Journal of Animal and Veterinary Advances 11 (12): 2138-2142, 2012
ISSN: 1680-5593

Effect of Paroxetine on Developmental Time, Hatching Egg, Viability and Fertility of *Drosophila melanogaster*

[1]Mohammad Reza Fakoorziba, [2]Mohammad Hossein Eghbal and [3]Fariba Eghbal
[1]Department of Medical Entomology, College of Health and Nutrition,
Research Center of Health Sciences,
[2]Shiraz Anesthesiology and Critical Care Research Center,
Shiraz University of Medical Sciences, Shiraz, Iran
[3]Department of Pediatrics, Medical College, Kazerun Branch,
Islamic Azad University, Kazerun, Fars Province, Iran

Abstract: The antidepressant drugs are long-term use administered to many patients and it can cause lot of complications and side effects. The extensive knowledge of the genetics, fitness parameters and morphology of *Drosophila melanogaster* has made it of the best usefulness in toxicity and genotoxicity researches. The pure strain of *Drosophila melanogaster* was applied for experiments. The culture was maintained under the laboratory condition. Wheat cream agar medium as standard food medium was used for maintenance of Drosophila flies. LC_{50} of paroxetine was estimated by the larval feeding method and sub lethal concentrations of paroxetine were selected. Viability, rate of development and sex ratio were three parameters employed to study the toxicity. The frequency of dominant lethals was scored to evaluate the genotoxic effect of this drug on male germ cell. The results have revealed that all three concentrations employed could reduce viability and significantly delay development ($p<0.01$). Sex ratio deviation was significant in the highest concentration (5 g L^{-1}) whereas result of dominant lethal test showed that paroxetine was able to induce significant lethality in concentrations 0.4 and 0.5 g L^{-1} ($p<0.05$).

Key words: Developmental time, viability, fertility, paroxetine, *Drosophila melanogaster*

INTRODUCTION

The World Health Organization report on the global burden of disease, placed major depression fourth among the leading cause of disease burden in the developing world in 1990 and predicated that it would rise to the second by the year 2020 (Murry and Lopez, 1996). In parallel with the increasing awareness of depression as an important health issue, the past decade has seen an increase in the pharmacotherapy option for managing depression with the arrival of several new classes of antidepressant (McManus *et al*., 2000; Chaudhari *et al*., 2010).

From the 2007 edition of the Martindale (2007) it can be inferred that 47 antidepressants are on the market and the majority of them are used in several countries (Brambilla *et al*., 2009).

Paroxetine in the class of serotonine specific reuptake inhibitor is in the list of the ten most commonly antidepressant drugs for managing depression as the most common form of mental disorder in the world (McManus *et al*., 2000; Kadioglu *et al*., 2011). This drug is a phenylpiperidine antidepressant and blocks serotonine reuptake. This process increases the amount of serotonin available to absorb by the receiver cell and it can help message transmission return back to normal. Paroxetine not only is used for treatment of depression but also used to relief other mood disturbances such as panic attack, obsessive-compulsive disorder and social anxiety disorder. In addition, it is used for treatment of diabetic neuropathy, chronic tension headache and premature ejaculation (Boyer and Nemeroff, 1996). This drug has lot of complications including nervousness, trouble sleeping, headache, drowsiness, fatigue, nausea, vomiting, diarrhea, loss of appetite, dry mouth, sweating, dizziness, muscle spasm, change in sexual function, rash and extrapyramidal effects (Baldessarini, 1996). With respect to various side effects of paroxetine and also numerous indications for prescribing in medicine, the researcher proposed to check the toxicity and mutagenicity of this drug in *Drosophila melanogaster*. This fly has been used as one of the best sub mammalian test systems for mutagenic and genotoxic studies and a number of mutagenic, carcinogenic chemicals and

Corresponding Author: Fariba Eghbal, Department of Pediatrics, Medical College, Kazerun Branch, Islamic Azad University, Kazerun, Fars Province, Iran

radiations have been tested for their mutagenic effect using this small tiny insect (Luning, 1966; Vogel and Sobels, 1976; Vasudev and Krishnamurthy, 1979; Abd-El-Samie *et al*., 2007; Li *et al*., 2011; Yu *et al*., 2011).

MATERIALS AND METHODS

Drosophila melanogaster with its well studied genetics and because of its metabolic activation system similar to mammals is acceptable for candidate of genotoxicity study of drugs. This research was done on the base of explanations of Nichols (2006) on life cycle of *D. melanogaster* and procedure of Delcour (1969). In the life cycle of *D. melanogaster* approximately 24 h takes time a newly laid egg takes to undergo embryogenesis and hatch as a first instar larva. After about 1 day, the larva feeds and molts into a larger second instar larva. After 24 h, the larva again feeds and undergoes a final molt to the third instar larva stage. After 2 days at this stage, the larva develops into a puparium. During the next 5-7 days, the pupa transforms into the adult form. The female is able to mate about 12 h after eclosion and lay hundreds of eggs within a few days. This short generation time and large number of progeny make it possible to detect numerous of mutation in a few weeks.

