA) using specific primers for Drosophila *hsp70* gene (Table 2). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) specific primers were used as internal control. The amplicons were separated on a 1.5% agarose gel containing ethidium bromide at 5 V/cm and visualized with a Fusion Gel Doc Imaging System Model SL 3500 X-Press (Vilber Lourmat, France).

**Table.2:** Primers sequence used for PCR

| | |
|---|---|
| *hsp70* | Forward (F) 5'- GAA CGG GCC AAG CGC ACA CTC TC-3' |
| | Reverse (R) 5'- TCC TGG ATC TTG CCG CTC TGG TCT-3' |
| GAPDH | Forward (F) 5'- AAT TCC GAT CTT CGA CAT GG-3' |
| | Reverse (R) 5'-GAA AAA GCG GCA GTC GTA AT--3' |

**Statistical Analysis:**

Statistical analyses were carried out at least in triplicate and expressed as mean ± SEM. The analyses were carried out using the GraphPad Prism software, version 5.0. The difference among control and wheatgrass fed flies were compared by one way analysis of variance (ANOVA) followed by Tukey's test. Kaplan-Meier log-rank test was used for survivorship assay.

## Results

**Effect of wheatgrass on survivorship of flies:**

It has been discussed above that wheatgrass plant has potent antioxidant activity. Thus to determine whether supplementation of wheatgrass powder with food, provides any nutritional support, survivorship assay of control (without wheatgrass) and wheatgrass fed flies was performed. For this, equal number of males and females flies were fed on control (CTL) and wheatgrass (WG, 10mg/mL) mixed food.

50% survival time of wheatgrass fed flies was found to be increased to 58 days as compared to 52 days in control flies (Table 3). A significant change in life span ($p < 0.05$) of wheatgrass fed flies was observed as compared to the control flies (Fig. 2).

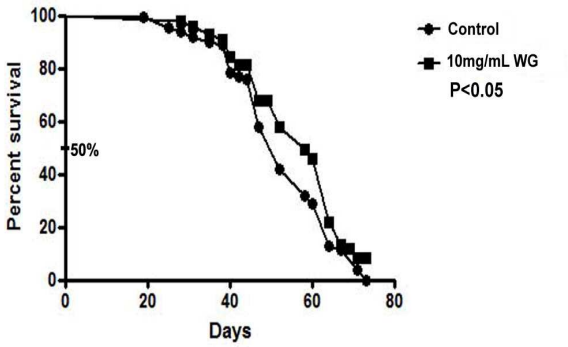


**FIG.2:** Lifespan curve of *Oregon R*$^+$ flies fed on normal diet (control) and wheatgrass diet (10mg/mL). The graph shows the maximum lifespan of last fly and 50% survival time of each group. The Kaplan-Meier (Log Rank test) test found wheatgrass supplementation could significantly extend the life span of flies ($p < 0.05$).

**TABLE.3:** Survival rate of *Oregon R*$^+$ flies fed on control diet (Normal Drosophila food media) and experimental diet (10mg/mL wheatgrass).

| Groups | Max Life span of last fly (days) | 50% survival rate (days) |
|---|---|---|
| *Oregon R*$^+$ (control) | 72 | 52 |
| *Oregon R*$^+$ (10mg/ml WG) | 76 | 58 |

**Gustatory Assay in Control and wheatgrass fed flies:**

Two main important factors that determine the life span in *Drosophila melanogaster* are its feeding behavior and nutritional constituents of the culture medium (Carvalho *et al*., 2005). To ascertain that any shifts in life span are imputed exclusively to the nutritional constituents of the food, we tested feeding behavior of adult flies (female & male) fed on wheatgrass supplemented medium (Fig. 3A & B). Gustatory assay of control and wheatgrass fed flies show no significant difference in the food intake of both the groups (Fig. 3C). Thus, It was confirmed that adult's feeding was not affected by supplementation of wheatgrass within the range used, indicating that any shifts in the longevity of flies are due to the nutritional constituents of the food rather than varied feeding behavior.

