TOXIC EFFECT OF AMITRIPTYLINE-AN ANTIDEPRESSANT DRUG ON DROSOPHILA MELANOGASTER

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ABSTRACT

Objective: *The objective of this study was to evaluate the genotoxic effect of amitriptyline on a fruit fly D. melanogaster and to make suggestions if any for its use.*

Material and Methods: *The pure strain of Drosophila melanogaster (Oregon K) was used for experiments. The pure culture was maintained under the laboratory condition at a temperature 24±2° C. Standard food medium (wheat cream agar medium) was used for maintenance of Drosophila flies in the laboratory. Eggs of the same age collected by procedure of Delcour. LC50 of amitriptyline for the larval feeding method was estimated (0.21%) then, sub lethal concentrations of 0.05, 0.1, 0.2 and 0.3% of amitriptyline were selected.*

Results: *Mean viability was reduced in different concentrations of amitriptyline. There is no significant difference in sex ratio of D. melanogaster treated with amitriptyline. On increase in concentration, not only there was delay in emergence but also reduction in the number of flies emerged. Amitriptyline in all concentrations employed prolong the developmental time even in the lowest concentration tested.*

Conclusion: *Amitriptyline has effect on viability, eclosion and developmental time but not on sex ratio of Drosophila melanogaster.*

Key Words: *Amitriptyline, Antidepressant drugs, Drosophila Melanogaster, Toxicology.*

INTRODUCTION

The fruit fly *Drosophila melanogaster* is one of the most intensively studied organisms in biology and serves as a model system for investigations of many developmental and cellular processes common to higher eukaryotes, including man. This fly is being used for genetic studies since almost a century. Considering these advantages *Drosophila* 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 radiations have been tested for their mutagenic effect using this small tiny insect.[1-3]

Antidepressant drugs are currently the mainstay of treatment for all but the mildest forms of depression. Their effectiveness in the management of depressive illness is undisputed and their effectiveness in preventing suicide, while not proven, may be assumed.[4] Nevertheless, of all the drugs that are taken in lethal overdose, antidepressants are the most common. Epidemiological studies from several countries have provided evidence of marked differences in overdose toxicity between drug classes and, in some cases, between individual drugs within a class. However, use of antidepressants in high concentrations may be associated with cellular toxicity.[5] A fatal toxicity index (deaths per million: National Health Service prescriptions) was calculated for antidepressant drugs on sale during the years 1975-84 in England, Wales, and Scotland. The tricyclic drugs introduced before 1970 had a higher index than the mean for all the drugs studied.[6] Amitriptyline {3 - (10,11-Dihydro - 5H Dibenzo - [a,d]cyclohepten- 5-yliden) N,N-dimethyl-1-propanamine}is included in a group of medications classified as tricyclic antidepressant. It was discovered in late 1930s before scientists had developed a concept of chemistry of brain. This drug was used to treat spontaneous endogenous depression and is very sedating. It is also helpful in the treatment of agitation, anorexia, adjunctive treatment of neurogenic pain, bulimia associated with depression, chronic hiccups, insomnia, major depression, or in a patient with chronic pain and other pain syndromes including ciguatera, post-herpetic neuralgia, neuropathic, and vulvodynia. In view of the excessive use of this

EFFECT OF AMITRIPTYLINE ON VIABILITY OF D. MELANOGASTER

Concentration (%)	Adults emerged / 1000 eggs	Viability (%)	Viability /vial M±SE
Control	926	92.60	23.15±0.21[a]
0.05	779	77.90	19.48±0.49[b]
0.1	634	63.40	15.85±0.51[c]
0.2	493	49.30	12.33±0.61[d]
0.3	378	37.80	9.45±0.56[e]

[a,b,c,d,e] Mean difference is significant at 0.01 level by One way ANOVA and DMRT (F value = 121.68).

Table 1

drug and absence of adequate information on genotoxicity, the present studies have been undertaken. The objective of this study was to evaluate the genotoxic effect of amitriptyline on *D. melanogaster* and to make suggestions if any for their use.

MATERIALS AND METHODS

The pure strain of *Drosophila melanogaster* (Oregon K) was obtained from *Drosophila* Stock Center, Department of Zoology, University of Mysore, Mysore, India, and the flies of this culture were used for experiments after 5-7 generation, when they were fully acclimatized to the laboratory condition. The pure culture was maintained under the laboratory condition at a temperature 24±2° C. Standard food medium (wheat cream agar medium) was used for maintenance of *Drosophila* flies in the laboratory according to the method described by Hegde *et al.*[7] For preparation of chemical treated media, appropriate quantity of amitriptyline was weight (0.05, 0.1, 0.2 and 0.3%) and separately added to 100 ml of food medium prepared as above before hardening. The medium was distributed to glass milk bottles of 200 ml capacity or 100 vials of 25 x 75 mm size. The mouth of the bottles / vials was kept closed with cotton. One day later, one or two drops of yeast solution were added to the food media. The medium was used after 24 hours. At every step heat sterilized bottles / vials were used for preparing medium. This was to prevent outbreak of pests and diseases. Similarly sterilized cotton was used to plug the bottles.

