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Toxicity of dimethyl sulfoxide against *Drosophila melanogaster*

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***Abstract*:**

Cvetković, V.J., Mitrović, T.Lj., Jovanović, B., Stamenković, S.S., Todorović, M., Đorđević, M., Radulović, N.: ***Toxicity of dimethyl sulfoxide against Drosophila melanogaster. Biologica Nyssana, 6 (2), December 2015: 91-95.***

Dimethyl sulfoxide (DMSO) is commonly used as a solvent for organic compounds but its toxic properties can affect both *in vitro* and *in vivo* studies. Thus, evaluation of DMSO toxicity must be performed on the model organism before its application as a solvent in the target studies. Present study aimed to determine the lethal concentration required to kill 50% of the population in a given period of time (LC_{50}), no observed effect concentration (NOEC), and lowest observed effect concentration (LOEC) values for DMSO on *Drosophila melanogaster* which is frequently used as a model for toxicity studies. Twelve different concentrations of DMSO were tested on three-day old *D. melanogaster* larvae for 12 days. At the end of the life cycle, number of live hatched adults and un-hatched pupae were counted. Based on probit analysis 12 days LOEC was 0.04% v/v, 12 days NOEC was < 0.04% v/v and 12 days LC_{50} was 0.42% v/v. The results indicate that DMSO may be more toxic to *D. melanogaster* than it was initially considered.

Key words: *Drosophila melanogaster,* NOEC, LOEC, LC_{50}, DMSO, toxicity

***Apstrakt*:**

Cvetković, V.J., Mitrović, T.Lj., Jovanović, B., Stamenković, S.S., Todorović, M., Đorđević, M., Radulović, N.: ***Toksičnost dimetil sulfoksida na modelu Drosophila melanogaster. Biologica Nyssana, 6 (2), December 2015: 91-95.***

Dimetil sulfoksid (DMSO) se često koristi kao organski rastvarač ali njegova toksičnost može da utiče na ishode *in vitro* i *in vivo* istraživanja. Zato se procena toksičnosti DMSO-a mora ispitati na model organizmu pre njegove primene kao rastvarača u željenom istraživanju. Ovo istraživanje ima za cilj da se za DMSO ustanovi letalna koncentracija koja ubija 50% populacije u posmatranom vremenskom periodu (*Lethal concentration 50* - LC_{50}), koncentracija koja ne uzrokuje detektabilne promene (*No observed effect concentration* - NOEC) i koncentracija koja uzrokuje najmanje detektabilne promene (*Lowest observed effect concentration* - LOEC) na modelu *Drosophila melanogaster* koji se veoma često koristi u toksikološkim istraživanjima. Larve *D. melanogaster* stare tri dana tretirane su sa dvanaest različitih koncentracija DMSO-a u periodu od 12 dana. Na kraju životnog ciklusa, prebrojani su adulti koji su se izlegli i lutke iz kojih se adulti nisu izlegli. Prema probit analizi, za 12 dana tretmana LOEC je 0.04% v/v, NOEC je < 0.04% v/v i LC_{50} je 0.42% v/v. Rezultati ukazuju da je DMSO toksičniji za *D. melanogaster* nego što se prvobitno smatralo.

Key words: *Drosophila melanogaster*, NOEC, LOEC, LC_{50}, DMSO, toksičnost

Introduction

DMSO originates from 19th century as a by-product in the process of paper production from a wood pulp (Capriotti & Capriotti, 2012; Horita & Weber, 1964). Since then, colorless liquid DMSO is characterized as a good aprotic solvent which can easily dissolve various non-polar and polar molecules (Capriotti & Capriotti, 2012; Horita & Weber, 1964). The concern regarding the toxicity of solvents is always a topical issue in toxicology studies. Thus, the pre-testing of solvents on certain model organism, which is planned to be used in further toxicology studies, is an imperative.