**Effect of wheatgrass on Cu-Zn SOD (Superoxide Dismutase) activity:**

Cu-Zn SOD activity assay was performed in control (*Oregon R*$^+$) and wheatgrass (10mg/mL) fed flies. For this, equal number of males and females of *Oregon R*$^+$ flies were cultured at 22$^o$C for 2 days. 10% homogenate of these were prepared and 25 *µL* of these were taken to measure the SOD1 activity. A significant reduction in SOD1 activity was observed in wheatgrass fed flies when it was compared with flies fed on only control diet (Fig. 4). This showed that feeding of flies on wheatgrass food reduces oxidative stress and SOD1 activity in flies. To further confirm this, flies were fed on 10mM Paraquat (PQ) in 1% sucrose solution for 16h (Fig. 5). Since, paraquat is a well-known oxidative stress inducer (Rzezniczak *et al*., 2011) thus it was observed that, flies fed on paraquat showed increased SOD1 activity due to the generation of free radicals. While, flies fed on wheatgrass first then on 10mm paraquat showed a significant reduction ($p<0.05$) in SOD1 activity as compared to the control (flies fed on PQ) but a higher SOD1 activity as compared to the flies fed on only wheatgrass mixed food (Fig. 4) further support our results.

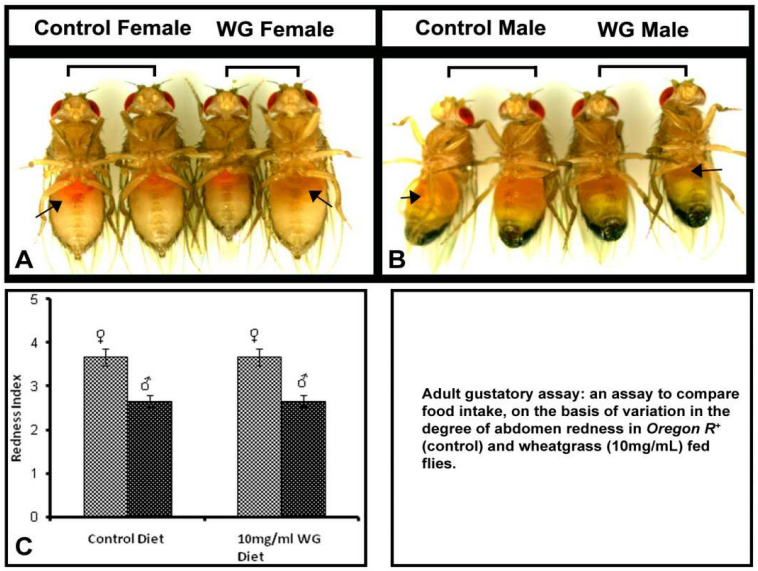


**FIG.3:** Gustatory assay (adult feeding behaviour) in control and wheatgrass fed Drosophila. Redness (arrow) seen in gut of female (A) and male (B) Drosophila show the food intake. This was measured on a basis of blind score. The graph (C) shows no significant difference in degree of abdomen redness indicating that adult feeding behaviour of flies were same in both diets.

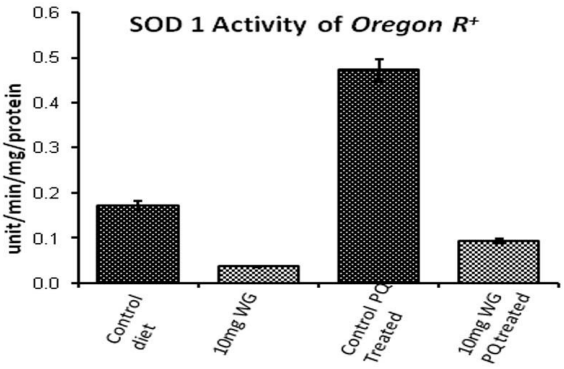


**FIG.4:** Cu-Zn superoxide dismutase (SOD1) activity in control and wheatgrass fed flies: The results showed a significant reduction in SOD1 activity in wheatgrass fed flies as compared to the control. Data are expressed as mean with different letter which differ significantly at $p<0.05$. One way analysis of variance (ANOVA) followed by tuckey test was applied to all parameters using Graph Pad Prism 5.0 software.

