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Original Research Article

Effect of Amitriptyline hydrochloride an antidepressant on courtship and reproducibility of *D. melanogaster*

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A B S T R A C T

Depression is the most common form of a mental disorder and the tricycles antidepressants are of most commonly suggested drugs for treatment. Antidepressants work by controlling the balance of the neurochemistry in the brain. Epidemiological studies from several countries have provided evidence of marked toxicity for overdose and long term administration. The present study is an attempt to understand the toxic effect of this antidepressant on the behaviour and fitness of *Drosophila melanogaster*. Does this antidepressant have toxic effect on the reproductive behaviour and fitness of the individuals? To address this question, *Drosophila melanogaster* a potential genetic model has been selected. The reproductive behaviour of flies is well established and it perform definite pattern of species specific behavioural patterns. Of the several, behavioural patterns, Mating Latency (ML) and Courtship durations (CD) have been chosen to observe the effect of antidepressant and the same flies were utilized to test fecundity and the fertility. The results revealed that there is increase in the courtship duration and increase in the mating latency compared to control. The fitness of the treated individuals reduced to 30% compared to control.

K e y w o r d s

Antidepressant, *Drosophila*, amitriptyline, fecundity mating latency courtship duration

Introduction

Model genetic organisms represent intact living systems where complex biological pattern and process can be readily examined. Further, the similarity between action, behaviour and gene response in *D. melanogaster* and mammalian systems combined with the power of genetics have recently made the fly a very attractive system to study fundamental neuropharmocological process relevant to human disease. When the drug is introduced to the market although some routine preliminary tests are conducted before the release of these drugs for commercial use, their effect on behaviour may be included in preclinical tests. According to Nazari (2004) the antidepressant drugs namely fuoxetine and amitriptyline when administered affect the male sexual behaviour, further he confirmed the sex specific action is

continued may also affect the fitness of individuals if it is treated long time. The present project is an attempt to understand the effect of amitriptyline hydroxide on the behaviour and fitness of *Drosophila*. Studies analyzed that the effect of antidepressant on sexual dysfunction and spermatogenesis appear to be due to changes in hormones level such as testosterone, LH, FSH,, Prolactin, Estrogen (Clyton and Montejo, 2006).

Fitness is an ability of an organism to produce the progeny and to leave the individuals of the species to the next generation. In the neo Darwininan thinking, a successful species/individual is the one which is able to reproduce successfully leave more viable offspring's. Natural selection favour organisms with higher fitness. Fitness is a complex polygenic trait produced the phenotype they are influenced by many factors either external or internal. Affleck *et al.* (2006) have worked on methotreseate which have influence on the fecundity and development of *Drosophila*. Nadd *et al.* (2005) have reported that parathyroid insecticide imbalance the reproductive performance of *Drosophila*, Further, Diethyl sulffoxide (Nazir *et al.,* 2003) mercurial fungicide (Gayathri and Krishnamurthy, 1981) have reported to affect the reproductive performance of *Drosophila* (Saxena and Ahuja 1998). Hence they must be evaluated for their toxic and mutagenic effect in the organism Clinical reports have suggested that antidepressant medication may contribute to the sexual dysfunction experienced by some depressed patients (Kowaiski *et al.,* 1985; Nazari, 2011). Sexual dysfunction has long been noted as both a symptom of depressive illness and as a side effect of many of the medications used to treat depression. Although most people suffering from a major depressive illness would like to be sexually active, half experience a decrease in desire or sexual performance. Antidepressant medications often interfere with several parts of the sexual response (Nazari, 2011). Further, Benelli *et al.* (2004) opinion is that impairment of sexual activity is one of the most frequent side effects of antidepressant drugs. Van Sheik and Graft 1991 have isolated wing somatic mutations and recombination tests of *Drosophila melanogaster* to understand the mutagenecity and genotoxicity of five tricyclic antidepressant drugs. The antichilinergic effects of tricyclic drugs may interfere with the courtship behaviour. As the courtship of *Drosophila* involves a series of sequential stereotyped elements by both male and female. Compared to the female, Male performs more number of elements. Relatively little is known about the sexual side effects of psychotropic drugs on animal.

