# PLANT EXTRACTS AFFECT DOSE-DEPENDENTLY THE HATCHING RATE OF DROSOPHILA MELANOGASTER BUT DO NOT INDUCE CHROMOSOME LOSS AND/OR ANEUPLOIDY

**Máthé Endre[1,2,4], Liana Moş[1], Zsombor Szőke-Kovács[2], Rita Szőke-Kovács[2], Szabolcs Vígh[2], Ciprian Pribac[1], Marika Czapár[2], Csaba Oláh[3]**

[1] "Vasile Goldis" Western University of Arad, Arad, Romania
[2] University College of Nyíregyháza, Nyíregyháza, Sostói út 1/B, 4400, Hungary
[3] Bosrsod-Abaúj-Zemplén County and University Hospital, Miskolc, Hungary
[4] corresponding author: endre.mathe64@gmail.com

***Abstract***

*Multiple studies on Drosophila melanogaster have led to many important discoveries during the past century, and this species developed into a powerful model organism that allows researchers to combine techniques and methods from many fields including genetics, molecular and cell biology, biochemistry, bioinformatics and developmental biology. Our research team has made further progresses by designing a Drosophila melanogaster based experimental system to study the effects, and ultimately the mechanism of action of bioactive compounds extracted from different fruits and vegetables on an individual and molecular levels. In this paper we report a nutritional genetics study that shows the beetroot, black current, blue berry and sour cherry extracts affecting in a dose-dependent fashion the hatching rate of Drosophila melanogaster individuals, and they do not lead to chromosomal loss and/or aneuploidy.*

**Key words:** *Drosophila*, nutritional genetics, bioactive compounds

## INTRODUCTION

Fruits and vegetables are of a great importance for the healthy nutrition of humans. Several studies have been conducted to elucidate the biological relevance of vegetables and fruits consumption for healthy and chronic disease affected humans (for review see Maiese et al., 2010; Vasanthi et al, 2012). Despite the fact that the majority of such experiments are converging to a general conclusion that the intake of fruits and vegetables is important for maintaining of health condition of humans and/or preventing diseases (Wiseman, 2005; Weng and Yen, 2012), very little is known about the direct effect of plant derived bioactive compounds on human organism (for review see Yu and Chung, 2006; Azad and Wright, 2012). Interestingly, using single bioactive compounds there have been several studies performed to follow the reactions of model organisms (Papaconstantinou et al, 2005; Xu et al., 2011;), though the scientific relevance of such experiments could be questioned in case someone tries to build an argument for the benefic effects of fruits and vegetables dietary intake based on the above mentioned studies. The dietary intake of fruits and vegetables leads to the simultaneous loading of several bioactive compounds into the humans or animal organisms. It seems therefore likely

that the plant derived multiple bioactive compounds could be are capable of acting simultaneously either enhancing or suppressing certain cellular phenomena in a dose-dependent manner. The concentration of individual bioactive compounds and the specific bioactive compound composition of a certain fruit and/or vegetable extract seem to be critical for the diet-related effects, and indeed, several empirical observations and controlled experiments related to plant based diets are suggesting the concentration dependent and cumulative effects of some bioactive compounds (for review see Kumar at al, 2012; Dutton and Turnbaugh, 2012; Lou-Bonafonte et al., 2012; Whiting et al, 2012). *Drosophila melanogaster* is considered to be the best known multicellular eukaryote model organism as we can study the interactions between genes and environmental conditions simultaneously. Recently, there have been successful attempts to use this species to investigate the effect of a certain type of diet on viability and lifespan (Khan et al, 2012; Li et al. 2007; Soh et al., 2012).