**Effect of wheatgrass on Catalase activity:**

Similar to SOD activity CAT activity was also observed in control and wheatgrass (10mg/mL) fed flies. For this, equal number of males and females of *Oregon* $R^+$ flies were cultured at 22°C for 2 days. 10% homogenate of these were prepared, and 25 *µL* was taken to measure the CAT activity. A significant reduction in CAT activity was observed in wheatgrass fed flies when it was compared with flies fed on only control diet (Fig. 6). This clearly showed that exposure of wheatgrass to *Drosophila melanogaster* reduces oxidative stress and CAT activity in flies. To further confirm this, flies were fed on hydrogen peroxide ($H_2O_2$) (Fig. 5), a well-known oxidative stress inducer (Davies, 1995). It was observed that flies fed on $H_2O_2$ showed increased CAT activity due to the generation of free radicals in flies, while flies fed on wheatgrass first then on $H_2O_2$ showed a significant reduction ($p<0.05$) in CAT activity as compared to the control (flies fed on $H_2O_2$) but a higher CAT activity as compared to the flies fed on only wheatgrass mixed food (Fig. 6).

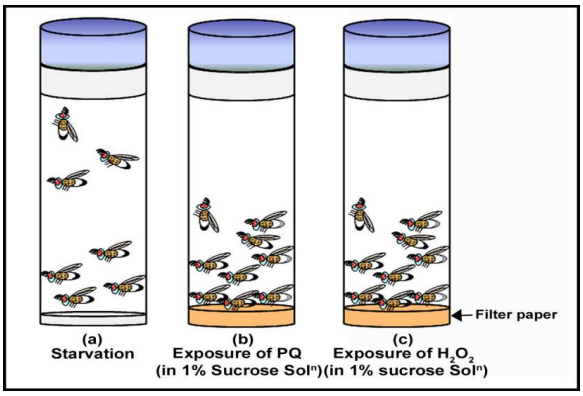


**FIG.5:** Pictorial representation of starvation (a) and treatment of flies using paraquat (b) and $H_2O_2$ (c).

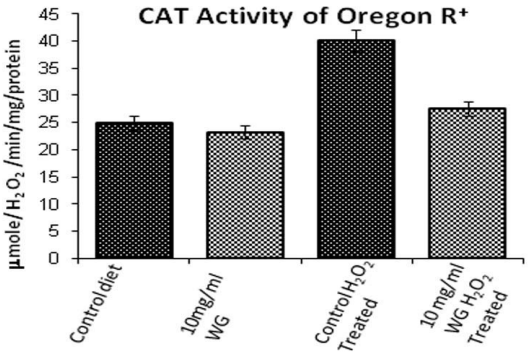


**FIG.6:** Catalase activity in control and wheatgrass fed flies: The results showed a significant reduction in CAT activity in wheatgrass fed flies as compared to the control. Data are expressed as mean with different letter which differ significantly at $p<0.05$. One way analysis of variance (ANOVA) followed by tuckey test was applied to all parameters using GraphPad Prism software, version 5.0.

**Effect of Wheatgrass exposure on Heat Shock Protein 70 (hsp70), a marker to measure cellular stress in Drosophila:**

As, it is well known that Hsp70 is the first indicator of cellular stress (Ryan *et al*, 1996, Mukhopadhyay *et al*., 2003). Thus, in order to find out whether feeding of flies reduces intracellular stress, the *hsp70* expression level was observed in control and wheatgrass fed flies. For this, flies, fed on control (CTL) and two experimental wheatgrass diets (5mg/mL and 10mg/mL) were transferred to vial containing filter paper soaked in 1% sucrose solution for 16h. The expression of *hsp7*0 was measured in 16h control and wheatgrass fed flies. A significant reduction in *hsp70* expression was observed in flies fed on 5mg/mL and 10 mg/mL wheatgrass as compared to the control flies, fed on 1% sucrose solution (Fig. 7). This, suggest that feeding of flies on wheatgrass reduces cellular stress. GAPDH was used as an internal loading control.