Materials and Methods

Fly stocks: Oregon strain of *Drosophila melanogaster* flies were obtained from the *Drosophila* stock centre and cultured in uncrowded conditions at 20°C±0.5°C and 75% relative humidity. These flies were used to treat antidepressant drug Amitriptyline hydrochloride. This antidepressant drug is obtained from the chemist and diluted in sucrose solution to get the two concentrations such as dose1 - 5µg/ml and dose2-10µg /ml sucrose solution added with known amount of yeast. This sub lethal dose was fixed after the pilot experiments.

Experiment: Flies were starved for 6-8 hours before treatment to ensure the feeding.

25 pairs of flies were treated with each concentration after starving. Four Experimental groups were made for the current study i.e.

Control Group: Untreated male and untreated female (only sucrose and yeast solution).

Treatment group1: Treated male crossed with untreated female

Treatment group2: Treated female crossed with untreated male

Treatment group 3: Male and Female treated crosses.

This has been done for both the dose 1 and dose 2

This grouping was made to facilitate appropriate behavioural analysis and to minimize sex-specific bias.

Each pair was then used to assess the mating behavioural parameter such as courtship duration and mating latency following the method of Hegde and Krishna (1997).

Virgin female and bachelor male flies of each experimental group were introduced into an Elens-Wattiaux mating chamber (5cm x 5 cm) circular glass chamber with a lid to facilitate easy observation). Since maximum mating occurs during morning hours, observation was made between 7 and 11 am.

Courtship duration (CD): It is the time between initiation and termination of copulation of each pair.

Mating latency (ML): Mating latency is defined as the time between introduction of males and females into mating chamber and initiation of copulation of pair.

Statistical analysis: The data were subjected to one way

Fecundity and fertility: To analyze the fecundity and fertility, we have used only the both treated group (treatment group 3) and followed the method of Shereen *et al.* (2013)

ANOVA followed by Duncan's Multiple Range test as post hoc test. by using SPSS software version 11.

Result and Discussion

Effect of amitriptyline hydrochloride on mating behaviour of *Drosophila melanogaster* is depicted in the table 1 and figure 1

Table 1 depicts that control male has a mating latency of 2.4 minute with a courtship duration of 15.9 minutes. Compared to control the treated groups showed increased mating latency and decreased courtship duration in the treated group. In Male treated group, mating latency is increased in both the doses. Copulation duration is also increased. The difference between four groups for both the doses is highly significant. CD: F=23.8, ML=42.14 with df=3 for dose =1 and F=10.2 and 5.567 for ML and CD df=3 respectively. Male treated groups showed increased mating latency compared to the control the other treated groups. Overall assessment for both the doses shows that there is significant difference between control and treated groups. F=6.9 df=2 in Mating latency and F=6.67 for CD. Post hoc test showed that difference between groups is significant. There is increase in the mating latency of the female treated groups in both the doses compared to all other groups. There is increased copulation duration was observed in the male treated groups compared to control and other groups. All other groups showed a one or two minute increase in the copulation duration compared to other

experimental groups. The table 3 revealed the fitness assessment is done only for male and female treated groups. The fecundity assessed for 10 days have showed high significance when compared to the control. Life time fertility has also revealed the significant difference between the treated and control group. F ratio= 11.8 df=2. So there is a reduction in the fecundity and fertility in a dose dependent manner.

