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Reduced mating activity and fitness of *Drosophila ananassae* on exposure to valproic acid.

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Abstract

Over the last few decades, *Drosophila* has been used as model for the study of toxic effect of drugs. The drugs play major implication as stress molecules that reduce the overall fitness in general. The present study is aimed to address the exposure of *D. ananassae* to variable doses of Valproic acid an anti epileptic drug (AED). In order to ascertain the biological potentiality in terms of life history traits such as mating propensity, fecundity, fertility and life span mating propensity, fecundity, fertility and life span. Interestingly the observation reveals that flies exposed to the higher dose of VAL have experienced increased courtship duration with low fecundity and fertility, while the differences are insignificant for longevity.

Key words: Valproic acid, *D. ananassae*, Mating propensity, Fecundity, Fertility, life span.

Introduction

Valproic acid (VAL) is an anticonvulsant drug used in the treatment of epilepsy and bipolar disorder, across AEDs, valproate was associated with the highest risk of reproductive toxicity. VAL has the highest risk of birth defects of any of the commonly used antiepilepileptic drugs. However, some epilepsy can only be controlled by valproate, and also the risk of birth defects with valproate is two to five times higher than other frequently used anti-epileptic drugs (Sander, 2010).

D. ananassae is used as a model organism for genetic studies because of its excellent viability, high mutability, and certain peculiarities in its cytological and genetic behavior. With the progress of research, it has become clear that it is unique among the species in the genus *Drosophila* (Singh and Chatterjee, 1985; Singh, 2010; Sisoda and Singh, 2006, 2009). *Drosophila* has emerged as one of the most powerful models for human diseases and toxicological research (Chowdhuri *et al*., 2005). VAL can affect each life stage in a different manner and may depend on a specific stage of life history traits. Pharmacological tools have introduced behavioral alterations in *Drosophila* reminiscent of human behavior. The fly can effectively be used for low- to high-throughput drug screens (Pandey and Nichols, 1982).

Materials and Methods

The fly stocks are routinely cultured in standard wheat cream agar medium. The test flies are cultured in wheat cream agar media along with the different concentrations of epileptic drug valproic acid (VAL) 0.2, 0.3, and 0.4 mg/ml. Both control and treated flies were cultured in un-crowded conditions at 22±1ºC (rearing temperature) and a relative humidity of 70%.

Antiepileptic drug – Valproic acid (*VAL*)

Valproic acid 98% (CAS no: 1069-66-9) was obtained from Sigma-Aldrich, soluble in water added to media. Standardization of lethal concentration was carried out on adult mortality for seven days and sub lethal (0.2 and 0.3 mg/ml) and lethal doses (0.4 mg/ml) were used to treat the flies (Mohammed *et al.*, 2009).

Experimental Crosses

D. ananassae virgin females and unmated males were collected and reared separately for 2 days. Further these flies were fed on wheat cream agar media with different concentrations (0.2, 0.3, and 0.4 mg/ml) of valproic acid and alongside control flies, fed on media for three days.

Four sets of crosses were made using about 30 pairs of flies for each cross, facilitating single pair mating, *i.e*., each pair in a separate vial. These crosses include untreated male × untreated female (Control-C), treated male × untreated female (T_1), untreated male × treated female (T_2), and treated male × treated female (T_3). A total of 120 pairs of flies were used to study mating propensity (courtship duration and copulation duration), reproductive fitness (fecundity and productivity) (Sisoda and Singh, 2009), and longevity (Luckinbill and Clare, 1985).

The mating propensity, *i.e*. courtship duration and copulation duration, was observed from 7 am to 9 am as maximum mating occurs during morning hours (Hegde and Krishna, 1997). Soon after mating, males from each pair were separated and females transferred into separate vial containing fresh food medium. Fecundity was assayed by counting number of eggs laid. Flies were successively transferred into fresh vials containing media every alternate day for 6 days. Eggs were allowed to hatch, and dilute yeast was added until pupation. Further, the same sets of vials were assessed for the emergence of the adult flies and likewise the fertility was recorded for the total productivity (Harini and Ramachandra, 2007). In addition to this, the treated and untreated flies were maintained until death to record lifespan of the flies.

