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Study of Genotoxic Effect of Lorazepam(ativan) on *Drosophila melanogaster*

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ABSTRACT. The mutagenic potentialities of psychoactive drug, lorazepam was tested in *Drosophila melanogaster* using two test systems, the sex-linked recessive lethals test (SLRL) and the estimation of the activities of the enzymes cholinesterase (CHE) and aliesterase (AliE). Org K, male flies of *Drosophila melanogaster* were reared on a medium containing two concentrations of the drug, 5 and 10 mg/100 ml medium, and screened for sex-linked recessive lethals.

The results indicated that significant number of recessive lethals were induced in *Drosophila* in all stages of spermatogenesis except in spermatozoa stage at the two doses of the drug, indicating that lorazepam(ativan) is capable of inducing sex-linked recessive lethal mutations in *Drosophila melanogaster*. Meanwhile, the drug showed a mutagenic effect on the genetic background of each of CHE and AliE, which proves the mutagenic potentiality of the drug.

Introduction

Several reports indicate that many chemical pollutants which are widely spread in the environment, such as pesticides and drugs, are mutagenic in various test systems. These findings reflect an urgent need to draw more attention to the examination of the possible genetic hazards of such pollutants to public health.

A number of psychoactive drugs are suspected of inducing chromosome aberrations, dominant lethals and micronuclei in mice (Prabhakar, 1978, Prabhakar and Sanfeeva, 1981, Sasaki *et al.*,1983 and Kar and Das, 1987).

Also, some tranquilizers induced changes in chromosome numbers and chromosome aberrations in chinese hamsters (Lafi *et al*., 1987 and Lafi and Parry,

1988). Tranquilizers are group of drugs introduced as psychotherapeutic agents, are being extensively used in human medicine to allay anxiety and muscle tension (Byck, 1974).

Lorazepam is widely used benzodiazepine class anxiolytic drug (Hietala, *et al.* 1977). Until recently, research indicated that all benzodiazepines impair explicit memory in humans, while only lorazepam impairs priming. Stewart and Teehan (1998), found that memory is impaired by lorazepam in humans.

The present study was carried out to evaluate the mutagenic response of Lorazepam the active ingredient of ativan using two test systems, the sex-linked recessive lethal test in *Drosophila melanogaster* which is known to be efficient in detecting chemical mutagens (Sobels and Vogal, 1976), and by estimating the effect of the drug on the activity of cholinesterase and aliesterase enzymes, which is a recent tool in mutagenicity testing. (Tobgy, *et al*., 1976).

Materials and Methods

1. Strains

Two strains of *Drosophila melanogaster* were used in the present study:

a. Oregon-K(O-K)

This stock is a wild type strain that has always been used in *Drosophila* laboratories. It was obtained from the department of Genetics, Ain Shams University, Cairo, A.R.E. This strain was repeatedly tested to determine its spontaneous sex-linked recessive lethals (S.L.R.L).

b. Mullar-5(M_5)

A marker strain of *Drosophila melanogaster* was used for the detection of sex-linked recessive lethal mutations. Its X-chromosome carries a dominant marker bar eye (B) and a recessive mutant eye color, white apricot (W^a). It has also two inversions, the first is scute (Sc^{8r}) inversion and the second designated (in-s), is included in the first inversion .

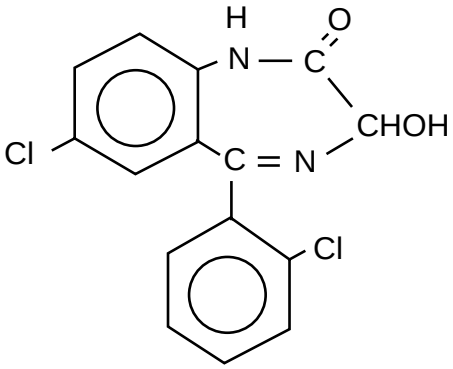
2. Chemicals

a . Lorazepam (ativan): tablets product by Wyeth-Pharma GMBH Munster/Westfalen, Germany.