Egg collection: Eggs of the same age (±3 hours) collected by procedure of Delcour (1969) were placed in vials containing normal or chemical supplemented medium at a density of 25 eggs / vial.[8] To prepare Delcour media, 100ml of distilled water and 3g of agar agar was added and boiled, while boiling, 1.5ml of acetic acid and 2.5ml of ethyl alcohol were added. This media was poured into rectangular plastic cup and allowed to cool to room temperature. The upper part of this media was then scraped by a razor blade to make the surface rough and then two drops of liquid yeast was added. Then the cup containing Delcour media was attached to one end of a long thick stick with the aid of a sticker and placed in the bottle containing starved flies. The batch of eggs laid within first 12 hours were discarded, because most of them are unfertilized and the second batch of eggs laid in the next 3 hours were collected from the surface of the media and seeded in each fresh vial of wheat cream agar media which served as control and also the different concentrations of treated with the drug.

In order to fix the concentrations for the study of effectiveness of the test compound, LC50

EFFECT OF AMITRIPTYLINE ON SEX RATIO OF D. MELANOGASTER

Concentration (%)	Adults emerged / 1000 eggs		Ratio	X^2 value
	Male	Female		
Control	469	457	1.03:1	-
0.05	363	416	0.87:1	2.777
0.1	302	332	0.91:1	1.367
0.2	238	255	0.93:1	0.724
0.3	180	198	0.91:1	0.985

All values are insignificant at 5% level, when compared to control.

Table 2

EFFECT OF AMITRIPTYLINE ON MEAN DEVELOPMENTAL TIME OF D. MELANOGASTER

Concentration (%)	Group	Males	Females
Control	12.41±0.05[a]	12.52±0.08	12.29±0.07
0.05	15.75±0.11[b]	15.71±0.17	15.79±0.15
0.1	16.53±0.13[c]	16.81±0.18	16.27±0.18
0.2	21.21±0.16[d]	21.06±0.23	21.35±0.23
0.3	22.88±0.16[e]	22.61±0.23	23.13±0.22

Values represent means (day) and their standard errors.

[a,b,c,d,e] Mean difference is significant at 0.01 level by One way ANOVA and DMRT (F value =1285.05).

Table 3

of amitriptyline for the larval feeding method was estimated (0.21%) by using log-dose/probit. Then, sub lethal concentrations of 0.05, 0.1, 0.2 and 0.3% of amitriptyline were selected.

Viability, Sex ratio and Developmental time

For viability (survival value) analysis, the number of flies emerged out of each vial are recorded every day until the last day of emergence. The pooled data is used to estimate the mean viability.

For analysis of sex ratio and developmental time, after emergence the flies were counted and sexed every day from the first to last day of eclosion. The data so collected were utilized to calculate the sex ratio and mean developmental time.

Statistical analysis: The data were compiled; means, standard errors and One-way ANOVA were calculated using SPSS software version 10. Since the F values for some of the groups were significant, the Post Hoc Test of DMRT (Duncan's Multiple Rang Test) was also carried out for each of the parameters analyzed. Chi-square test (X^2) was applied to sex ratio.

RESULTS

The LC50 value of amitriptyline fed through larval feeding was 0.21%. In the present investigations mean viability per vial was reduced in different concentrations of amitriptyline (Table 1). The viability has been reduced from 92.60% (control) to 37.80% in 0.3% of amitriptyline. The lowest mean viability per vial was produced in the highest concentration of chemical tested. Therefore, it is clear that this antidepressant drug has toxic effect on *D. melanogaster*. As there is no such study conducted earlier, this represents the first report of the effect of amitriptyline on viability. The extent of viability was indirectly