Statistical Analysis

One-way ANOVA was performed for the said life history parameters, namely courtship duration, copulation duration, fecundity, fertility, and longevity. Post-hoc Duncan's multiple range test (DMRT) was conducted to record the significant differences. The analysis was performed using the statistical presentation system software package SPSS 15.0 for MS Windows.

Results

Mating propensity

Figure 1a reveals that the exposure to higher doses the courtship time taken compared to low dose was significantly more and this was similar for both treated females and treated males, *i.e*., T_1, T_2, and T3 than C significant value for sublethal dose $P < 0.001$ and for lethal dose is $P < 0.001$

(Table 1). Insignificant difference in copulation duration was determined (Figure 1b) between control and treated trials in different concentrations.

Table 1. Mean mating propensity of *D. ananassae* treated with Valproic acid

Traits→	Courtship Duration			Copulation duration		
Trials*↓ Doses→	0.2	0.3	0.4	0.2	0.3	0.4
C	5.40 ± 0.54	4.80 ± 0.55	4.80 ± 0.48	4.30 ± 0.98	4.20 ± 0.29	4.30 ± 0.30
T_1	4.34 ± 0.45	7.60 ± 0.70	18.90 ± 2.12	4.30 ± 0.94	4.40 ± 0.37	4.20 ± 0.35
T_2	6.10 ± 0.60	8.80 ± 1.11	21.80 ± 1.94	4.10 ± 0.99	3.82 ± 0.41	3.80 ± 0.41
T_3	5.80 ± 0.42	11.20 ± 1.22	32.80 ± 2.47	4.44 ± 0.88	3.80 ± 0.32	3.28 ± 0.32
ANOVA	F = 115 d.f. = 3,116 P > 0.951	F = 7.98 d.f. = 3,116 P < 0.001	F = 3.302 d.f. = 3,116 P < 0.001	F = 0.229 d.f. = 3,116 P < 0.876	F = 0.717 d.f. = 3,116 P > 0.548	F = 0.564 d.f. = 3,116 P > 0.648
DMRT	C/T_1, C/T_2, T_1/T_2, $T1/T_3$, T_2/T_3	C/T1, C/T_2, C/T_3, T_1/T_2, T_1/T_3, T_2/T_3	C/T_1, C/T_2, C/T_3, T_1/T_2, T_1/T_3, T_2/T_3	C/T_3, T_1/T_2, T_1/T_3, T_2/T_3	C/T_1, C/T_2, C/T_3, T_1/T_2, T_1/T_3	C/T_2, C/T_3, T_1/T_2, T_1/T_3,

*Note: C- Untreated♂ x Untreated♀; T_1-Treated♂ x Untreated♀; T_2- Untreated♂ x Treated; T_3-Treated♂ x Treated♀

Fecundity

The mean fecundity (Table 2) indicates significant reduction in mid dose $P < 0.001$ and high dose $P < 0.001$ of T_3 than T_2 and T_1. When compared to control, fecundity has been reduced in T_1 and T_2 while differences are insignificant at low dose for both treated and control (Figure 2a).

Fertility

Table 2 indicates that the fertility has reduced significantly in mid and high dose treatments. Reduction in the fertility was observed for 0.3 mg/ml and 0.4 mg/ml, $P< 0.001$ and $P < 0.001$, respectively. There was no difference between low dose and controlled treatments. A significant difference was observed in higher dose in T3. A difference was also seen in high dose treatment of T_1 and T_2 than the control experimental trial (Figure 2b).

Longevity

Treated and controlled flies in Figure 2c have not shown differences in lifespan with all three concentrations. Thus, the present study shows that the exposure of flies to valproic acid at variable concentration has left no effect on the lifespan.

Discussion

Fertility is dependent on sexual activity, mating season, and on semen quality. Very few studies have addressed the issue of sexual activity in animals after AED treatment; however, it was reported that sexual desire was reduced in rats treated with VAL at very low doses (Tauboll *et al.,* 2008). The present study confirms that time taken to court the females is maximum in treated flies

Table 2. Mean life history traits of *D. ananassae* treated with Valproic acid.