The active ingredient of ativan is lorazepam (molec. From $C_{15}\ H_{10}\ Cl_2\ N_2\ 0_2$; Molec. Wt. 321-2.

Chemical name: 7-chloro-5-(chlorophenyl)-1, 3-dihydro-3-hydroxy-2H 1,4-benzodiazepine-2-one.

Chemical formula:



Lorazepam

b. Reagents: The following reagents were utilized throughout enzyme activity evaluation.

- Phosphate buffer, (M/15 at pH = 7.2).
- Acetyl choline (0.04 M).
- Hydroxylamine hydrochloride (2 M).
- Sodium hydroxide (3.5 M).
- Alkaline hydroxylamine solution.
- Diluted hydrochloric acid.
- Ferric chloride (0.37 M)

3. Methods

Two methods were carried out in the present study to assess the mutagenic effect of the psychoactive drug (lorazepam). They are used according to the following publications:

– Mullar (1972) and Brusick (1980) for *Drosophila* sex-linked recessive lethal (SLRL) assay.

– Augustinson (1961) for detecting enzyme activities (ChE and AliE) in *Drosophila.*

In this investigation, Oregon-K of *D. melanogaster* males were treated with two concentrations of drug was used, 5.0 and 10.0 mg/100ml of corn meal food medium and detection of SLRL.

Also, three categories were analyzed for enzyme activities: F_1 females heterozygous, F_2 females and F_2 wild type males.

Statistical Analysis

For sex-linked recessive lethal, the Kasten baum and Bowman test was used to test significance of the results. (Wurgler *et al*., 1975).

As significance test for enzyme estimation the F test was used.

Results and Discussion

1. Induction of Sex-linked Recessive Lethals

As can be seen in Table 1 the spontaneous SLRL percentage (control) ranged from 0.13 to 0.27.

TABLE 1. Identification of sex-linked recessive lethals occurring spontaneously and after treatment with two concentrations of ativan in three broods of *Drosophila melanogaster.*

Experiment	Broods	No. of chromosome	Lethals	
			Total	%
Control	1	740	2	0.27
	2	745	1	0.13
	3	740	0	0.00
	Total	2225	3	0.13
Ativan 5%	1	693	6	0.87*
	2	718	21	2.92**
	3	722	19	2.63**
	Total	2133	46	2.16
Ativan 10%	1	744	2	0.27**
	2	740	9	1.22**
	3	742	23	3.10**
	Total	2226	34	1.53

*P0.05 **P0.01

The results obtained from the SLRL test after treatment with the two-concentrations ativan (5.0 and 10.0 mg/ml of medium) are summarized in Table (1) and presented graphically in Figure (1). The data show that the two-concentrations of ativan mainly induced SLRL mutation in the second and third broods. According to Demerec, (1950), the first brood represents thes-permatozoa and the second brood represents spermatids, while the third brood represents the spermatocytes.

