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EFFECT OF LAMOTRIGINE-ANTIEPILEPTIC DRUG ON LOCOMOTOR ABILITY IN *DROSOPHILA MELANOGASTER*

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KEYWORDS

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ABSTRACT

Drosophila is a suitable model organism in the field of behavioral science. The objective of this study was to explore the effect of lamotrigine, an antiepilepticdrug on behavior (locomotion) of *Drosophila melanogaster*. Lamotrigine is a commonly used anticon vulsant with a profile of adverse-effects. The *Drosophila* model hassensed an inverse relationship between metabolic rate, and locomotion. Interestingly, the behaviour traits observed generally decreased with increasing dose of Lamotrigine in *Drosophilame lanogaster*. Dose dependent action of Lamotrigine has an important role in regulating behaviour which is use full for understanding the multiple effects on Behaviour and health.The observation revealed that the survival benefits displayed by this Drug would be associated with deleterious effects on health with reference to depression in locomotion. There by the use of lamotrigine has led to decreased locomotoractivity (Climbing ability).

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INTRODUCTION

Epilepsy is a chronic brain disorder in which spontaneous recurrent seizures with a prevalence of 0·5-1% in the developed world and an incidence of 70-80/100,000 per Year. A complex interaction between epileptic seizures during pregnancy, antiepileptic drugs (AED), and its adverse impacts on the developing fetus, congenital malformations are all being related (Arthma, 2007).

AED treatment options have improved significantly during the last two decades (Beghi and Perucca, 1995). Older AEDS include drugs introduced before the 1990s (Duncan *et al.*, 2006). Some of these drugs introduced are still widely used as a primary medical treatment of epilepsy, but many new AEDS have been introduced since, 1990 (Brazil and Padeley, 1998). The newer AEDS are mainly for the treatment of partial epilepsy and most of them are used as adjuvant medication with the older AEDS. However, some of them (lamotrigine, levetiracetam, gabapentin and Oxocarbamezepine) are also licensed as monotherapy (Brodie and Kwan, 2001).

Lamotrigine is the FDA approved most commonly used newer AED for treatment of bipolar I disorder. It is effective both as monotherapy and add on therapy for partial and generalized epilepsies andit effects sodium channels (Ettinger, 2006). The manufacturer reported teratology testing in mice, rats, and rabbits at oral doses. Maternal and fetal toxicity (delayed ossification and decreased weight) were seen at the high doses in the rodent studies (Micromedex® 2.0, 2011).

Drosophila melanogaster has been used as a model system in genetic and biochemical research. This is because it offers many advantages, including a short generation time of approximately 14 days and it is easy and economical to maintain in large quantities and under controlled conditions. Fruit flies also possess a relatively small genome with only four chromosomes and many easily observable mutant phenotypes. Recent advances in fruit fly biology and the development of resources such as the complete genome sequence, SNP databases and the availability of mutant lines have only increased the importance of *Drosophila* as a model organism. It also been widely used to study various diseases and also the adverse effects in *Drosophila melanogaster*can be extrapolated to human system.

At the forefront of behavioral genetics research, *D. melanogaster* has provided important insights into the molecular, cellular and evolutionary basis of behavior (Sokolowski, 2001). Simple behavioral assays are widely applicable for studying the role of genetic and environmental factors on fly behavior on exposure to few AEDs (Sharma *et al.*, 2010). The newly hatched adult fly will rapidly acquire characteristic behaviors of flight, chemotaxis, phototaxis, and geotaxis, foraging and mating (Truman *et al.*, 1993). In many cases the explicit circuits controlling visual (Ting and Lee, 2007), olfactory (Hallem and Carlson, 2004), mechanosensory (Kernan, 2007) and chemosensory (Stocker, 1994) inputs from the peripheral organs (eye, antennae, bristle organs and maxillary palps) have been mapped both physically and functionally. In addition, the central 'mushroom body' of the brain has been elucidated as a center for memory and conditioned behaviors.

