The Bioscan 7(1) : 31-34, 2012
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

ADVERSE EFFECT OF VALPROIC ACID ON MATING BEHAVIOUR AND FERTILITY IN *DROSOPHILA MELANOGASTER*

D. S. GAYATHRI[1] AND B. P. HARINI*
Department of Zoology, Jnana Bharathi, Bangalore University, Bangalore - 560 056, INDIA
[1]Centre for Applied Genetics, Jnana Bharathi, Bangalore University, Bangalore - 560 056, INDIA
E-mail: dns.gayathri@gmail.com

KEY WORDS
D. melanogaster
Fecundity
Fertility
Mating propensity
Valproic acid

Received on :
19.12.2011

Accepted on :
07. 02.2012

***Corresponding author**

ABSTRACT
Drosophila melanogaster, the model organism that has been widely used to study various diseases and also the adverse effects. *D. melanogaster* can be extrapolated to human system which could facilitate the effect of drugs on reproduction. Valproic acid is a major antiepileptic drug with a broad spectrum of antiepileptic activity, the drugs given to treat epilepsy alongside therapeutical use, also has a lot of side effects which mainly effects reproduction. In view of this, the present study is aimed to address the effect of valproic acid on *Drosophila* life history traits. Interestingly, the observation reveals that flies exposed to the higher dose of VAL have experienced significant increase in courtship duration with decreased copulation duration, fecundity and fertility, while the longevity has not been affected on exposure to VAL at any experimental dose. Thus the result implies significant effect of valproic acid on reproduction.

INTRODUCTION

Epilepsy, the most common seizure disorder, is a chronic condition that briefly interrupts the normal electrical activity of the brain to cause unpredictable and recurrent seizures (Fisher, 2010). Valproic acid (VPA) is the most frequently used antiepileptic drug (Sander, 2009). Valproic acid is a broad spectrum AED effective over the complete range of seizure types. The use of antiepileptic drugs (AEDs) can lead to decreased fertility and increased incidence of reproductive endocrine disorders in both men and women (Lofgren, 2007). In women menstrual disorders reflecting ovulatory dysfunction are affected which reduces fertility (Isojärvi *et al.,* 2005). AEDs may reduce sperm motility, induce sperm abnormalities and decrease testicular volume (Roste *et al.,* 2003; Isojarvi *et al.,* 2004). Reasonable evidence indicates valproate is a significant teratogen during therapeutic use and there is an urgent need to evaluate the risks of these drugs (Barrett and Richens, 2003). Valproic acid is associated with the occurrence of polycystic ovary syndrome when used in young adulthood or adolescence. Once a molecule is identified as a potential drug, the detection of adverse drug reactions is one of the key components of drug development. Due to immense complexity of higher organisms, systems biology approaches are however currently focused on simpler organisms (Joyce and Palsson, 2006). Thus, the use of *D. melanogaster* to screen for reproductive adverse drug reactions using non-mammalian model. *D. melanogaster* has many similarities to the mammalian reproductive system, including putative sex hormones and conserved proteins involved in genitourinary development. Furthermore, the *D. melanogaster* model would present significant advantages in time efficiency and cost-effectiveness compared with mammalian models (Avanesian *et al., 2009*). The *Drosophila* model offers an excellent system to efficiently screen agents with potential therapeutic (Sharma *et al*., 2009). Functional analysis of human disease genes including high-throughput pharmacological screens as well as behavioral assays have become available in *D*. melanogaster (Jeibmann and Paulus, 2009).

To understand the toxic effect of valproic acid on life history traits the drug was administered to adult flies and reproductive toxicity was evaluated on the basis of mating propensity, the number of eggs laid, adult's eclosed and longevity in treated flies.

MATERIALS AND METHODS

Antiepileptic drug - Valproic acid (VAL)

Valproic acid 98% (CAS no: 1069-66-9) was obtained from Sigma-Aldrich, soluble in water and added to wheat cream agar media. The modified protocol of Mohammad (2009) has been used for drug standardization. Standardization of lethal concentration was carried out on adult mortality for seven days and low dose (0.2mg/mL), mid dose (0.3mg/mL) and high dose (0.4mg/mL) were used to treat the flies.

Experimental crosses

The fly stocks were routinely cultured in standard wheat cream agar medium. *D. melanogaster* virgin female and unmated

male were collected and reared separately for 2 days. Further these flies were fed on wheat cream agar media with different doses (0.2, 0.3 and 0.4 mg/mL) of valproic acid and alongside control flies, fed for three days. Both control and treated flies were cultured in un-crowded conditions at 22 ± 1ºC (rearing temperature) and a relative humidity of 70%.