The anti cholinergic effects of tricycle drugs are of interest because increasing use of these drugs in recent years. Several studies in human and other organisms have showed the side effects on usage of antidepressants. The production of offspring's in *Drosophila* and many organisms is followed by a series of courtship act. The courtship and mating despite are genetic; they are also controlled by different aspects such as acoustic, visual chemical and tactile signals (Edwin, 1983). Therefore, signals are also species specific. Many studies on *Drosophila* courtship behavior have revealed interesting courtship act performed by both the partners namely, tapping, licking, scissoring, courtship song, decamping, rejection kicking etc., compared to females males perform more courtship elements.. In the present study to observe the negative effect of amitriptyline hydrochloride on the courtship duration (CD) and Mating latency has been taken into consideration. Courtship duration is an important parameter because longer the courtship duration the sperm transfer will be more the shorter the duration the sperm ejaculation is reduced it certainly reflected in the fecundity of the individuals. Guru Prasad *et al.* (2011) opines that longer duration of copulation permits the transfer of more number of sperms by male to female, It is further supported by Pankaj *et al.* (2011), Eastwood and Bunet (1977), Markow (1998), therefore extension of copulation duration enhances the fitness of male. It can also enhance the fitness of female in terms of the number of fertilized eggs. Hence this parameter sounds better measurement of fitness. In the present study, the number of groups studied and with two doses of antidepressant is represented the reduction in the courtship duration and increase in the mating latency of the treated individuals. Mating latency of the male treated group and female treated group was increased to 3 minutes compared to control. It reveals that the reduction in the male vigor. It represents the time between introduction of male and female flies into observation chamber up to initiation of the copulation. A male with high vigor reacts quickly in the presence of female while a male with less vigor reacts slowly. In the present study, the more the mating latency represents the reduction in the male vigor due to the antidepressant treatment. Probably antidepressant interferes with the male vigor. A contrasting study by Suchitra and Shakunthala (2014) mating latency was shorter than the control and increased courtship duration was observed after the treatment with *Mucuna pruriens* extract an aphrodisiac by nature. The copulation duration of the treated group is increased for 1 or two minutes in treated groups of both the concentration. When the same group of antidepressant treated flies was analyzed for the fecundity and fertility studies showed 30% reduction in the fecundity and fertility compared to control groups. When compared to male treated groups, the female treated groups showed highest effect in terms of increased mating latency and took long time for copulation without increase in the fecundity. When their offspring' were tested with the same parameters just to know there efficacy to carry any such adverse effect to the next generation. Compared to parent groups F1 generation flies have showed difference in the mating latency and copulation duration only in the Female

treated group progeny, non significant in other groups. Hence, females carry a little effect on the progeny. It should further validated by assessing several generations.

It is well known fact that when courtship is affected the copulation is altered, during copulation the sperm transfer from male to female takes place. Hence the time of CD play a very important role in the number of viable sperms transfer to the female tract. The antidepressant treated flies have showed increased mating latency, it is inversely proportional to male vigor. Drug treatment has not influenced much on the copulation duration. However, there is reduction in the fertility and fecundity of the treated groups compared to the control. Our findings are similar to the work done by Nazari and Hegde (2006) and Nagabhushan (2002), on sexual behaviour of fluoxetine treated flies. In *Drosophila* the sensory-CNS-motor circuits is modulated by 5-HT which also play an important role in behaviour and developmental process. It has been show that 5-HT uptake is necessary for the normal development and declination of the same neurotransmitter results in altered and delayed development. As amitriptyline hydrochloride blocks the uptake of serotonin it may lead to the dumping of 5-HT which in turn effects the larval development (Dasari, 2007).

In conclusion amitriptyline hydrochloride has serotonin inhibitor interfere with the behaviour and also development process, administration of these drugs for long time perhaps interfere with the development and neuron function.

Table.1 Effect of Amitriptyline hydrochloride on Copulation duration and Mating Latency of *D. melanogaster* in control and treated groups

Groups	Mating Latency	F ratio	Copulation duration	F ratio
Control*	2±0.25	6.89	15.09 ±0.4	6.67
5 mg*	3±0.02		16.96±0.96	
10mg*	4±0.32		18.29±0.62	

Table.2 Effect of Amitriptyline hydrochloride on Copulation duration and Mating Latency of *D. melanogaster* in control and 5mg treated groups

