



A combination of dimethyl sulfoxide (DMSO) and methyl paraben (nipagin) in *Drosophila* food affects survival rate.

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Introduction

Huntington's disease (HD) is a dominant, late-onset neurodegenerative disease caused by an expansion of a homopolymeric polyglutamine (polyQ) tract within the disease-specific huntingtin (Htt) protein (The Huntington's Disease Collaborative Research Group). HD portrays a clinical condition, which is characterised by the selective and progressive loss of neurons, eventually leading to cognitive, behavioral, and physical defects that can ultimately cause the death of the diseased individual (Beal *et al.*, 2005).

At present, there is no cure or effective therapeutic strategy for HD or other neurodegenerative diseases. Administration of plant-derived compounds or 'phytochemicals' that can target multiple cellular functions and processes are anticipated to achieve better therapeutic efficacy with minimal or no side effects as opposed to mono-targeted agents or synthetic drugs in the treatment of multi-faceted neurodegenerative diseases.

In our attempt to test the efficacy of phytochemicals in alleviating disease symptoms, we performed pilot experiments using a *Drosophila* model of Huntington's disease to investigate if both dimethyl sulfoxide [(CH₃)₂S; DMSO] and methyl paraben (HOC₆H₄CO₂CH₃; nipagin) at intended concentrations can be administered without any undesirable effects. DMSO is an organosulfur, polar, aprotic compound, which is commonly used as a solvent for the dissolution of a wide range of polar and nonpolar molecules (Szmant, 1975). DMSO has the unique capability to penetrate living tissues without causing significant damage. Due to its broad solubilising property, DMSO is used as a solvent for many drug molecules and is also employed as the vehicle control-of-choice for both *in vitro* and *in vivo* studies. The phytochemical we wanted to test was dissolved in DMSO which was thoroughly mixed with regular *Drosophila* food containing nipagin also. Nipagin is one of the member of family of parabens (methyl, ethyl, butyl, heptyl, and benzyl parabens) and is commonly used in *Drosophila* food as an anti-microbial agent. Parabens are particularly active against bacteria, yeast and moulds. Their key mechanisms of action include inhibition of membrane transport and mitochondrial function.

It has been reported that increasing doses of DMSO exhibit toxicity above which survival is reduced (Agrawal *et al.*, 2005). However, we observed that a safe dose of DMSO in combination with nipagin is toxic as revealed by significantly reduced eclosion rate of wild type flies (*Canton S*). Similar results were observed in our experiments in which the control male flies not expressing mHtt (internal control) and female flies expressing the mutant protein also displayed reduced survival.

In order to check the reason for the reduced eclosion, we conducted a series of experiments and found that safe dose of DMSO alone did not affect the survival rate of the flies but if added along with the safe dose of nipagin then it resulted in significant reduction in eclosion rate. Although DMSO is an excellent solvent for a wide variety of drugs employed in biomedical research, caution is required when added with food ingredients of *Drosophila* while designing and interpreting experiments.

Results

A non-toxic dose of DMSO in combination with nipagin causes lethality in transgenic flies Transgenic *Drosophila* models of neurodegenerative diseases, including Huntington's disease mimic most of the major phenotypes associated with the disease, such as reduced eclosion rate, reduced longevity, impaired mobility, and neurodegeneration (Steffan *et al.*, 2001; Richards *et al.*, 2011). During the attempt to test effect of

phytochemical, we found that 0.1 % DMSO (safe dose) in combination with 0.03% nipagin (safe dose to prepare regular *Drosophila* food) displayed only 6% eclosion of flies expressing the first exon of Htt with 93 glutamine residues (Httex1p Q93, mHtt), *i.e.*, diseased flies and 56.67% eclosion of control flies not expressing Htt (Figure 1a). On the contrary, normal eclosion rate of both Httex1p Q93 as well as control flies was observed when transgenic flies were grown in standard food (Figure 1a) or food without nipagin but with DMSO (data not shown).