PATTERN OF EMERGENCE OF D. MELANOGASTER IN CONTROL AND DIFFERENT CONCENTRATIONS OF AMITRIPTYLINE

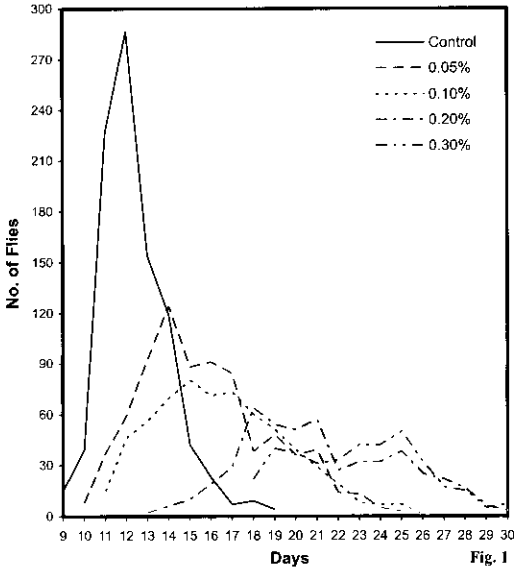

Fig 1

proportional to the concentration. Furthermore, the analysis of variance (ANOVA), as well as post hoc test of DMRT shows that this drug induced significant effects in all concentrations ($P<0.05$).

Table 2 shows that there is no significant difference in sex ratio of *D. melanogaster* treated with amitriptyline. Therefore, it is clear that amitriptyline treatment does not bring about sex ratio deviation.

Graphic representation of the pattern of emergence of flies in control and in different concentrations of amitriptyline is shown in Fig 1. Eclosion of flies in control group started on 9th day and terminated on 19th day, while in the lowest concentrations of amitriptyline emergence started on 10th day and ended on 25th day. In the highest concentration of amitriptyline (0.3%) emergence started on 18th day and extended up to 30th day. Thus, with increase in concentration not only there was delay in emergence but also reduction in the number of flies emerged.

Amitriptyline in all concentrations employed prolong the developmental time even in the lowest concentration tested. Longest mean developmental time was noticed in the highest concentration of drug employed. It was prolonged from 12.41±0.05 (days) in control to 22.88±0.10 in 0.3% of amitriptyline. There was a linear relationship between mean developmental time and the chemical concentrations used. ANOVA and post hoc test of DMRT computed to compare the mean developmental time between different concentrations of amitriptyline and that of control have shown significant differences ($P<0.05$). It is also clear from Table 3 that there was no significant difference in the mean developmental time of the two sexes in any of the chemical treatments or control ($P>0.05$). Such a type of effect on the developmental time by different chemicals in *D. melanogaster* have been shown by other workers.[3,3-9,11,14,15,20]

DISCUSSION

Viability is one of the adaptive traits of any population and determines the rate of increase or decrease of population in an environment. Therefore, it is one of the fitness parameters, which could be used to analyze the toxicity of any drug or chemical. Any change in viability reflects the somatic effect induced by them provided the analysis is made in a uniform environment[1,9]. Environmental factors which would affect viability mainly include components such as food, temperature, space and population density[10]. In the present experiments, temperature and space were uniform for both control and treated batches, same number of eggs were allotted to vials, same strain of flies were used for all the experiments, thus leaving the food medium supplemented with antidepressant drug.

Although, no reports on the effect of amitriptyline on viability is available until now, change in viability due to incorporation of other chemicals have been demonstrated by earlier workers in *D.* Melanogaster.[3,9,11-15] The present observations agree with the work of the above authors.

The subject of sex ratio in animals is very much debated by many biologists. According to Fishers theory[16], sex ratio between male and female of any species must be 1:1 which is favored by natural selection to get an equal expenditure of the parents on the offspring. If the sexes are in equal proportion, that population is expected to be well adapted. It will have maximum productivity. This contrasts the work of Chinnici *et al.*[14], Rajasekarasetty *et al.*[11], Shabana *et al.*[13], Shamim[17], where they have noticed significant variation in sex ratio of *D. melanogaster* treated with different chemicals. According to Reddy and Krishnamurthy (1972-73) the sex ratio imbalance may be caused due to certain genetic and environmental factors[18]. The sex ratio imbalance occurs if the X or Y bearing gametes are affected. In the present studies, the antidepressant drug used is not able to affect the gametes of any specific type; hence there was no sex ratio imbalance.

Change in pattern of emergence of *D. melanogaster* has been noticed by Jacobson *et al.*[19], Shabana *et al.*[13], Nazir *et al.*[20], Driver and Georgeou[21], when they used divalent metal ions, carbamate pesticide (Dunet), fungicide captan and vitamin E, respectively. The present observation of the author is an example of the effect of antidepressant drug on the pattern of emergence.

Rate of development is another parameter, which is used to analyze the toxicity of the chemicals under study. In the present experiments the genetic constitution, number of eggs per vial, amount of food, temperature and space were kept constant. Obviously the differences in the developmental time must have been determined by the chemical concentrations used and not by other factors. The author has observed variation in the developmental time at different concentrations of amitriptyline used (Table 3).

Thus the study shows that the antidepressant drug amitriptyline has toxic effect and hence recommends the prescription of the drug in small doses.

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