Paroxetine hydrochloride ($C_{19}H_{20}F NO_3 HCl. ½H_2O$), manufactured by Cardial Healthcare Ltd. India was employed in this study. To assess the effect of paroxetine on *D. melanogaster*, larval feeding technique was employed. For preparation of chemical treated media, appropriate quantity of paroxetine was weight and separately added to 100 mL of food medium before hardening. The medium was distributed to glass milk bottles of 200 mL capacity or 100 vials of 25×75 mm size. The mouth of the bottles/vials was kept closed with cotton. About 1 day later, one or two drops of yeast solution were added to the food media. The medium was used after 24 h. At every step heat sterilized bottles/vials were used for preparing medium. Similarly sterilized cotton was used to plug the bottles. Eggs of the same age were collected following the procedure of Delcour (1969) and equal numbers of eggs were placed in each vial containing wheat cream agar media as control and media with different concentrations of drug as treated media. About 20 replicates of each set were maintained. The LC_{50} value of paroxetine on *D. melanogaster* was calculated and three significant concentrations below LC_{50} (0.3, 0.4 and 0.5 g L^{-1}) were selected for this research. In order to preparing different concentrations of treated series, paroxetine was mixed with distilled water and added to standard wheat cream agar media after cooking. Number of flies emerged and their sexes were recorded every day from the first to the last day of eclosion. According to these data, viability, developmental time and sex ratio of *D. melanogaster* in each concentration and control were analyzed.

For mutagenicity screening of paroxetine, dominant lethal test was carried out following the procedure of Sankaranarayanan (1967). Male flies emerged out of the control and different concentrations of treated media were collected and aged them for 3 days then crossed to untreated virgin females flies of the same age. After 24 h, male flies were discarded and females transferred to fresh vials containing normal media every day for 10 days consequently. Every day the number of eggs was laid by each female recorded and after 48 h, the numbers of unhatched eggs were checked. All of the experiments were conducted at constant temperature of 20±1°C.

Statistical analysis: Viability (survival value) and developmental time were analyzed by using one way Analysis of Variance (ANOVA) with Duncan's Multiple Range Test (DMRT). Chi-square was used for analyzing of sex ratio and dominant lethal test data was analyzed by using 2×2 contingency χ^2-test.

RESULTS AND DISCUSSION

Table 1 shows the effect of different concentrations of paroxetine on viability of *D. melanogaster*. Observations reveal that the viability decreases with increasing dose of tested drug. This parameter is significant for all of concentrations ($p<0.01$). Viability for control has been recorded 80.4% whereas this parameter decreased to 68, 53.6 and 50.8% in concentrations 0.3, 0.4 and 0.5 g L^{-1}, respectively.

The effect of paroxetine on developmental time of *D. melanogaster* could be shown in Table 2. It reveals

Table 1: Effect of paroxetine on viability of *Drosophila melanogaster*

Concentrations (g L^{-1})	Total number of eggs placed	No. of adult emerged	Viability (%)	F (df)
Control	500	402	80.4	4194.666 (3)*
0.3	500	340	68.0	-
0.4	500	268	53.6[a]	-
0.5	500	254	50.8[a]	-

*Values significant ($p<0.01$). The viability with the same letter in the parenthesis is not significantly different at 5% level according to DMRT

Table 2: Effect of paroxetine on developmental time of *Drosophila melanogaster*

Concentration (g L^{-1})	Eggs	N	Range (Min.-Max.)	Mean±SD (Days)	F (df)
Control	500	402	14-21	16.44±1.68	368.034* (3)
0.3	500	340	16-30	20.86±2.72	-
0.4	500	268	17-31	22.14±3.15[a]	-
0.5	500	254	17-31	22.21±3.13[a]	-

*Values significant by one way ANOVA test ($p<0.01$). Developmental time with the same letter in the parenthesis are not significantly different at 5% level accordig to DMRT; N: Number of emerged flies

that developmental time significantly increased in all treated series compared to the control ($p<0.01$). This trait for control was 16.44±1.68 whereas it has increased to 20.86±2.72, 22.14±3.15 and 22.21±3.13 in concentrations of 0.3, 0.4 and 0.5 g L^{-1}, respectively. There is linear relationship between the increase in developmental time and the concentrations employed in treated series. Such results have also been reported by Nazir *et al*. (2000) following exposure of *D. melanogaster* to chlorpyridos an organophosphate insecticide. These results are also in line with the observations of Choudhary (2004) that used fenvulerate as a contact poison against larval development and viability.

Table 3 and 4 show the effect of paroxetine on sex ratio of *D. melanogaster* and the result of induction of dominant lethality by paroxetine. As it is presented in this table, lethality for control was 8.44% while for concentrations 0.3, 0.4 and 0.5 g L^{-1} this parameter has increased to 16.20, 18.29 and 20.88%, respectively. Statistically analysis of these results demonstrated that this value is significant at concentrations 0.4 and 0.5 g L^{-1} ($p<0.05$). Van Schaik and Graf (1991) have reported that imipramine and sipramine as two antidepressant drugs are clearly mutagenic at concentrations of 10 and 1 mμ, respectively in the standard Drosophila wing spot test.