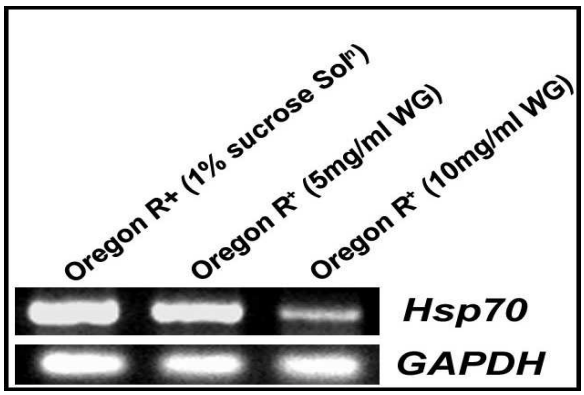


**FIG.7:** Expression of *hsp70* in 16h *Oregon* $R^+$ flies fed on 1% sucrose solution, 5.0mg/ml wheatgrass & 10mg/mL wheatgrass diet respectively.

## Discussion

Reactive oxygen species (ROS) is one of the major causes of ageing and there are several studies which have suggested the use of diet-derived antioxidant to extend the life span in *Drosophila melanogaster* (Bauer *et al*., 2004; Driver *et al*., 2004). In the present study, using survivorlship assay, oxidative stress assay and by measuring cellular stress, we have shown that feeding of fruit flies on wheatgrass diet reduces SOD1, CAT levels, improves longevity and reduces cellular stress in *Drosophila melanogaster*.

As mentioned in Table 1, 100gm of wheatgrass powder is contains most of the essential amino acids, vitamin's (A, $B_{12}$, C, E, $B_{17}$, $B_9$), minerals and nutrients. The reduced SOD1, CAT activity and extended life span seen in wheatgrass fed flies is likely due to the high nutritious value and great antioxidant property associated with wheatgrass plant (Calzuola *et al*., 2004).

The reduced expression of *hsp70* level in 5mg/mL and 10mg/mL wheatgrass fed flies suggested that feeding of flies on wheatgrass reduces cellular stress that further supported our oxidative stress results.

Further, no significant difference seen in gustatory assay of control and wheatgrass fed flies confirmed that reduced SOD1, CAT levels, extended lifespan and other changes was not because of changes in food intake but it was due to wheatgrass diet. In conclusion, to our knowledge, it is the preliminary study which shows that supplementation of wheatgrass (*Triticum aestivum L.*) reduced oxidative stress, improves longevity in *Drosophila melanogaster*. Therefore, results provide opportunities to explore additional tests by which the use of wheatgrass could be more beneficial to mankind.

## Acknowledgements

The authors are thankful to the Director, Indian Institute of Advanced Research (IIAR), Koba, Gandhinagar, India, Prof. J. K. Roy, Banaras Hindu University, Varanasi, India, for *Oregon* $R^+$ fly stock, Dr. S. Acharya, CERW, Gujarat, India for providing wheatgrains samples. Mr. Anurag Sharma and Ashutosh Pandey, IITR, Lucknow for inputs regarding enzymatic assays. The work was partly supported by funds from Gujarat State Biotechnology Mission (SSA/456/2010-11), DBT Programme Support, India and Puri Foundation to AKT.

## References

1. Kirkwood TB, Understanding the odd science of aging. Cell, 2005, 120, 437-447.

2. Vijg J, Campisi J, Puzzles, promises and a cure for ageing. Nature, 2008, 454, 1065-1071.

**3.** Jacobson J, Lambert AJ, Portero-Otín M, Pamplona R, Magwere T, Miwa S, Driege Y, Brand MD, Partridge L, Biomarkers of aging in Drosophila. Aging Cell 2010, 9, 466-477.