## MATERIAL AND METHOD

**Fly Strains and Culture Conditions.** The wild-type Canton-S and the C(2)EN, *bw sp* strains were maintained and mated on standard yeast–agar–cornmeal medium, or this standard medium was supplemented with plant derived extracts. In fact, the total volume of medium in a culture vial for *Drosophila melanogaster* was 5 ml, and when plant extracts were incorporated in the medium at different amounts (0.3 ml, 0.5 ml, 1 ml, 1.5 ml), the volume of normal media was reduced accordingly. All experiments were performed at 25°C. All genetic markers and mutations used are described in Flybase (www.flybase.org). The hatching rate was estimated by comparing the number of hatched F1 individuals for non-treated and plant extract treated wild-type

**Detection of nondisjunction:** To test whether fruit and vegetable extracts can induce chromosome loss and/or nondisjunction during spermatogenesis, we made use of the compound second chromosome C(2 Canton-S flies obtained from crosses between five virgin female and males of the same age. )EN, *bw sp* (Gonzales et *al.,* 1989). In this system, *wild-type* males were mated with C(2)EN, *bw s p* females. These crosses were carried out either on normal or on plant extract containing yeast–agar–cornmeal medium, and the crosses monitored for F1 offspring.

**Preparation of plant extracts.** Crude extracts have been made of beetroot, black currant, blue berry and sour cherry by pressing out the liquid content that later was made more concentrated by reducing the water content through evaporation.

## RESULTS AND DISSCUSIONS

### The plant extracts affect specifically the vitality of *Drosophila*

We have investigated the effect of beetroot, black currant, blue berry and sour cherry extracts on the viability of a wild-type *Drosophila melanogaster* strain called Canton S. The viability of F1 individuals can be determined by estimating the hatching rate comparing the number of offspring for non-treated or control and treated individuals. Actually, the parents were raised on normal yeast–agar–cornmeal medium, and as the parental cross was set, the flies were put on media containing different amounts of plant extracts, and for the control experiments the parental cross was carried out in normal medium containing culture vials. This it means that the so-called treated F1 progeny throughout the larval period had the possibility to utilize plant extracts that were incorporated into the fly media at different concentrations. The number of the treated and hatched F1 adults was determined for every concentration, and compared to the total number of non-treated hatched individuals observed in the control experiments. This estimation of the results allows us to conclude on the dose-dependent effect of the plant derived extracts (see Figure 1.). The beetroot, black currant, blueberry and sour cherry extracts influence differently but in a dose-dependent manner the hatching rate of wild-type *Drosophila* individuals. In the case of the beetroot extract type of experiments we observed the increasing tendency of hatching rate as the concentration of the extract was growing, so the concentration of beetroot extract in the fly food looked to be directly proportional with viability. However, in the case of sour cherry type of experiments we were able to observe an opposite tendency to what we have seen for the beetroot type of experiments. Surprisingly, the less concentrated sour cherry containing medium had a more decisive effect on the hatching rate of F1 individuals, while the increasing amount of sour cherry extract containing medium seemed to affect negatively the viability by reducing the number of hatched F1 individuals. Therefore we can conclude that the concentration of the sour cherry extract in the fly food appears to be inverse proportional with viability.

In the case of *Drosophila* individuals raised on black currant and blue berry extracts containing medium again dose dependent effects were observed, though the normal medium specific hatching rate was observed at different concentrations of extracts. In the case of black currant the lowest concentration of plant extract containing medium had similar effect like the normal medium, while the 0.5g of plant extract containing fly food seemed to increase the most significantly the viability of F1 individuals. The effects of higher amounts of extracts (1g and 1.5g) seemed to be inverse proportional with the hatching rate, yet they remain in the domain of

increased viability as compared to the normal medium specific outcome. In the case of blue berry containing extract, the lowest concentration (0.3g) reduced significantly the hatching rate, while the higher concentrations (0.5g, 1g, 1.5g) produced a hatching rate that corroborates with normal medium raised flies.