Table 3: Effect of paroxetine on sex ratio of *Drosophila melanogaster*

Concentration (g L^{-1})	Male	Female	Ratio (Male/Female)	χ^2(df)
Control	197	205	1/1.04	0.159 (1)
0.3	157	183	1/1.17	1.988 (1)
0.4	131	137	1/1.05	0.134 (1)
0.5	107	147	1/1.37	6.299 (1)*

*Values significant by χ^2-test ($p<0.05$)

Table 4: Frequency of dominant lethal induced after feeding *Drosophila melanogaster* larvae on paroxetine supplemented medium

Concentration (g L^{-1})	No. of egg counted	No. of egg unhatched	Dominant lethal (%)	χ^2 (df)
Control	1304	110	8.44	-
0.3	1500	243	16.20	3.030 (1)
0.4	1077	197	18.29	4.421 (1)*
0.5	1815	379	20.88	6.816 (1)*

*Values significant at 5% level compared to control by χ^2 cross tabs 2×2 test

Brambilla *et al*. (2009) in their review article about genotoxic and carcinogenic effects of antipsychotics and antidepressant drugs have reported most of researches and their results that were carried out on paroxetine by scientist in the world (Table 5). In their review, there was not any test system on effects of paroxetine on dominant lethal and life cycle test on *D. melanogaster*. So, this investigation was done to increase knowledge of effects of paroxetine that was applied long term in several countries.

Viability and the rate of development represent two parameters for evaluating the toxicity of chemicals (Luning, 1966). It has been demonstrated in *D. melanogaster* that the rate of development results from the compound effect several causes both genetics and environmental factors (Bonnier, 1960). In the present experiment, the genetic composite, the density of eggs in each vial, the temperature and the space were not variable. So, significant difference in developmental time and viability must be due to drug employed. Although, no reports on the effect of paroxetine on viability is available until now, change in viability due to incorporation of other chemicals have been demonstrated by earlier workers in *D. melanogaster* (Vasudev and Krishnamurthy, 1979; Sorsa and Pfeifer, 1973; Rajasekarasetty *et al*., 1979; Lopez-Fanjul and Villaverde, 1989; Basheer *et al*., 1999; Chinnici *et al*., 1976). This investigation also agrees with the most work of them.

Sex ratio is one of the adaptive traits of any population which determines the rate of increase or decrease of a sexual population in an environment (Choudhary, 2003). Environmental factors are known to affect the sex ratio (West *et al*., 2002). Statistically analysis of data in present study on the effect of paroxetine on sex ratio of *D. melanogaster* revealed that female to male ratio has significantly increased in the last concentration 0.5 g L^{-1} ($p<0.05$). It means that in the highest concentration of paroxetine females germ cells compared to males are more resistant in exposure to this drug.

Table 5: Genotoxic and Carcinogenic effects of paroxetine as antidepressant drug (Duplay, 2005)

Test system	Results without exogenous metabolic system	Results with exogenous metabolic system	Lowest effective dose
Long-term carcinogenesis assay (mice)	Equivocal (2.4)		25 mg/(kg day)
Long-term carcinogenesis assay (male rats)	Positive (reticulum cell sarcomas)		20 mg/(kg day)
Long-term carcinogenesis assay (female rats)	Negative (3.9)		20 mg/(kg day)
S. typhimurium, reverse mutation	Negative	Negative	Not reported
UDS, mammalian cells *in vitro*			
Gene mutation, mouse lymphoma15178y cells, TK locus			
Chromosomal aberrations, human lymphocytes *in vitro*			
Chromosomal aberrations, mouse bone-marrow cells *in vivo*			
Dominant lethal test (rats)			

The number in parentheses indicates the ratio [high animal dose (mg/m^2)/maximum recommended human dose (mg/m^2)]

There is considerable evidence with the idea that dominant lethality is related to chromosomal breakage (Sankaranarayanan, 1967; Serres and Shelby, 1977). So, the frequency of dominant lethals is an evidence for the clastogenic effect of a compound. The researchers's result tallies with the finding of Vijayan (1982) by using dithranol as an antipsoriatic drug and Serres and Shelby (1977) while assessing procarbazin as an anticancer drug.

CONCLUSION

The scrutiny of the overall results of these studies on *D. melanogaster* reveals that paroxetine has toxicity and genotoxicity. So, prescribing of this drug in medicine should be done with more caution.

ACKNOWLEDGEMENTS

The researchers thank to the colleagues of Department of Medical Entomology, College of Health and Nutrition, Shiraz University of Medical Sciences (SUMS) for reading the manuscript. Researchers also, appreciate the deputy of research affairs of SUMS and Kazerun Branch, Islamic Azad University for providing the facilities.

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