Some of these well documented developmental and behavioral aspects of *Drosophila* make it an especially informative and adaptable model to investigate a

wide variety of toxicological endpoints relevant to human biology and behavior. Flies exhibit a wide array of behaviors relevant to understanding human response to environmental challenges. These behaviors include locomotion, circadian rhythm, sleep patterns, courtship and mating, aggression, and grooming. Many of these are under the control of genetic and molecular mechanisms in Drosophila (Sokolowski, 2001; Greenspan and Dierick, 2004). Furthermore, at a physiological level the underlying neurotransmitter systems in the fly are conserved including serotonin, dopamine, GABA, glutamate and acetylcholine (Nichols, 2006).

To date, behavioral endpoints in Drosophila have been used primarily to isolate genes that specifically support a given trait rather than as a tool for screening vast numbers of chemicals (Moore *et al*., 1998). Locomotor activity is a complex behavior and different neural systems may influence in fly (Fleming and Copp, 1998). It can be assessed in transgenic and mutant flies through longevity assays, locomotor and climbing assays. Progressive locomotor decline can be observed in transgenic *Drosophila* through climbing assays (Greene *et al*., 2003). The duration of climbing is determined by Rapid Iterative Negative Geotaxis (RING) (startle induced vertical climbing) as an assay for evaluating the sedative effects of AEDs (Sharma *et al*., 2010). A climbing or negative geotaxis assay measuring the ability of the organisms to climb up the walls of a plastic vial was used and several genotypes or drug treatments can be tested for screening experiments (Nichols *et al*., 2012).

Climbing ability is a frequently used assay to measure locomotor activity in D. melanogaster model of Parkinson's disease (Todd and Staveley, 2004). The climbing response of wild-type flies remained essentially unchanged as reported by Feany and Bender (2000). The climbing response for subsequent anti-parkinson drug studies has an effective in decreasing fly locomotorfunction (Pendleton *et al*., 2000). The low levels of accumulated ethanol stimulate locomotion and high levels depress it (Heberlein *et al*., 2004). These behavioral assays are widely applicable for studying the role of genetic and environmental factors on fly behavior. In light of the above studies the present work determines the acute behavioral responses to commonly used conventional AED-lamotrigine with respect to adult geotaxis in *Drosophilame lanogaster* at different doses. The present study has been assessed for the dose response relationship between lamotrigineand their behavior in *Drosophilamelanogaster*.

MATERIALS AND METHODS

Antiepileptic drug

Lamotrigine (LTG) 3, 5-diamino-6-(2, 3-dichlorophenyl)-1,2,4-trigine was obtained from Sigma-Aldrich, soluble in Ethanol 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 (5mg/mL), mid dose (10mg/mL) and high dose (15mg/ml) were used to treat the flies.

Drosophila culture at variable doses of Lamotrigine

The *Drosophila melanogaster*stocks were obtained from National *Drosophila* Stock Centre, Mysore, India. The fly stocks were routinely cultured in standard wheat cream agar medium in uncrowded condition at 22 ± 1ºC (rearing temperature), 12 : 12h light and darkperiods and relative humidity of 70%. The test flies were cultured in wheat cream agar medium along withdifferent concentrations of the epileptic drug lamotrigine 5, 10 and 15 mg/mL of the medium.

Climbing ability

To record the climbing ability, Virgin females and unmated males of *Drosophilamelanogaster*, were isolated, collected and aged for 5days (Pendleton *et al*., 2002). All flies used in individual experiments were grown, collected and handled in parallel. Adult flies (5 days old) were aspirated, transferred to fresh food vials containing different doses of lamotrigine (5mg/ml, 10mg/mL and 15 mg/mL) and treated for 3 days (Gayathri and Harini, 2012). 20 flies were selected from treated and placed in a 100 mL glass graduated cylinder (length 25 cm and diameter 3 cm) to climb. The cylinder was sealed with parafilm at the top to prevent escape. The flies were gently knocked to the bottom of the cylinder and were allowed to climb for 30 sec. The number of flies crossing the 60 ml line (9 cm) was recorded. Ten such trials were conducted for each dose of lamotrigine. The locomotor activity of *Drosophila* without drug administration *i.e.*, control was compared to treated (modified protocol of Greene *et al*., 2003). The number of flies climbed in the given time for each dose was averaged for statistical analysis.