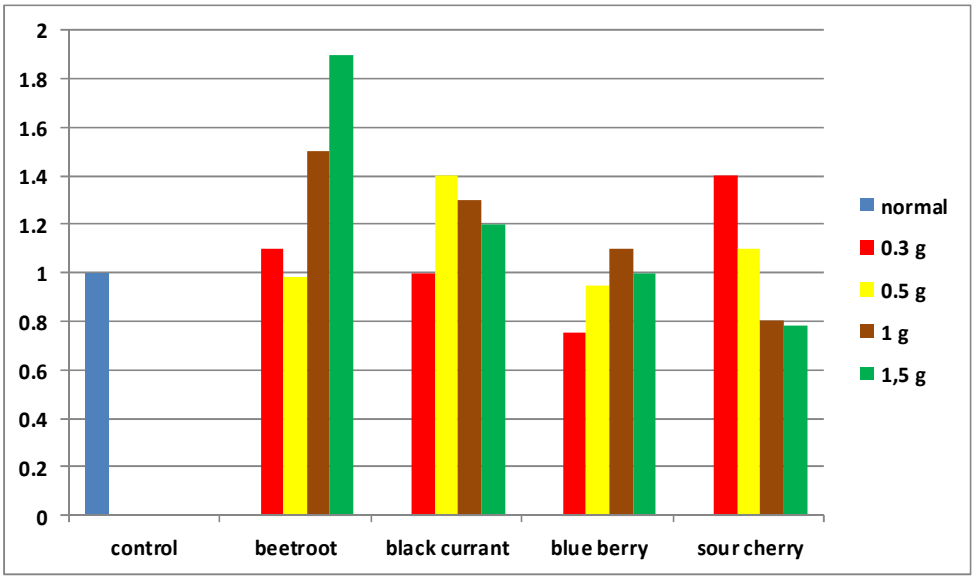


**Figure 1.** The dose-dependent effect of plant extracts influencing the hatching rate of wild-type Drosophila melanogaster. The hatching rate represents the ratio of hatched individuals obtained on plant extract containing and normal fly foods.

Our observations clearly demonstrate that beetroot, black currant, blue berry and sour cherry extracts affects viability in a concentration dependent manner, but the so-called normal non-treated fly food specific hatching rate was achieved at different concentrations. Therefore all the studied extracts have specific action profiles that argue for the necessity of determination of the optimal concentration and optimal biological effect type of correlations for every single plant extract in any nutrition based treatment.

**The plant extracts do not induce chromosome loss and/or aneuploidy.**

To test whether the beetroot, black currant, blue berry and sour cherry extracts can induce chromosome loss and/or nondisjunction during spermatogenesis, we made use of the compound second chromosome C(2)EN, *bw sp* (Gonzales et *al.,* 1989). In this system, *wild-type* males were mated with C(2)EN, *bw s p* females. Oocytes of the C(2)EN, ***bws p*** females carry either two or none of the second chromosomes. In normal circumstances viable offspring does not result when such gametes are fertilized by regular sperm. However, viable offspring develop when the

diplo-2 and nullo-2 oocytes fuse with aneuploid nullo-2 or diplo-2 sperm, respectively. Aneuploid nullo-2 or diplo-2 sperms result when during male meiosis nondisjunction occures. The phenotype of the descending F1 progeny allows conclusions to be made about the mechanism generating the aneuploid sperm during the two successive divisions of male meiosis. Three sets of independent experiments were carried out using normal and different concentration of plant extract containing yeast–agar–cornmeal medium at 18°C and 25ºC. The F1 individuals were not observed to hatch in either the normal or the plant extract containing media type of experiments suggesting that the beetroot, black currant, blue berry and sour cherry derived plant extracts do not induce chromosome loss and/or aneuploidy during the male meiosis. In fact these experiments are strongly indicating that the above mentioned plant extracts do not have a mutagenic effect, and does not affect negatively the chromosomal stability during male meiosis in *Drosophila melanogaster*.

**Acknowledgments**

The authors wish to thank to the all members of the Agricultural and Molecular Research Institute from University College of Nyiregyhaza, and the Life Science Institute of "Vasile Goldis" Western University of Arad for their scientific support and helpful comments. The research activities described in this paper where supported by the "Food-Energ Regional Knowledge Center" Grant (Péter Pázmány Program, NKTH; HU) and a HU-RO CrossBorderCooperation Grant (*In vitro* study of some plant extracts of natural origin with emphasis on their anti-tumor effects).