One of the widest and frequently used model organisms in toxicity and genotoxicity studies is fruit fly, *Drosophila melanogaster* (Stamenković-Radak et al., 2008; Patenković et al., 2009; Vales et al., 2013). *Drosophila* is a remarkable model for *in vivo* assays in toxicology because of high number of progeny and its short lifespan. In addition, culturing and maintaining of *Drosophila* is more economical compared to other model organisms. It was demonstrated that administration of the test substances through the feeding medium is simple (Nazir et al., 2003; Stamenković-Radak et al., 2008) making toxicology assays on *Drosophila* even easier.

The adverse effects of DMSO in *Drosophila* were noticed in the 1980's (Massie et al., 1985; Brodberg et al., 1987) when the authors recommended that DMSO must be used carefully as a solvent. Therefore, present study aimed to determine the lethal concentration required to kill 50% of the population in a given period of time (LC_{50}), no observed effect concentration (NOEC), and lowest observed effect concentration (LOEC) values for DMSO on a three-day-old *D. melanogaster* larvae of the wild-type strain.

Material and methods

Test organism

In this experiment the *D. melanogaster* Oregon-RC (wild-type flies, stock no. 5) ("Bloomington Drosophila Stock Center", Indiana University, USA) were used. Flies were cultivated on a standard feeding medium for Drosophila (9% sugar, 10% corn meal, 2% agar, 2% yeast) with addition of fungicide 2.50 mg mL^{-1} methyl 4-hydroxybenzoate (Nipagin®, Alfa Aesar GmbH & Co KG, Germany) diluted in 95% EtOH (ethanol). The flies were cultivated in a standard cultivating 200 ml vials on 25 ºC, 60% humidity, with 12 h/12 h day-night cycle. Adults were allowed to lay eggs during the eight hour period, after that they were removed from the vials. After three days larvae which were 72±4 h old, have been removed from the cultivating medium, washed with distilled water and used in further procedure.

Test substance and treatment schedule

Twelve concentrations of DMSO (Carl Roth, Germany) were tested in duplicates and each experimental group consisted of 30 three-day-old larvae. Therefore, final concentrations of DMSO in 3ml of standard feeding medium were: 0.16%, 0.32%, 0.48%, 0.64%, 0.80%, 0.97%, 1.13%, 1.29%, 1.45%, 1.61%, 2.42% and 3.23% v/v. Negative, water control, was also included. At the end of incubation time, which lasted up to 12 days, numbers of live hatched units (adults) and un-hatched units (at pupal stadium) were counted.

Statistical analysis

Mean values were calculated and used for further analysis. Risk Assessment software tool (RA V1.0; Pensacola, Florida, USA) and probit analysis were utilized in order to calculate values for LC_{50}, LOEC, and NOEC. Also, the confidence intervals (95%) were calculated.

Results and discussion

The toxic effects of DMSO, particularly antimicrobial and virucidal properties, were noticed in the 1960's. DMSO was shown to inhibit bacterial and fungal growth at concentration of 30-50% and at concentration of 80% inactivated infectivity of some RNA and DNA viruses (Basch & Gadebusch, 1968; Chan & Gadebusch, 1968; Wagner et al., 2002). Even nowadays, the adverse properties of DMSO, which is used as a solvent in toxicology studies of aquatic model organisms, was noticed (Máchová et al., 2009). Máchová et al. (2009) tried to establish general value for the non-toxic concentration of DMSO as a solvent that could be applied across different conditions in aquatic model organism such as fish. But they found that effect of

DMSO on fish model depends on fish age, species, type of test and indicators observed so they concluded that general value for the non-toxic concentration of DMSO can't be found and every experiment should include solvent control (Máchová et al., 2009). It implies that DMSO must be pre-tested on certain model organism if it is planned to be used in experiment as solvent.

