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Differential Sensitivities of Three Strains of *Drosophila melanogaster* (Diptera: Drosophilidae) to Imipramine as an Antidepressant Drug

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ABSTRACT

In developing world, with progression in modernity and industrialization and synthesis of new chemicals and drugs, man, animal and their ecosystem are constantly threatened by wild range of chemical substances. Several of which are known to be mutagenic, carcinogenic and teratogenic. The antidepressant drugs like imipramine and paroxetine are long-term use administered to many patients and they can cause so many complications and side effects. So, the differential sensitivity of three strains of *Drosophila melanogaster* for imipramine hydrochloride, an antidepressant drug has been tested by larval feeding. Wheat cream agar medium as normal food medium was used for breeding of *Drosophila* flies. LC_{50} of imipramine was estimated by the larval feeding method and sub lethal concentrations of imipramine were selected. The effect of drug on viability, rate of development and sex ratio was analyzed. The results revealed that all three concentrations could reduce viability and significantly delay development. Among the three strains, Oregon-K, the wild type was found to be the most resistant. Sex ratio deviation was significant in concentration of 1 and 2 g L⁻¹ of imipramine in Oregon-K but in two mutant strains the deviation was insignificant.

Key words: Toxicity, imipramine, antidepressant, *Drosophila melanogaster*

INTRODUCTION

Depression is the most common form of mental disorder in the world. Imipramine, in the class of tricyclic antidepressant is one of the most commonly used drugs for treatment of this symptom in adult and also in pediatric age group (McManus *et al.*, 2000). It was reported that 47 antidepressants are on the market and most of them are used in many countries (Brambilla *et al.*, 2009).

This drug enhances the action of norepinephrine by blocking its reuptake and in lesser extent, it also inhibits serotonin reuptake. So, imipramine has anticholinergic and antihistaminic properties. In generally, selective serotonin re-uptake inhibitors such as paroxetine and imipramine are used in the treatment of many mental disorders and also diabetic neuropathy (Kadioglu *et al.*, 2011). Lot of complications have been recognized for this drug both in therapeutic dosage and over dosage including effect on central and peripheral nervous system, cardiovascular, endocrine, ocular, hematologic, genitourinary and gastrointestinal system (Shannon *et al.*, 1995). In addition, it also seems to have genotoxic effect (Saxena and Ahuja, 1988; Van Schaik and Graf, 1991).

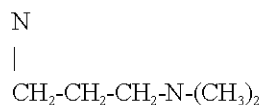
Although, considerable information is available about side effects and toxicity at both therapeutic doses and over dosage, much less attention has been paid to their possible genotoxicity. However, these drugs, since they are extensively used; need to screen both on the somatic and genetic system in the interest of human health, welfare and posterity. Hence, they must be evaluated for their toxicity and mutagenicity in appropriate test system to understand their somatic and genetic effects. It is such studies that can make us to understand the toxic and mutagenic potential, if any, of the given drug and in cases of need, one could caution the society of any probable genetic hazards, whether the drug need to be used or not, if used, the safe level of concentration that can be employed should be prescribed.

Although, direct evidence in support of mutation or other chromosomal changes leading to origin of somatic diseases in man and animals is limited, experimental evidences from other organism give strong support of fact that some human somatic diseases originate from the mutation in the somatic cells. Since, study of mutagenicity of drugs or other chemical substances on human are highly expensive and time consuming and also not safe, so the test system employed is the fruit fly, *Drosophila melanogaster* which is a very good submalian assay system due to it has several advantages both for toxicity screening and genotoxicity evaluation (Sobels and Vogel, 1976; Vogel and Zijlstra, 1987; Graf *et al.*, 1983; Wurgler *et al.*, 1984; Frolich and Wurgler, 1989; Tripathy and Patnaik, 1991; Carolino *et al.*, 2005; Abd-El-Samie *et al.*, 2007; Yu *et al.*, 2011). In addition, Li *et al.* (2011) reported a method to identify species unknown animals by using of *Drosophila*.