4. Boyd O, Weng P, Sun X, Alberico T, Laslo M, Obenland DM, Kern B, Zou S, Nectarine promotes longevity in Drosophila melanogaster. Free radic Biol Med, 201150, 1669-1678.

5. Harris DM, Phytochemicals in Health and Disease. Marcell Dekker Inc, New York, 2004.

6. Liu RH, Potential synergy of phytochemicals in cancer prevention: mechanism of action. J Nutr, 2004, 134,3479S–3485S.

7. Joseph JA, Shukitt-Hale B, Lau FC, Fruit polyphenols and their effects on neuronal signalling and behavior in senescence. Ann N Y Acad Sci, 2007, 1100, 470–485.

8. Pan MH, Lai CS, Dushenkov S, Ho Tang C, Modulation of inflammatory genes by natural dietary bioactive compounds. J Agric Food Chem, 2009, 57, 4467–4477.

9. Porter CL, Taxonomy of flowering plants. W.H. Freeman Company San Fransisco and London 1959, 1 ed, 1-452.

10. Kulkarni SD, Acharya R, Nair AGC, Rajurkar NS, Reddy AVR, Determination of elemental concentration profiles in tender wheatgrass (*Triticum aestivum L.*) using instrumental neutron activation analysis. Food Chem, 2006, 95,699–707.

11. Hanninen O, Rauma AL, Kaartinen K, Nenonen M: Vegan diet in physiological health promotion. Acta Physiol Hung, 1999, 86,171-180.

12. Peryt B, Szymczyk T, Lesca P, Mechanism of antimutagenecity of wheat sprout extracts. Mutat Res, 1992, 269,201-215.

13. Aydos OS, Avci A, Ozkan T, Karadag A, Gürleyik E, Altinok B, Sunguroğlu A: Antiproliferative, apoptotic and antioxidant activities of wheatgrass (Triticum aestivum L.) extract on CML (K562) cell line. Turk J Med Sci, 2011, 41, 657-663.

14. Shah K, Sheth D, Tirgar P, Desai T, Anti-ulcer activity of Triticum aestivum on ethanol induced mucosal damage (cytoprotective activity) in wistar rats. Pharmacologyonline, 2011, 2, 929-935.

15. Falcioni G, Fedeli D, Tiano L, Calzuola I, Mancinelli L, Marsili V, Gianfranceschi G, Antioxidant activity of wheat sprout extracts *in vitro*: Inhibition of DNA oxidative damage. J Food Sci, 2002, 67, 2918-2922.

16. Sidow A, Thomas WK: A molecular evolutionary framework for eukaryotic model organisms. Curr Biol, 1994, 4, 596-603.

17. Rubin GM, Lewis EB, A brief history of *Drosophila*'s contributions to genome research. Science, 2000, 287, 2216–2218.

18. Pathak P, Prasad BR, Murthy NA, Hegde SN, The effect of *Emblica officinalis* diet on lifespan, sexual behavior, and fitness characters in Drosophila melanogaster. Ayu, 2011, 32, 279-284.

19. Tiwari AK, Pragya P, Ravi Ram K, Chowdhuri DK, Environmental chemical mediated male reproductive toxicity: Drosophila melanogaster as an alternate animal model. Theriogenology, 2011, 76, 197-216.

20. Dwivedi V, Anandan EM, Mony RS, Muraleedharan TS, Valiathan MS, Mutsuddi M, Lakhotia SC, In vivo effects of traditional ayurvedic formulations in Drosophila melanogaster model relate with therapeutic applications. PLoS ONE, 2012, 7, doi:10.1371/journal.pone.0037113.

21. Jafari M, Zarban A, Pham S, Wang T: *Rosa damascena* decreased mortality in adult Drosophila. J Med Food, 2008, 11, 9–13.

22. Altun D, Ayar A, Uysal H, Kara AA, Unal EL, Extended longevity of *Drosophila melanogaster* by water and ethanol extracts of Stachys lavandulifolia. Pharm Biol, 2010, 48, 1291–1296.