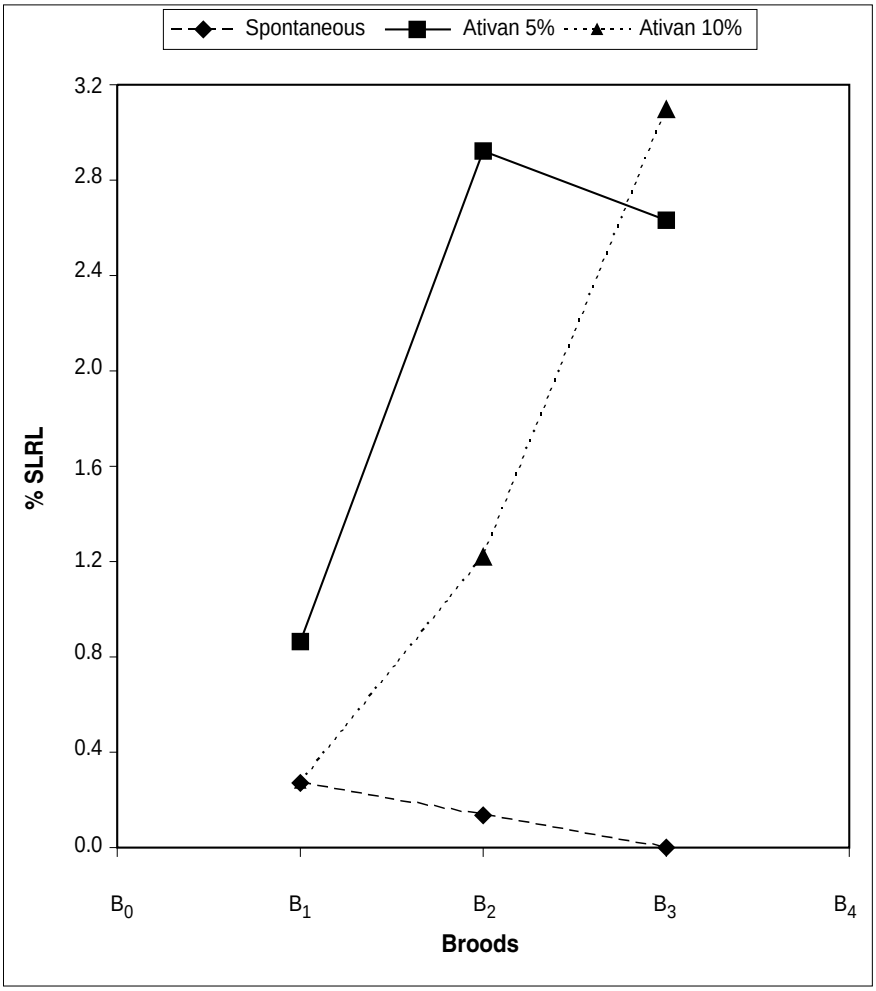


FIG. 1. Sex linked recessive lethals in three broods of *Drosophila melanogaster* occurring spontaneously and after treatment with two concentations of ativan.

Accordingly, ativan at 5% concentration induced lethality with a frequency of about 2.92% in the spermatid stage, and in the spermatocytes about 2.63%. Since the lethal frequency reached only 0.86% in the spermatozoa stage treatment with ativan 10% showed a significant increase of lethals in second and third broods, while it was not significant in the first brood. This indicates that ativan 10% did not induce SLRL's in the spermatozoa stage. Also the mutagenic potentiality of it appeared to be higher in the spermatocytes than in the spermatids. It is of interest to note here that spermatids of this strain showed higher sensitivity to the mutagenic effect of ativan 5% than ativan 10%, while the spermatocyte stage showed higher sensitivity for mutagenic effect of ativan 10% than ativan 5%. This result suggests a mutagenic effect of lorazepam in the induction of sex linked recessive lethals in *Drosophila melanogaster.* This is in agreement the results obtained by Sanjeeve and Pratap, (1974) and Susheela, (1975, 1981), who found that the benzodiazepine group of tranquilizers induces SLRLs in *Drosophila melanogaster.* Meanwhile, these results disagree with the results obtained by Degraeve *et al.* (1985), who found that the benzodiazepines showed a weak mutagenic effects in mice. Also, Husun *et al.* (1985), failed to observe mutagenic effects of diazepam in human lymphocytes.