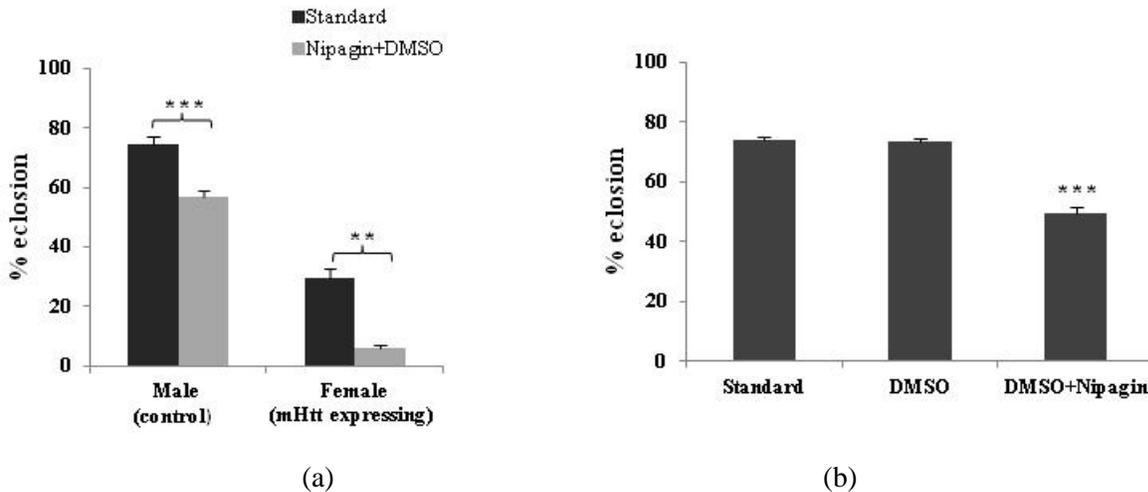


Figure 1. Administration of DMSO in combination with nipagin results in toxicity. (a) DMSO in combination with nipagin significantly reduces viability in control (male) as well as mutant Htt (mHtt) expressing flies (female). (b) The eclosion of wild type flies was affected by DMSO and nipagin in combination, while DMSO alone did not affect their survival. Flies were grown at $25 \pm 0.5^\circ\text{C}$. ***, $P << 0.001$; **, $P < 0.01$.

DMSO with nipagin reduces the survival of wild type flies

Our results clearly indicate that feeding DMSO in combination with nipagin results in detectable toxic effect in control as well as in mHtt (diseased) flies. To further validate our results with the diseased flies, we then investigated if a combination of DMSO (0.1%) and nipagin (0.03%) produces any unwanted toxic effect in wild type flies (Figure 1b). We found similar reduced eclosion of wild type flies reared in food with both DMSO and nipagin. Eclosion remained unaffected when flies were grown in food devoid of nipagin but containing DMSO at 0.1%. Therefore, DMSO by itself at a dose of 0.1% does not cause toxicity and can be safely administered to flies without adding nipagin in the food.

Materials and Methods

Drosophila stocks

The polyglutamine expressing transgenic line used in the present study was $w; P\{UAS-Httex1p Q20\}4F1$. These flies were mated with the pan-neuronal elav driver $w; P\{w^{+mW.hs}=GawB\}elavC155$. The wild type line used for the experiments was *Canton S*.

Survival assay

UAS-Httex1p Q93 females were mated with elav-GAL4 males and the eggs were transferred to standard *Drosophila* food containing 0.03 % nipagin (39231, SDFCL), secondly with 0.1 % DMSO ((D5879, Sigma) alone, and third with nipagine and DMSO. The ratio of eclosed adults to eggs of the same genotype (males and females) was taken as a measure of survival. For every condition, at least 6 vials with 100 eggs in each were scored.

Statistical procedure

Throughout this paper, error bars indicate standard error of the mean (SEM = standard deviation / square root of n). Student's t-test was performed for pair wise comparisons.

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Silver nanoparticle affects flying ability of fruit flies.

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Introduction

With the growing knowledge and advancement of nanotechnology, nanoparticles are being ubiquitously utilized in a wide array of applications including consumer goods, construction, food industry, and medicine. The extraordinary feature that makes nanoparticles act differently than bulk counterparts is their relative size in the scale of nanometers, which offers larger surface area for enhanced reactivity (Nam *et al.*, 2008). Silver nanoparticle (SNP) is one of the commonly used nanoparticles that is known for its anti-microbial property (Sharma *et al.*, 2009; Chen *et al.*, 2008). However, several reports using *Drosophila* as a model system suggest that higher dose of SNP compromise behavioral activities such as climbing of *Drosophila* (Key *et al.*, 2011). Flight is the integral behavior found in insects and is vital for performing various activities such as mating, migrating in search of food, and so forth. The influence of SNP on the basic fundamental behavior of *Drosophila*, *i.e.*, flight is largely unknown at this stage. In view of this, the impact of silver nanoparticles on flight of *Drosophila melanogaster* has been successfully monitored by modifying the experimental set up from the existing ones (Sadaf *et al.*, 2012; Sherwood *et al.*, 2004; Wojtas *et al.*, 1997).

Methodology

The flight assay was performed by placing a set up where an empty vial containing flies was positioned in the center of the beaker (14.5 cm in diameter). The beaker was half filled with water to create a barrier between the vial containing flies and the wall of beaker. The inner side of the beaker was coated with yeast paste in order to attract the flies. The beaker containing the vial was placed in a large enclosure (23 cm in diameter) for 40 minutes. Flight ability was determined by releasing flies from the vial and counting the numbers of flies that flew and crossed the water barrier and sitting on the walls of beaker or enclosure were scored as flyers. However, the flies still wandering in the vial or drowned in the water surrounding the vial were considered as non-flyers. Flies were collected in batches of 30 and starved for 1 hour before conducting this assay (Figure 1).