Traits→ / Trials*↓ Doses→	Fecundity			Fertility			Longevity		
	0.2	0.3	0.4	0.2	0.3	0.4	0.2	0.3	0.4
C	102.40 ± 2.37	98.02 ± 2.73	104.02 ± 2.71	96.80 ± 2.77	98.50 ± 3.14	101.40 ± 2.87	85.06 ± 0.462	89.24 ± 0.802	92.64 ± 0.262
T_1	101.70 ± 4.08	99.60 ± 3.95	98.80 ± 2.86	96.90 ± 3.86	94.30 ± 4.18	76.40 ± 2.56	88.02 ± 0.321	84.24 ± 0.654	82.10 ± 0.942
T_2	95.70 ± 3.66	95.50 ± 4.70	94.10 ± 5.32	90.50 ± 3.95	87.60 ± 4.25	68.60 ± 4.25	82.34 ± 0.824	79.28 ± 0.464	86.98 ± 0.246
T_3	96.30 ± 2.94	86.80 ± 3.16	67.70 ± 3.39	94.17 ± 2.76	65.20 ± 4.11	43.20 ± 5.04	84.12 ± 0.234	89.68 ± 0.456	84.02 ± 0.102
ANOVA	F = 2.016	F = 4.348	F = 17.962	F = 1.617	F = 14.090	F = 14.083	F = 1.265	F = 2.454	F = 0.986
	d.f. = 3,116	d.f. = 3,116	d.f. = 3,116	d.f. = 3,116	d.f. = 3,116	d.f. = 3,116	d.f. = 3,116	d.f. = 3,116	d.f = 3,116
	P > 0.129	P < 0.001	P < 0.001	P > 0.202	P < 0.001	P < 0.001	P > 0.268	P > 0.432	P > 0.864
DMRT	C/T_1, C/T_2, C/T_3, T_1/T_2, T_1/T_3, T_2/T_3	C/T_2, C/T_3, T_1/T_2 T_1/T_3, T_2/T_3	C/T_1, C/T_2, C/T_3, T_1/T_2, T_1/T_3, T_2/T_3	C/T_2, C/T_3, T_1/T_2, T_1/T_3, T_2/T_3	C/T_1, C/T_2, C/T_3, T_1/T_2, T_1/T_3, T_2/T_3	C/T_1, C/T_2, C/T_3, T_1/T_2, T_1/T_3, T_2/T_3	C/T_1, C/T_2, C/T_3, T_1/T_2, T_1/T_3, T_2/T_3	C/T_1, C/T_2, T_1/T_2, T_1/T_3, T_2/T_3	C/T_1, C/T_2, C/T_3, T_1/T_2, T_1/T_3, T_2/T_3

*Note: C- Untreated♂ x Untreated♀; T_1-Treated♂ x Untreated♀; T_2- Untreated♂ x Treated♀; T_3 -Treated♂ x Treated♀

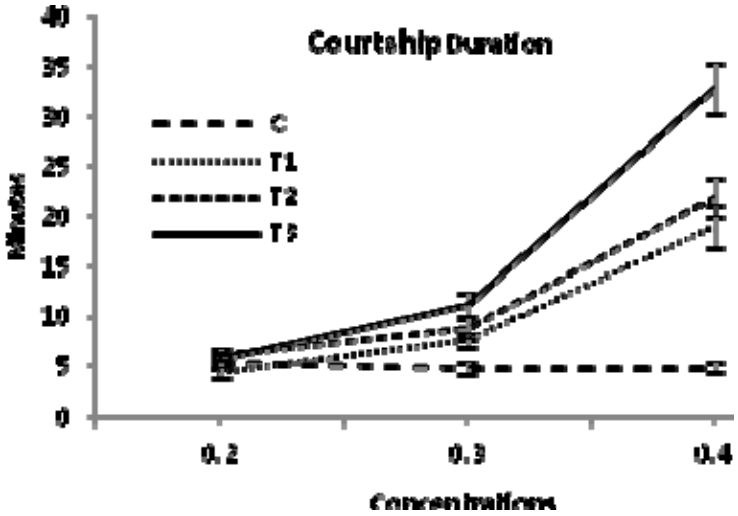

Figure 1a. Mean ± SE of courtship duration for three concentrations of valproic acid in different experimental crosses: C, T_1, T_2, and T_3.