The **Fig. 1a** represents survival plot of *D. melanogaster* units exposed to different concentration of DMSO which unambiguously shows that number of hatched flies decreased in dose-depended manner. **Fig. 1b** represents probability of *D. melanogaster* units mortality exposed to DMSO which was calculated by Risk Assessment software tool. 12 days LC_{50} value is 0.42% v/v (**Tab. 1**) and total number of adults hatched from pupal stadium drastically decreased from 0.32% v/v where almost every unit developed into adult to 0.97% v/v where none of the adults hatched. In addition, pupal mortality (un-hatched units) increased drastically in this concentration range, respectively (**Fig 2**). Concentration of DMSO in substrate, from 0.97% v/v up to the 3.23%, v/v caused increase of larval mortality (Fig. 2). Concentrations above 0.32% v/v decreased hatchability of adults. These observations are in accordance with similar study (Nazir et al., 2003).

In genotoxicity and toxicity studies larvae, or adults, of *D. melanogaster*, as a model organisms, are widely used (Nazir et al., 2003; Adamski et al., 2009; Castañeda-Partida et al., 2011; Miyazawa et al., 1998; 2000; Graf et al., 1984). There are many references on DMSO toxicity, but only a few papers are on DMSO toxicity in *Drosophila*. For instance, Graf et al. (1984) used DMSO in final concentration of 2% in feeding medium for *Drosophila* in somatic mutation and recombination test (Graf et al., 1984). But recent research by Nazir et al. (2003) showed that DMSO should be used very cautiously as solvent because it could results with false conclusions in the screening

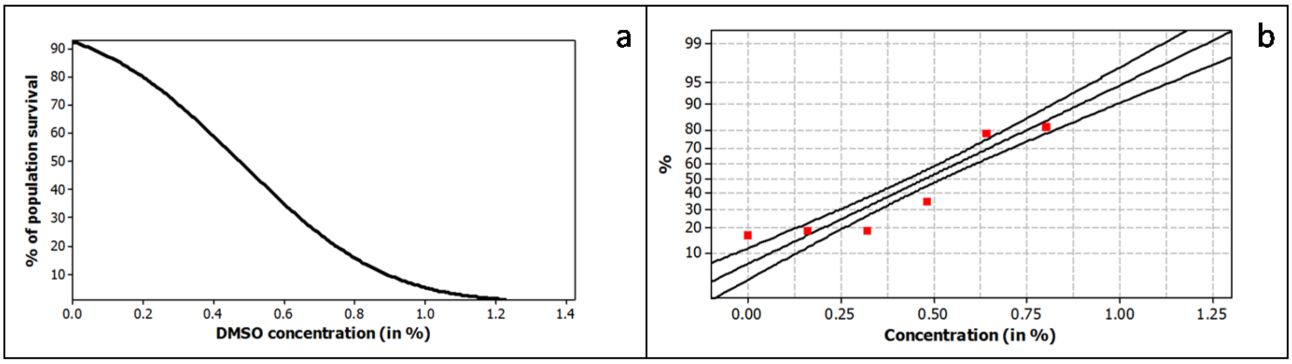

Fig 1. Survival plot of *D. melanogaster* units (a) and probability of the units mortality exposed to DMSO with 95% confidence intervals (b).

Table 1. Probit analysis: LOEC, NOEC and LC50 values with confidence intervals after 12 days of exposure.

	% of population response	**Concentration in % (v/v)**	**Lower limit 95%**	**Upper limit 95%**
LOEC 12 days	0.01%	**0.04555**	0.00780	0.09803
	0.10%	0.06642	0.01461	0.12884
	1.00%	0.10502	0.03122	0.18004
	5.00%	0.15806	0.06122	0.24374
	10.00%	0.19656	0.08741	0.28734
	20.00%	0.25598	0.13387	0.35252
	30.00%	0.30967	0.18096	0.41093
	40.00%	0.36435	0.23254	0.47149
LC_{50} 12 days	50.00%	**0.42404**	0.29133	0.54068