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 x untreated female (Control-C), treated male x untreated female (T_1), untreated male x treated female (T_2) and treated male x 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) (Harini and Ramachandra, 2007) and longevity.

The mating propensity *i.e.*, courtship duration and copulation duration was observed from 7 to 9 am as maximum mating occurs during morning hours. Soon after mating, males from each pair were separated and a female was transferred into separate vial containing fresh media. 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 till 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**.** In addition to this, the treated and untreated flies were maintained till 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

Table1. represents the mean mating propensity on exposure to three different doses of valproic acid. The time taken to court the female is significant in high dose ($p < 0.003$) and time taken to court was increased in T_3 (20.78min) in which both sexes are treated while in T_2 trail mean time was 14.49 minutes but in low and mid dose the courtship time remains insignificant even when both males and females treated trail (T_3).

The significant copulation duration was observed for 0.4mg/mL ($p < 0.001$) and mean time in T_3 (16.35 min), T_2 (18.67min) and T_1 (17.28min) while, results were insignificant for trails of 0.2mg/mL and 0.3mg/mL of valproic acid exposure with $p > 0.738$ and $p > 0.761$ respectively. The copulation duration between both treated sexes was significantly decreased when compared with either the treated male or treated female were used in experimental crosses.

Life history traits

The mean life history traits were observed on exposure to different doses of valproic acid is represented in Table 2. The fecundity and fertility were found to be reduced in T_3 treated trail for all the doses of drug assessed. The egg production and adult emergence have shown contrasting result to the mating propensity. The significant differences observed among T_3, T_2 and T_1 experimental trials for fecundity ($p < 0.012$), fertility ($p > 0.002$) with high dose, according to ANOVA.

Fecundity was significantly reduced in 0.4mg/mL and when compared to T_1 trail (140.61eggs) and T_2 trail (154.35), T_3 trail shows decreased fecundity (112.41eggs) in which both males and females were treated. But significant difference also exists between low dose (180.03 eggs) and mid dose (156.70 eggs) for T_3 trail. Fertility was significantly reduced in high dose for all treated trails T_1 (123.34 adults), T_2 (131.03 adults) and T_3 (92.56 adults) have shown in fig 2b. The number of adults eclosed was significantly less since treated males and treated females were involved in cross (T_3) of high dose, while slightly reduced in 0.2mg/mL (164.38 adults) and 0.3mg/mL (144.10 adults). Statistical analysis showed significant difference in mating propensity, number of eggs laid and number of adult's eclosed between different treated trails with high dose.

There was no difference in longevity of both control and treated flies of all the three doses and in all experimental crosses.

DISCUSSION

Valproate has a significant influence on reproductive endocrine hormones in non-epileptic animals of both genders (Craig, 2009). Very few studies have addressed the issue of sexual activity in animals after AED treatment. Few studies reported that sexual desire was reduced in rats treated with

Table 1: Mean and S.E of effect of Valproic acid on mating behavior of *Drosophila melanogaster*

Traits→	Courtship duration			Copulation duration		
Doses→ 0.2	0.3	0.4	0.2	0.3	0.4	
Trails*↓						
C	5.01 ± 0.55	5.26 ± 0.55	5.40 ± 0.55	23.80 ± 0.81	24.80 ± 0.81	20.80 ± 0.81
T_1	5.00 ± 0.63	7.58 ± 0.58	14.49 ± 1.20	20.60 ± 0.95	21.80 ± 0.92	17.28 ± 1.23
T_2	6.50 ± 0.85	7.20 ± 0.82	9.80 ± 0.89	22.60 ± 0.12	20.00 ± 0.44	18.67 ± 0.74
T_3	5.86 ± 0.93	6.00 ± 0.93	20.78 ± 1.19	20.26 ± 0.98	19.78 ± 0.86	16.35 ± 1.32
ANOVA	F = 1.969d.f=3, 116P > 0.136	F = 2.012d.f = 3, 116P > 0.130	F = 5.70d.f = 3, 116P < 0.003	F = 0.422d.f = 3, 116P > 0.738	F = 0.389d. f = 3, 116P > 0.761	F = 2..250d.f = 3, 116P < 0.001
DMRT	C/T_2, T_1/T_2, T_3/T_2 T_1/T_3, T_2/T_3	C/T_1, C/T_2, C/T_3, T_2,T_1/T_3, T_2/T_3	C/T_1,C/T_2, C/T_3,T_1/ T1/T2, T_2/T_3	C/T_1,C/T2,C/T_3, 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

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

Table 2: Mean and S.E of effect of Valproic acid on Reproductive fitness of *Drosophila melanogaster*