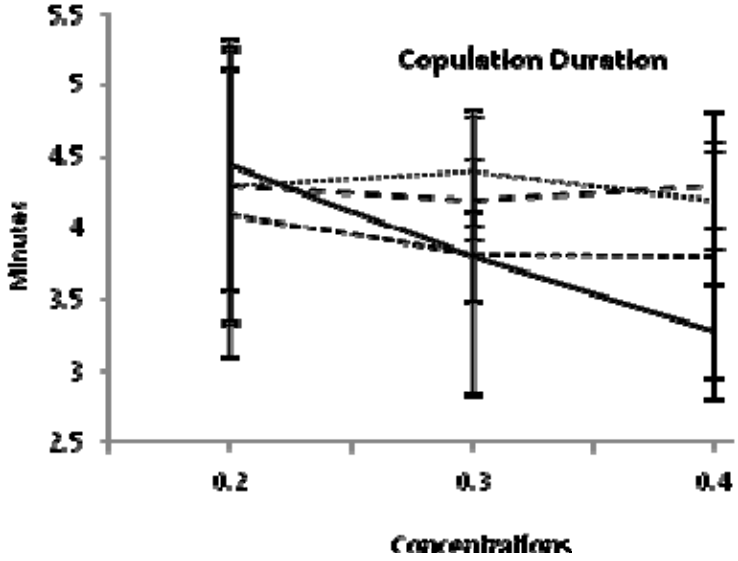

Figure 1b. Mean ± SE of copulation duration for three concentrations of valproic acid in different experimental crosses: C, T_1, T_2, and T_3.

indicated in Figure 1a than control in 0.3 mg/ml and 0.4 mg/ml of drug, while a difference was not observed between 0.2 mg/ml and control. The differences were insignificant for copulation duration between treated and control flies (Figure 1b) for all the three concentrations. It has been claimed that fertility rates have been reduced after long-term treatment with different AEDs; fertility rates in rats after 60 days low-

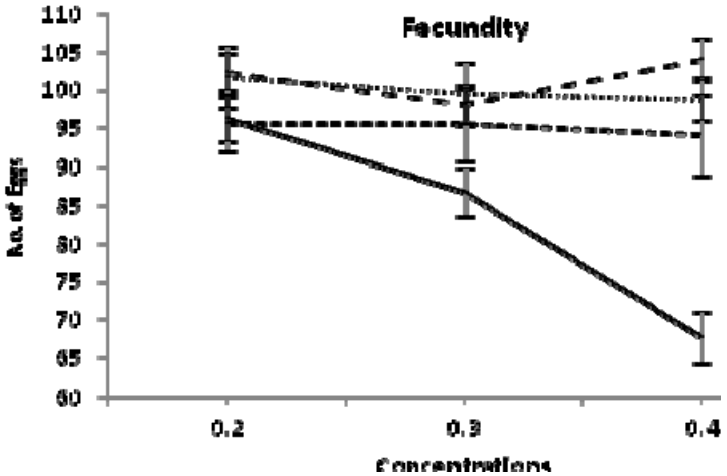

Figure 2a. Mean ± SE of fecundity for three concentrations of valproic acid in different experimental crosses: C, T_1, T_2, and T_3.