2. Mutagenic Effect of Ativan (lorazepam) on the Enzyme Activities

The second part of this investigation was carried out to estimate the activities of the two enzymes ChE and AliE in some insects of two generations of SLRL: F_1 females, F_2 bar eye females and F_2 wild type males. Tables 2 and 3 showed that ativan caused changes in ChE and AliE activities due to its mutagenic potentiality and it can be easily notice that lowest values of the activities of the two enzymes were observed with the two concentrations of the drug. The mean values of ChE activities in both males and females were for the control 559.2 units, while in the treated experiments with ativan they dropped to 524 units in the 5% concentration and 464.5 units in the 10% respectively. The AliE activities were 154.4 units in the control group while they decreased to 100.4 units and 92.6 units under the effect of 5% and 10% ativan treatment respectively. Statistical analysis indicated that the differences of F_1 females, F_2 females and F_2 males with the control were significant in the ativan 5% experiment of ChE. Moreover, the differences between ChE activities after ativan 10% and control were highly significant, this results are presented graphically in Figure 2. Also, as shown in Table 3 and Figure 3, AliE activity in both ativan treatments, 5% and 10%, was less than in the control experiment. Statistical analysis indicated that the differences between control and treatment with ativan 5% in AliE activities of the three categories were significant, while in ativan 10% experiment the decrease in the activities of AliE was highly significant. This results is in agreement with Loewenstein *et al.* (1993), who observed that carbamate com-

pounds led to a drop of ChE in *Drosophila*. Data for ChE in F_2 females were more variable than those in F_1 which was noticed in both concentration of ativan. This would suggest that ChE activity is a polygenic trait, which is in accordance with the findings of Salam *et al.* (1995), who mentioned that the genetic background of different steps of acetylation and liberation of the enzyme differentiality of the chemicals used. Also, the results of AliE activity due to its mutagenic potentiality. The variable For substrates of this enzyme suggest that AliE is a group for enzymes and also, that even in the presence of a major structural gene, the presence of some modifiers or minor genes is highly expected (Tobgy *et al.* 1976), Nagy *et al*. (1986),and Salam, *et al.* (1995). This conclusion would facilitate the illustration of the variable occurrence of mutational events. Furthermore the data of the two test systems for mutagenicity indicated that the drug lorazepam (ativan) of benzodiazepine derivative used in the present study had mutagenic potentiality on the genetic background. On the other words, ativan exhibited a high mutagenic effect on *Drosophila melanogaster.*

TABLE 2. Effect of ativan 5% and ativan 10% on ChE activity in three categories of *Drosophila melanogaster.*

Category		ChE activity (units)*		
		Control	Ativan 5%	Ativan 10%
F_1	B_1	566.7	537.6**	450.0***
	B_2	522.2	499.6**	443.2***
	B_3	544.9	494.0**	428.7***
♀	Total	1633.8	1531.2	1321.9
	Mean	544.6	510.4	440.6
F_2	B_1	570.3	557.7**	494.0***
	B_2	537.6	522.2**	479.0***
	B_3	537.6	506.9**	434.2***
♀	Total	1645.5	1586.8	1407.2
	Mean	548.5	528.9	469.1
F_2	B_1	577.6	559.5**	508.6***
	B_2	574.0	530.4**	479.5***
	B_3	572.0	508.6**	463.3***
♂	Total	1723.6	1598.5	1451.4
	Mean	574.5	532.8	483.8

*one unit of ChE activity is expressed as one Ug of acetylcholine (Substrate) reacting with ChE in on ml of 100 flies homogenate in one hour incubation at 37ºC (Nagy, 1986).