Traits→	Fecundity			Fertility			Longevity		
Doses→ / Trails*↓	0.2	0.3	0.4	0.2	0.3	0.4	0.2	0.3	0.4
C	186.50 ± 2.43	180.50 ± 2.43	182.50 ± 1.23	180.30 ± 3.10	178.30 ± 3.10	179.01 ± 0.97	89.12 ± 1.63	93.14 ± 1.28	86.30 ± 0.36
T_1	180.70 ± 3.35	177.50 ± 3.61	140.61 ± 5.73	172.90 ± 4.34	166.30 ± 5.67	123.34 ± 4.08	93.56 ± 0.86	88.94 ± 1.97	89.20 ± 1.87
T_2	171.70 ± 5.01	174.20 ± 4.49	154.35 ± 5.57	161.80 ± 4.79	158.40 ± 4.19	131.03 ± 4.34	87.04 ± 1.76	85.28 ± 1.49	86.40 ± 0.43
T_3	180.03 ± 4.30	156.70 ± 6.42	112.41 ± 5.03	164.38 ± 4.78	144.10 ± 7.02	92.56 ± 5.52	90.14 ± 1.43	90.76 ± 0.65	92.56 ± 1.74
ANOVA	F = 1.789d.f=3, 116P > 0.167	F = 0.575d.f=3, 116P > 0.635	F = 7.298d.f=3, 116P < 0.012	F = 1.299d.f=3, 116P > 0.290	F =0143d. f=3, 116P > 0.934	F =4.910d.f=3, 116P < 0.002	F =0.802d.f=3, 116P > 0.103	F =0.675d.f=3 , 116P > 0.342	F=1.232d.f=3, 116P >0.421
DMRT	C/T_1, C/T_2, C/T_3, T_1/T_2, 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_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, C/T_3, T_2/T_1, T_2/T_3, T_3/T_1	C/T_1, C/T_2, C/T_3, T_2/T_1, T_2/T_3, T_3/T_1	C/T_1, C/T_2, C/T_3, T_2/T_1, T_2/T_3, T_3/T_1

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

VPA at very low doses (Cohn *et al.*, 1982). Invertebrate models like *Drosophila melanogaster* would provide useful insights into the mechanisms of drug action (Wolf and Heberlein, 2003). Interestingly significant increase in courtship duration was observed for high dose ($p < 0.003$) experimental trails (T_1 and T_3) in which treated male is involved rather than treated female. While courtship duration is not affected for low and mid dose. Copulation duration was decreased in 0.4mg/mL of all the treated trails where both sexes are treated (T_3) or either of the sex is treated (T_1 and T_2) trails as the doses were increased. Thus the present observation has revealed that the lethal dose exposure of *D. melanogaster* to valproic acid shows reduction in mating propensity.

The risk of reproductive endocrine disorders is higher in men with epilepsy treated with AEDS. Men on AEDS have been reported to have more sperm abnormalities and lower sperm motility, and also fertility rates have been reduced after long-term treatment with different AEDs (Isojarvi *et al.*, 1990). Antiepileptic medication affects reproductive endocrine function, but its impact on fertility is not well known (Arthma, 2007). The number of children was lower in patients on AEDS than in untreated patients, or subjects without epilepsy. Women with epilepsy, particularly those taking AEDs, are at increased risk for endocrine dysfunction and infertility. Recent studies suggest that the prevalence of birth defects might be higher with exposure to valproate (Kaplan, 2004). The present study conducted on *D. melanogaster* model has also proved that the treated males and females are prone to toxic effects with lesser reproductive fitness. Fecundity and fertility has shown reduction in treated trails (T_1, T_2 and T_3) therefore, reduction in the mating behavior reduces the productivity with increased dose. Longevity in *Drosophila* was found to be increased when treated with 4-phenylbutyrate (PBA) throughout adulthood, without diminution of locomotor vigor, resistance to stress, or reproductive ability and also in *C. elegans* when treated with anticonvulsant medicines (Kang *et al.*, 2002). The data provides insignificant values for longevity in the valproic acid treated flies when compared to control.

Thus, the cost of reproductive success has been significantly reduced with the use of antiepileptic drug VAL, but it has no adverse effect on life span**.** The present study confirms that the toxic effect of valproic acid on the life history parameters with significantly decreased values. Thereby the result reveals that the life history traits are affected by valproic acid is dose dependent. In addition to this, the range of effect of valprioc acid toxicity as per DMRT is as follows for all the life history parameters $T_3 > T_1 > T_2 > C$.

Therefore, a critical need for systematic evaluation at the preclinical level is not only the anticonvulsant effects to be studied, but also the side effects induced by such novel drugs are also the facts to be considered for the better health.