23. Bahadorani S, Hilliker AJ, Cocoa confers life span extension *in Drosophila melanogaster*. Nutr Res, 2008, 28, 377–382.

24. Lee KS, Lee BS, Semnani S, Avanesian A, Um CY, Jeon HJ, Seong KM, Yu K, Min KJ, Jafari M, Curcumin extends life span, improves health span, and modulates the expression of age-associated aging genes in Drosophila melanogaster. Rejuvenation Res, 2010, 13, 561–570.

25. Peng C, Chan HY, Huang Y, Yu H, Chen ZY, Apple polyphenols extend the mean lifespan of *Drosophila melanogaster*. J Agric Food Chem, 2011, 59, 2097–2106.

26. Peng C, Zuo Y, Kwan KM, Liang Y, Ma KY, Chan HY, Huang Y, Yu H, Chen ZY, Blueberry extract prolongs lifespan of *Drosophila melanogaster*. Exp Gerontol, 2012, 47, 170-178.

27. Chandrashekara KT, Shakarad MN, Aloe vera or resveratrol supplementation in larval diet delays adult aging in the fruit fly, *Drosophila melanogaster*. J Gerontol A Biol Sci Med Sci, 2011, 66, 965-971.

28. Boyd O, Weng P, Sun X, Alberico T, Laslo M, Obenland DM, Kern B, Zou S, Nectarine promotes longevity in Drosophila melanogaster. Free radic Biol Med, 2011, 50, 1669-1678.

29. Nazir A, Mukhopadhyay I, Saxena DK, Siddiqui MS, Chowdhuri DK, Evaluation of toxic potential of captan: Induction of hsp70 and tissue damage in transgenic *Drosophila melanogaster (*hsp70-lacZ*) Bg*$^{9}$. J Biochem Mol Toxicol, 2003, 17, 98-107.

30. Nishikimi M, Appaji N, Yagi K, The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. Biochem Biophys Res Commun, 1972, 46, 849–854.

31. Gupta SC, Siddique HR, Saxena DK, Chowdhuri DK, Hazardous effect of organophosphate compound, dichlorvos in transgenic *Drosophila melanogaster* (hsp70- lacZ): induction of hsp70, anti-oxidant enzymes and inhibition of acetylcholinesterase. Biochim Biophys Acta, 2005, 1725, 81–92.

32. Sinha A K, Colorimetric assay of catalase. Anal Biochem, 1972, 47, 389–394.

33. Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem, 1976, 72, 248–254.

34. Carvalho GB, Kapahi P, Benzer S, Compensatory ingestion upon dietary restriction in *Drosophila melanogaster.* Nat Methods, 2005, 2, 813-815.

35. Rzezniczak TZ, Douglas LA, Watterson JH, Merritt TJ, Paraquat administration in Drosophila for use in metabolic studies of oxidative stress. Anal Biochem, 2011, 419, 345-347.

36. Davies KJ, Oxidative stress: The paradox of aerobic life. Biochem Soc Symp, 1995, 61, 1–31.

37. Ryan JA, Hightower LE, Stress proteins as molecular biomarkers for environmental toxicology. Exs 1996, 77, 411-424.

38. Mukhopadhyay I, Nazir A, Saxena DK, Chowdhuri DK, Heat shock response: hsp70 in environmental monitoring. J Biochem Mol Toxicol, 2003, 17, 249-254.

39. Bauer JH, Goupil S, Garber GB, Helfand SL, An accelerated assay for the identification of lifespan-extending interventions in Drosophila melanogaster. Proc Natl Acad Sci, U S A, 2004, 101, 12980-12985.

40. Driver C, Georgiou A, Georgiou G, The contribution by mitochondrially induced oxidative damage to aging in Drosophila melanogaster. Biogerontology, 2004, 5, 185-192.

41. Calzuola l, Marsili V, Gianfranceschi GL,Synthesis of antioxidants in wheat sprouts. J Agric Food Chem, 2004, 52, 5201-5206.

**Source of support:** GSBTM, DBT PS and Puri Foundation
**Conflict of interest:** None Declared