In this regard Balbi *et al.* (1980) have reported that the effect of imipramine is negative in the *Salmonella* and on the other hand, it shows positive effects in *Bacillus subtilis*. In human lymphocyte culture, imipramine has been reported to increase chromosomal aberration at concentration above 4 times plasma level and at upper plasma levels, respectively by Saxena and Ahuja (1988). Whereas, Fu and Jarvik (1977) have found no effect of imipramine at similar concentrations. Thus these reports are controversial. In view of such controversial reports, the author proposed to check the toxic effect of imipramine. Three genetic stocks of *D. melanogaster*, the best submalian test system has been used in the present investigation.

MATERIALS AND METHODS

Imipramine hydrochloride, the material used for this study manufactured by Sarabhai Pirmal Ltd. India, has the following structural formula:



To assess the effect of imipramine, three strains of *D. melanogaster*, including one wild type (Oregon-K) and two mutant strains (*Muller-5* and *bw/st*) have been used. Larval feeding technique was employed for assessing the differential sensitivity. This investigation was done on the base of procedure of Delcour (1969) and explanations of Nichols (2006) on life cycle of *D. melanogaster*. Eggs of the same age were collected and equal numbers of eggs were placed in vials containing either wheat cream agar media or media with different concentrations of drug. Twenty replicates of each set were maintained. The LC₅₀ was calculated for wild type and then three concentrations below this (1, 2 and 4 g L⁻¹) were selected and used for comparison of

sensitivity of these strains. For preparing different concentrations of treated series, imipramine was mixed with distilled water then added to standard wheat cream agar media after cooking. The number of flies emerged and their sexes were recorded every day from the first to the last day of eclosion. All the experiments were conducted at constant temperature of 20±1°C.

Statistical analysis: Viability and developmental time of three strains were analyzed by using of one way analysis of variance (ANOVA) with DMRT (Duncan's Multiple Range Test) whereas sex ratio was analyzed by chi-square (χ^2).

RESULTS

Table 1 shows the effect of different concentrations of imipramine on viability of three strains. Observation reveals that the viability decreases with increasing dose of test chemical. This parameter is significant for all of concentrations (p<0.01). It also represents that in all concentrations of imipramine there is significant difference between three strains but in the 1 and 4 g L⁻¹ concentrations, this value is not significant between two mutant strains (p<0.01).

In the highest concentration (4 g L⁻¹), viability of Oregan-K is 57.20 % while, for *bw/st* and *Muller-5* it is zero. It means these two mutant strains could not tolerate this high concentration and they are more sensitive to imipramine compared to the wild type.

The effect of imipramine on developmental time of three strains could be seen in Table 2. It reveals that developmental time significantly increased in all treated series compared to the control (p<0.01). There is linear relationship between the concentrations employed in treated series and increase in developmental time. Such results have also been reported by Mahmood *et al.* (1990) in *Drosophila* while assessing toxicity of a pesticide, furadan and also by Choudhary (2004) who used fenvalerate as a contact poison against larval development and viability.

Table 1: Viability of three strains of *Drosophila melanogaster* in different concentrations of imipramine

Strain	Total No. of eggs placed	Control		1 g L ⁻¹		2 g L ⁻¹		4 g L ⁻¹		F(df)
		No. of adult emerged	Viability (%)	No. of adult emerged	Viability (%)	No. of adult emerged	Viability (%)	No. of adult emerged	Viability (%)	
Oregan-K	500	405	81.00 ^b	365	73.00 ^{ad}	353	70.60 ^a	286	57.20	22.646(3)*
Muller-5	500	430	86.00 ^b	337	67.40 ^d	240	48.00	0	0.00 ^e	288.967(3)*
bw/st	500	366	73.20	320	64.00 ^e	117	23.40	0	0.00 ^e	243.186(3)*
F(df)			8.959(2)*		4.623(2)*		92.969(2)*		315.622(2)*	

Valves significant (p<0.01), *The viability with the same letter in the parenthesis are not significant at 5% level according to DMRT

Table 2: Comparison of effects of imipramine on developmental time of three strains of *Drosophila melanogaster*

Concentration (g L ⁻¹)	<i>D. melanogaster</i> strains			F(df)	Sig.
	Oregan-k	Muller-5	bw/st		
Control	18.05±2.08 ^a	18.07±1.91 ^a	17.02±1.11	7.632(2)*	0.001
1	19.09±2.36	21.68±2.79	19.90±2.48	97.454(2)*	0.000
2	24.42±2.68	28.23±4.52	26.05±2.87	80.506(2)*	0.000
4	26.30±3.28	0.00±0.00 ^b	0.00±0.00 ^b	23440.9(2)*	0.000