ACKNOWLEDGEMENT

The authors are thankful to Centre for Applied Genetics, Bangalore University, Bangalore for providing research facilities.

REFERENCES

Arthma, M. 2007. Reproductive health of patients with epilepsy: birth rate and malformations in offspring. *Academic Dissertation, Tampere School of Public Health, University of Tampere, Finland.*

Avanesian, A., Semnani, S. and Jafari, M. 2009. Can *Drosophila melanogaster* represent a model system for the detection of reproductive adverse drug reactions? *Drug Discovery Today*. **14(15-16):** 761-766.

Barrett, C. and Richens, A. 2003. Epilepsy and pregnancy: report of an Epilepsy Research Foundation workshop. *Epilepsy Res.* **52:** 147-87.

Cohn, D. F., Homonnai, Z. W. and Paz, G. F. 1982. The effect of anticonvulsant drugs on the development of male rats and their fertility. *J. Neurol Neurosurg Psychiatry.* **45:** 844-846.

Craig, J. J. 2009. Epilepsy and women, in *Epilepsy: From benchside to bedside. A practical guide to epilepsy* edited by J W Sander, M C Walker and J E Smalls (International League Against Epilepsy (UK Chapter) and The National Society for Epilepsy). Chapter 44.

(http://www.epilepsysociety.org.uk/Forprofessionals/Articles/Specialgroups/main_content/Chapter44Craig.pdf)

Fisher, R. 2010. Definition of epilepsy, in *Overview of Epilepsy*. 2.

Harini, B. P. and Ramachandra, N. B. 2007. Newly evolved races of *nasuta- albomicans* complex of *Drosophila* live better than their

parents as revealed by life-history traits analysis at three different temperatures. *Curr. Sci.* **93:** 348-356.

Isojarvi, J. I. T. Lofgren, E., Juntunen, K. S. T., Pakarinen, A. J., Paivansalo, M., Rautakorpi, I. and Tuomivaara, L. 2004. Effect of epilepsy and antiepileptic drugs on male reproductive health. *Neurology*. **62:** 247-253.

Isojarvi, J. I. T., Pakarinen, A. J., Ylipalosaari, P. J. and Myllyla, V. V. 1990. Serum hormones in male epileptic patients receiving anticonvulsant medication. *Arch. Neurol.* **47:** 670-676.

Isojarvi, J. I. T., Tauboll, E. and Herzog, A. G. 2005. Effect of antiepileptic drugs on reproductive endocrine function in individuals with epilepsy. *CNS Drugs*. **19:** 207-223.

Jeibmann, A. and Paulus, W. 2009. Drosophila melanogaster as a model organism of brain diseases. *Int. J. Mol. Sci.* **10:** 407-440.

Joyce, A. R. and Palsson, B. O. 2006. The model organism as a system: integrating 'omics' data sets *Nat. Rev. Mol. Cell. Biol.* **7(3):** 198-210.

Kang, H. L., Benzer, S. and Min, K. 2002. Life extension in Drosophila by feeding a drug, *PNAS*. **99(2):** 838-843.

Kaplan, P. W. 2004. Reproductive health effects and teratogenicity of antiepileptic drugs. *Neurology*. **63(10:4):** S13-23.

Lofgren, E. 2007. Effects of epilepsy and antiepileptic medication on reproductive function. Academic Dissertation. *University of Oulu, Finland.*

Mohammad, F., Singh, P. and Sharma, A. 2009. A *Drosophila* systems model of pentylenetetrazole induced locomotor plasticity responsive to antiepileptic drugs. *BMC Systems Biology*. **311:** (doi:10.1186/1752-0509-3-11)

Roste, L. S., Tauboll, E., Haugen, T. B., Bjornenk, T., Saetre, E. R. and Gjerstad, L. 2003. Alterations in semen parameters in men with epilepsy treated with valproate or carbamazepine monotherapy, *Eur. J. Neurol.* **10 (5):** 501–506.

Sander, J. W. 2009. Overview of established antiepileptic drugs. in *Epilepsy : From benchside to bedside. A practical guide to epilepsy*, J. W. Sander, M. C. Walker and J. E. Smalls Eds, (International League against Epilepsy (UK Chapter) and The National Society for Epilepsy), Chapter 28.

Sharma, A., Mohammad, F. and Singh, P. 2009. Gender differences in a *Drosophila* transcriptomic model of chronic pentylenetetrazole induced behavioral deficit. *Nature Proceedings*. hdl:10101/npre.2009.3460.1

Wolf, F. W. and Heberlein, U. 2003. Invertebrate models of drug abuse. *J. Neurobiol.* **54(1):** 161-178.