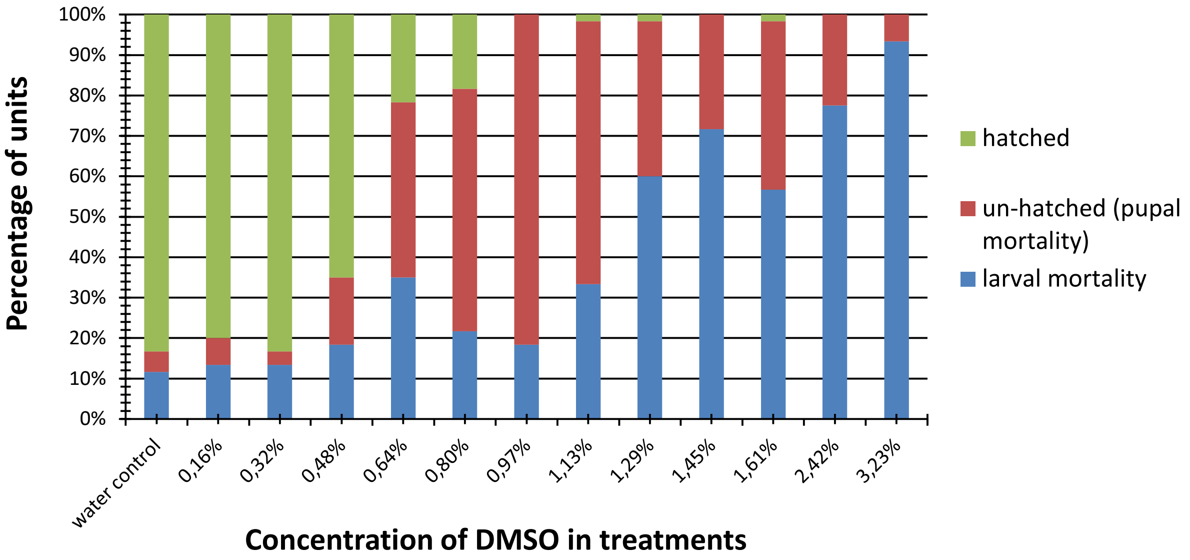

Fig 2. Percentage of hatched (alive adults), un-hatched (non-survived units at pupal stadium) and non-survived units at larval stage (larval mortality) influenced with different concentration of DMSO (showed in % v/v) in feeding media and in water (negative) control.

of test substances because of its toxicity to *D. melanogaster* (Nazir et al., 2003). Our results confirmed observation that DMSO is toxic and can impact normal larval development in concentration greater than 0.04% v/v (LOEC value) in feeding medium. The present study was performed with twelve concentrations which provided better resolution of obtained results than in the study by Nazir et al. (2003) where six concentrations were tested. Also, we treated three-day-old larvae of the wild-type strain of *D. melanogaster* which differs from the study by Nazir et al. (2003) whose treatment started at the egg stadium of the transgenic strain of *D. melanogaster* and lasted for 10-14 days. Finally, probit analysis was performed as a common analysis in toxicology, in order to determine NOEC, LOEC and LC_{50}. Nazir et al. (2003) considered DMSO at 0.3% v/v as NOAEL (No Observed Adverse Effect Level) value which can be used as dietary concentration in toxicity studies with *D. melanogaster*. However, according to present research 12 days LOEC is 0.04% v/v so the absolutely safe dietary concentration of DMSO in feeding medium for *D. melanogaster* larvae of wild-type strain, should be less than 0.04% v/v. One of the reasons for higher toxicity (and lower value of LOEC) observed in the present study compared to the Nazir et al. (2003) may be the lower sensitivity of *D. melanogaster* eggs to DMSO since the eggs do not actively feed on the exposure media thus the cumulative toxicity during same exposure time period is lower. Therefore, a more realistic scenario would be to start the exposure during the larval stage, as it was performed in the present study.

Conclusion

The probit analysis showed that 12 days LOEC was 0.04% v/v, NOEC was < 0.04% v/v and LC_{50} was 0.42% v/v. Pupal mortality reached maximum at 0.97% v/v of DMSO in substrate while concentration greater than 0.32% v/v reduced hatchability of adults at the end of lifespan. These results indicate that DMSO is more toxic to *D. melanogaster* larvae than it was initially thought. Obtained LC_{50}, LOEC and NOEC values for DMSO in this experiment should be considered seriously in further toxicity testing with *D. melanogaster* larvae as experimental model.

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