Value significant by ANOVA test (p value 0<0.01), *The developmental time with the same letter in the parenthesis are not significant at 5 % level according to DMRT

DISCUSSION

The genotoxic and carcinogenic effects of antipsychotics and antidepressant drugs have been reported by most of researches (Brambilla *et al.*, 2009). In this review of Brambilla *et al.* (2009) there was not any test system on effects of imipramine on dominant lethal and life cycle test on *D. melanogaster*. So this study was done to increase knowledge of effects of imipramine that was applied long term in several countries.

The rate of development time and viability are two factors for evaluating the toxicity of chemicals (Luning, 1966). Bonnier (1961) has demonstrated that change in rate of development is due to compound effects of genotype and environment. Uysal *et al.* (2007) have reported recovering effects of some plant extracts on the teratogenic effects during the development of *D. melanogaster*. Smith *et al.* (2011) have also investigated sexual dimorphism in the effect of a Taurine supplemented diet on biology of adult *D. melanogaster*. The present study also revealed significant difference in developmental time and viability that must be due to the drug employed and also different genetic system of the three strains. In this investigation, Oregon-K is more resistant to imipramine compared to other two mutant strains, perhaps because it is a wild strain which is having certain advantage of being in man made as well as in natural environments. Sex ratio is one of the adaptive traits of any population which identifies the rate of changes of a sexual population in an environment.

Statistical analysis of the data in the present investigation on the effect of imipramine on sex ratio of three strains revealed that, the female to male ratio of Oregon-K (wild type) has significantly increased in concentrations 1 and 2 g L⁻¹ (Table 3). It means female germ cells compared to male are more resistant to exposure to this drug. In contrast to this, among mutants, sex ratio deviation was significant only in 2 g L⁻¹ concentration for *bw/st* and in 1 g L⁻¹ for *Muller-5* (p<0.05). This difference is perhaps due to absence of flies at the highest concentration of 4 g L⁻¹. Thus, this research shows that imipramine has toxic effect on *Drosophila* and different strains have different sensitivity. Although, no enough data on the effect of imipramine on viability, developmental time and sex ratio is available until now, changes in these biological data due to incorporation of other chemicals have been demonstrated by earlier workers in *D. melanogaster* (Sorsa and Pfeifer, 1973; Chinnici *et al.*, 1976; Vasudev and Krishnamurthy, 1979; Rajasekarasetty *et al.*, 1979; Lopez-Fanjul and Villaverde, 1989; Basheer *et al.*, 1999). This study also agrees with the most work of them in some aspects. Finally, the scrutiny of the overall results of this investigation on *D. melanogaster* reveals that imipramine has toxicity and genotoxicity.

Table 3: Effect of imipramine on sex ratio of three strains of *Drosophila melanogaster*

Concentration (g L ⁻¹)	Oregon-K				Muller-5				bw/st			
	σ	♀	σ/♀	x ²	σ	♀	σ/♀	x ²	σ	♀	σ/♀	x ²
Control	194	211	1/1.1	0.60	210	220	1/1.0	0.19	175	192	1/1.1	0.70
1	138	222	1/1.6	10.14*	151	214	1/1.4	10.53*	149	171	1/1.1	1.38
2	134	202	1/1.5	13.36*	114	90	1/0.8	2.59	78	39	1/0.5	12.34*
4	137	151	1/1.1	0.59	N	N	-	0.00	N	N	-	0.00

No. of eggs for each concentration = 500, *Differences are statistically significant (p<0.05)

CONCLUSION

With respect to overall picture of data of this investigation on *D. melanogaster*, it is clear that imipramine is genotoxic both in larval and adult feeding in dominant lethal test. Also according to the report of previous workers, this drug is mutagenic in somatic cells of *Drosophila*. On the other hand this antidepressant drug associates with development of breast cancer. So, it was suggested that, due to possible genotoxic and carcinogenic potential of imipramine as antidepressant drug, prescription in medicine should be done with more caution especially in pediatric age group, during pregnancy and lactation.

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