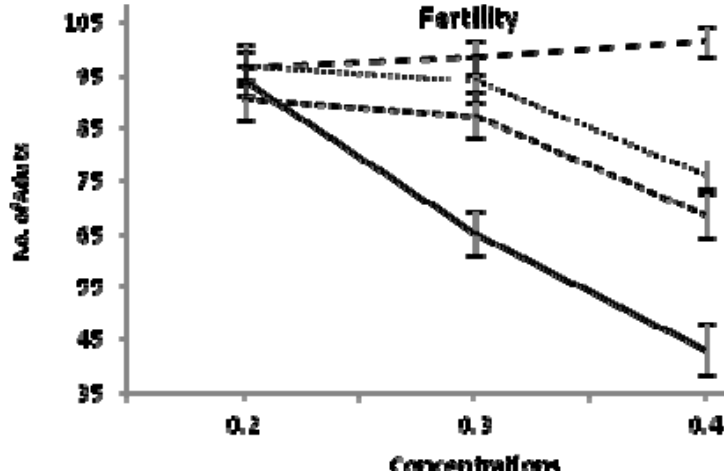

Figure 2b. Mean ± SE of fertility for three concentrations of valproic acid in different experimental crosses: C, T_1, T_2, and T_3.

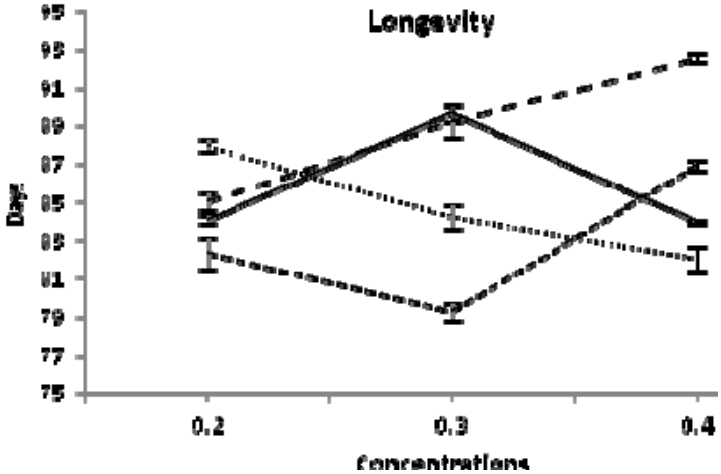

Figure 2c. Mean ± SE of longevity for three concentrations of valproic acid in different experimental crosses: C, T_1, T_2, and T_3.

dose drug treatment with valproate were reduced from 90% in controls to 40% and 30%, respectively. A significantly reduced fecundity and fertility rate was also observed for valproic acid in *D. ananassae* with increased concentration of valproic acid whether male or female is treated (Figures 2a-2b). Valproic acid is more commonly associated with reproductive endocrine disorders characterized by an ovulatory dysfunction than some other AEDs (Morrell *et al*., 2002; Betts *et al*.,

2003). A decrease in the prostate weight was found in the valproic acid treated rats. Sperm content and motility were decreased and the fertility rate diminished by 60%. It is important to remember that valproic acid has been found in the semen of treated rabbits and that chronic toxicity studies in animals showed testicular damage, including degeneration of the interstitial cells (Cohn *et al.,* 1982). VAL caused a significant effect on steroidogenesis in both unstimulated and gonadotropin-stimulated porcine ovarian follicular cells. These findings showed a direct effect of VPA on steroidogenesis, independent of epileptic activity (Aktas *et al.,* 2010).

The present study implies that the males and females of *D. ananassae* are prone to toxic effects with lesser reproductive ability. Fecundity and fertility are the fitness parameter that are used to assess the fitness in different species of *Drosophila*. Valproic acid caused significant effects on mating activity and reproductive fitness with the increase of courtship duration led to significant reduction in fecundity and fertility of treated flies. Thus fecundity and productivity of treated flies showed contrasting results with mating propensity when compared to the control with increased concentration of drug. Courtship duration is less in control and increased for copulation duration. An insignificant difference was observed for copulation duration for both treated and control experimental trial but maximum time consumed for courtship in treated trials. Fecundity and fertility are reduced in treated flies compared to control, while the differences for lifespan are insignificant in both control and treated flies. Statistical analysis and DMRT showed significant differences in mating activity and fitness parameters. Thus, the cost of reproductive success has been significantly reduced with the use of antiepileptic drug valproic acid, but it has left least adverse effect on life span. Therefore, the effect of toxicity on life history traits is dose dependent.

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