**REFERENCES**

1. Azad, M.A., Wright, G.D. (2012). Determining the mode of action of bioactive compounds. *Bioorg. Med. Chem*. **20(6)**:1929-39.
2. Dutton, R.J. and Turnbaugh,P.J. (2012). Taking a metagenomic view of human nutrition. *Curr. Opin. Clin. Nutr. Metab. Care.* **15(5)**:448-454.
3 Gonzalez, C.J., J. Casal and P. Ripoll (1989). Relationship between chromosome content and nuclear diameter ine arly spermatids of *Drosophila melanogaster. Genet*. Res. **54:** 205-212.
4 Khan, S., Jyoti, S., Naz, F., Shakya, B., Afzal, M., Siddique, Y.H. (2012). Effect of L-Ascorbic Acid on the Climbing Ability and Protein Levels in the Brain of Drosophila Model of Parkinson's Disease. *Int. J. Neurosci.*
5 Kumar, H., More, S.V., Han, S.D., Choi, J.Y., Choi, D.K. (2012). Promising therapeutics with natural bioactive compounds for improving learning and memory - a review of randomized trials. *Molecules*. **17(9):**10503-39.
6 Li YM, Chan HY, Yao XQ, Huang Y, Chen ZY (2007). Green tea catechins and broccoli reduce fat-induced mortality in *Drosophila melanogaster*. *J. Nutr. Biochem*. **19(6):**376-83.
7 Lou-Bonafonte, J.M., Arnal, C., Navarro, M.A., Osada, J. (2012). Efficacy of bioactive compounds from extra virgin olive oil to modulate atherosclerosis development. *Mol. Nutr. Food. Res.* **56(7)**:1043-57.

8 Maiese, K., Chong, Z.Z., Hou, J., Shang, Y.C. (2010). Oxidative stress: Biomarkers and novel therapeutic pathways. *Experimental Gerontology*, **45**: 217-234.
9 Papaconstantinou, M., Wu, Y., Pretorius, H.N., Singh, N., Gianfelice, G., Tanguay, R.M., Campos, A.R. and Bédard,P-A. (2005). Menin is a regulator of the stress response in *Drosophila melanogaster*. *Molecular and Cellular Biology*. 25(22): 9960-9972.
10 Soh, J.W., Marowsky, N., Nichols, T.J., Rahman, A.M., Miah, T., Sarao, P., Khasawneh, R., Unnikrishnan, A., Heydari, A.R., Silver, R.B. and Arking, R. (2012). Curcumin is an Early-acting Stage-specific Inducer of Extended Functional Longevity in *Drosophila*. *Exp Gerontol*. S0531-5565.
11 Xu, H.L., Tang, W., Du, G.H., Kokudo, N. (2011). Targeting apoptosis pathways in cancer with magnolol and honokiol, bioactive constituents of the bark of Magnolia officinalis. *Drug. Discov. Ther.* **5(5)**:202-210.
12 Yu, B.P. and Chung, H.Y. (2006). Adaptive mechanisms to oxidative stress during aging. *Mechanisms of Ageing and Development.* **127(5):**436-43.
13 Vasanthi HR, ShriShriMal N, Das DK.(2012). Phytochemicals from plants to combat cardiovascular disease. *Curr. Med. Chem*. 19(14):2242-2251.
14 Weng, C.J. and Yen, G.C. (2012). Flavonoids, a ubiquitous dietary phenolic subclass, exert extensive in vitro anti-invasive and in vivo anti-metastatic activities. *Cancer Metastasis Rev.* **31(1-2)**:323-51.
15 Wiseman, A. (2005). Functional-food protected by biomonitoring of reactive oxygen species(ROS)? *Trends in Food Science and Technology*, **16**:166-168.
16 Whiting S, Derbyshire E, Tiwari BK. (2012). Capsaicinoids and capsinoids. A potential role for weight management? A systematic review of the evidence. *Appetite*. **59(2)**:341-348.