**P0.05 ***P0.01

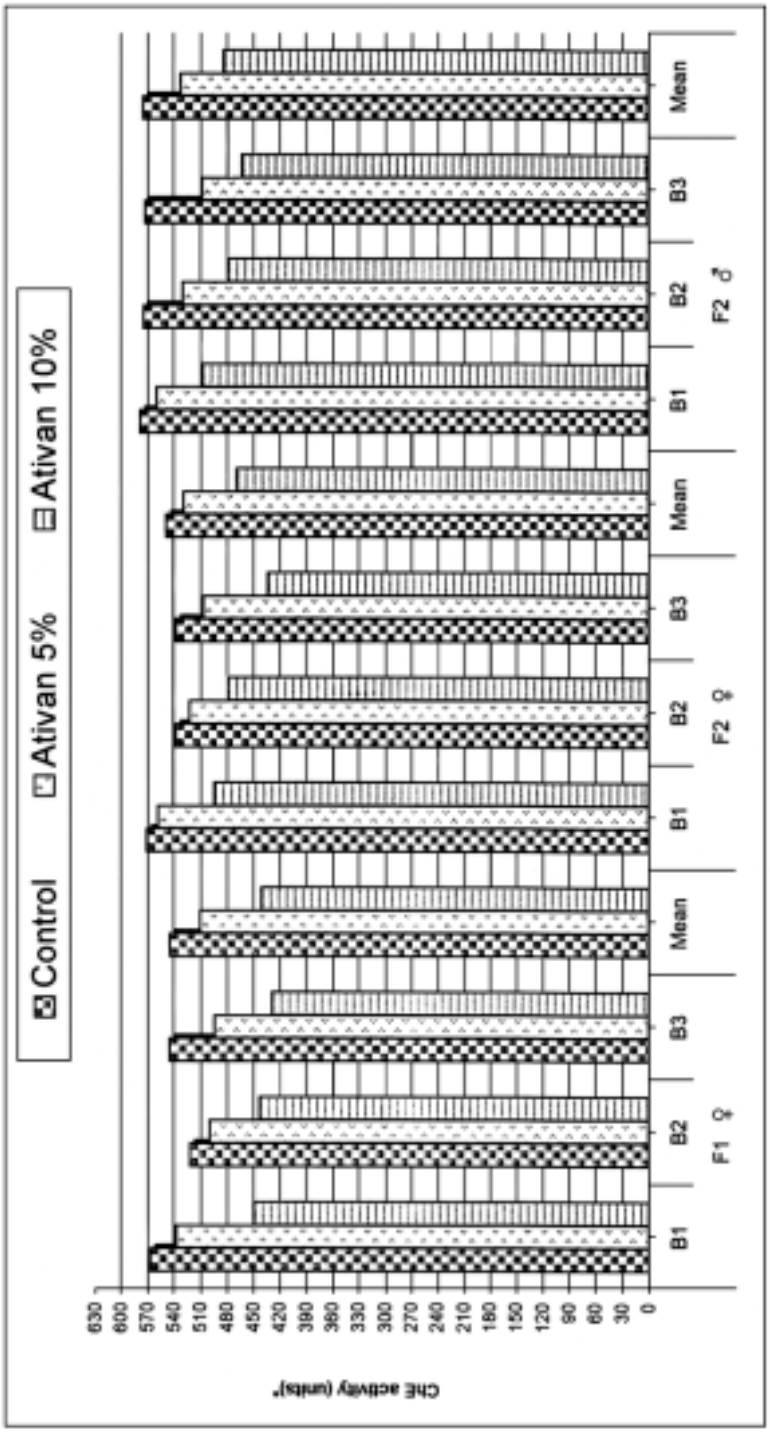


FIG. 2. Effect of ativan 5% and ativan 10% on ChE activity in three categories of *Drosophila melanogaster*.

TABLE 3. Effect of ativan 5% and ativan 10% on AliE activity in three categories of *Drosophila melanogaster.*

Category		AliE activity (units)*		
		Control	Ativan 5%	Ativan 10%
F_1	B_1	190.0	104.0**	72.0***
	B_2	168.0	84.0**	64.0***
	B_3	132.0	80.0**	64.0***
♀	Total	490.0	268.0	200.0
	Mean	163.3	89.3	66.7
F_2	B_1	136.0	104.0**	112.0***
	B_2	132.0	104.0**	104.0***
	B_3	128.0	96.0**	96.0***
♀	Total	396.0	304.0	312.0
	Mean	132.0	101.3	104.0
F_2	B_1	184.0	116.0**	112.0***
	B_2	168.0	112.0**	108.0***
	B_3	152.0	104.0**	100.0***
♂	Total	504.0	332.0	320.0
	Mean	168.0	110.7	106.7
Total		1390.0	904.0	832.0
Mean		154.4	100.4	92.4

*one unit of AliE activity is expressed as one Ug of methyl butyrate (Substrate) react with AliE in one ml of 100 flies homogenate in 15 minutes incubation at 37ºC.