Treatment groups	Mating Latency	F ratio	Copulation duration	F ratio	Mating Latency	F ratio	Copulation duration	F ratio
	5 µ/ml				10 µ/ml			
Control	2±0.25	23.88	15.09 ±0.4	42.14	2.4±0.25	10.02	15.9±0.40	5.567
Male treated	2.3±0.3		18.7±0.49		4±0.61		20.6±1.38	
Female treated	5.5±.02		15.6 ±.0.40		4.8±0.44		18.6±0.49	
Both treated	2.4±.03		17.1±1.01		2.5±0.30		16.7±0.69	

Table.3 Effect of Amitriptyline hydrochloride on fecundity and fertility of *D. melanogaster* in control and treated group (both treated)

Groups	Fecundity	F ratio	Fertility	F ratio
Control	298.0± 7.08	108.8 Difference between groups highly significant	379.9± 4.72	11.99 Difference between groups highly significant
5 mg	209.7± 5.66		326.7± 5.08	
10mg	185.2± 3.84		260.1± 3.52	

Fig.1 Mating latency and Courtship duration in *D. melanogaster* for control and Amitriptyline treated groups (5µg/ml)

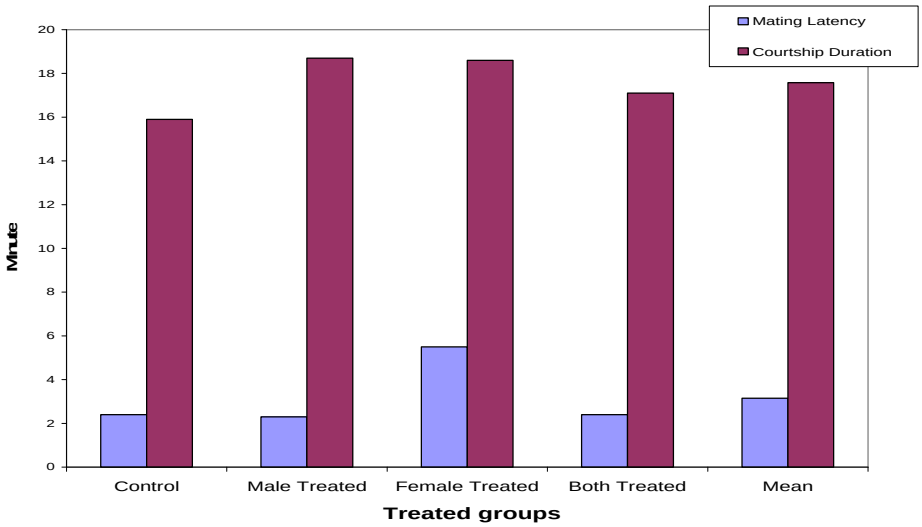
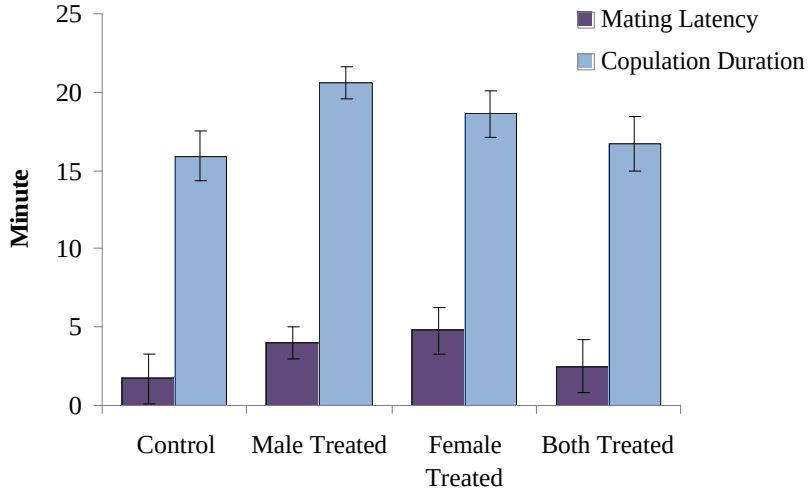


Fig.2 Mating latency and Courtship duration in *D. melanogaster* for control and Amitriptyline treated groups (10µg/ml)



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