RESULTS

Climbing assay performed on adults of *Drosophila* exposed to different doses of lamotrigine is presented in Fig 1. The response to increase dose has resulted with decrease climbing activity of flies for all the three different doses of lamotrigine (5mg/mL, 10mg/mL and 15 mg/mL). The percentage of flies exhibiting about 50% negative geotaxis was observed at mid and high doses. At 10 and 15 mg/mL, the climbing responses were 54% and 40% in *D. melanogaster*was recorded.

DISCUSSION

The *Drosophila* flies were exposed to varying doses of lamotrigine-Antiepileptic drugs (AED).

For three days to determine its effect on behaviors. In preclinical studies on animals, AED produce acute adverse effects such as sedation, ataxia, tremor and impairment of motor coordination, disturbance in locomotor activity and alterations in skeletal muscular strength. Strength test is able to evaluate the acute adverse effect potential of AED at high (neurotoxic) doses with respect to the reduction of muscular strength (Zadroniak *et al*., 2009).

The animals exposed to PHT showed significant increase in locomotor activity measures. These results confirm a small but growing body of literature that demonstrates that PHT is a behavioral teratogen (Pizzi and Jersey, 1992). The observed mean values of locomotor activity were dose dependent and significantly different among different doses oflamotrigine exposure on *Drosophila*. The present study revealed that the climbing ability in *Drosophila melanogaster* is significantly varied on exposure to different doses of lamotrigine.

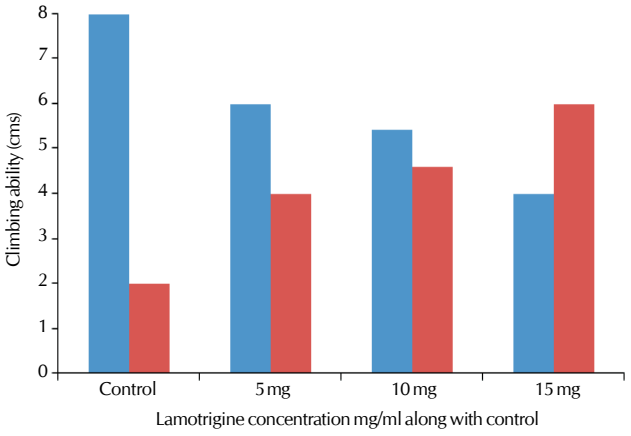

Figure 1: Mean (± SE) Climbing ability in *D.melanogaster* on exposure to lamotrigine

Theresponse to increase dose has resulted with decrease climbing activity of flies for all the three different doses of lamotrigine.The percentage of flies exhibiting about 50% negative geotaxis i.e., 54% and 40% was observed at mid and high doses respectively. Climbing activity was decreased at mid and high doses with less than 50% of the flies climbing within 30 sec while 95% of the flies climbing within 10 sec in control.

Interestingly, the behavioural traits observed were generally dose dependent. The nervous system, the most crucial system in the elicitation of behaviour, is formed during development by networks of interacting genes and the physiological structures necessary to generate these behaviour patterns. Despite the sources of complexity, the amount of research accomplished has pushed the fruit fly to the forefront of behavioural genetics research (Sokolowski, 2001). Dose dependent action of different AEDs produced a maximal effect on behaviours of Drosophila species and provides an efficient system to study genetic, neurological, and behavioural mechanisms mediating these effects. AED has an important role in regulating behaviour through metabolism; such studies should be useful for understanding the multiple effects on behaviour

So, from the foregoing discussion it is seen that lamotrigine is toxic to *Drosophila*. Therefore, the fruit fly offers remarkable biological similarity to humans, as a sophisticated genetic tool to screen drugs and drug targets. Lamotrigine exhibitsadverse health effects at high doses or after chronic use by humans and is lethal when added to the diet of *Drosophila*.

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