**P0.05 ***P0.01

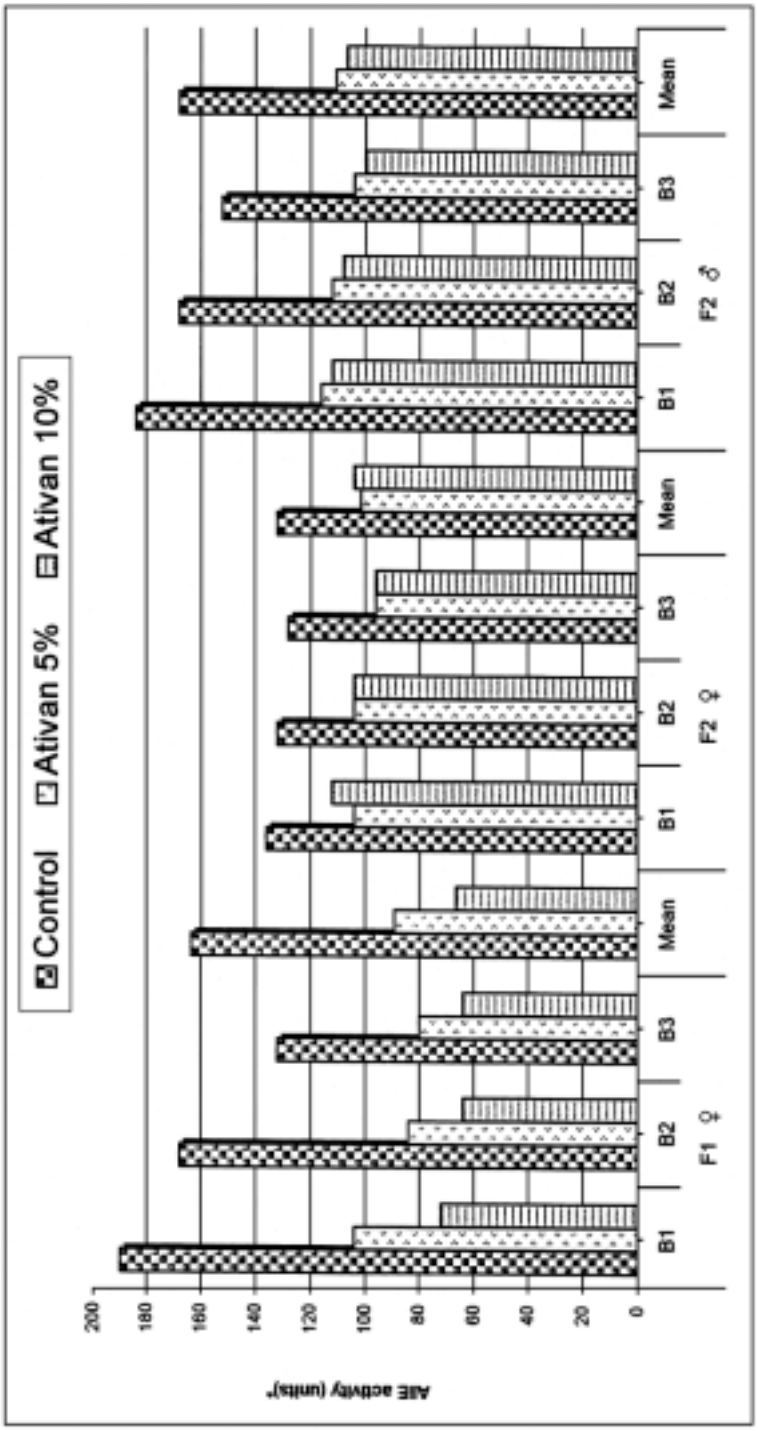


FIG. 3. Effect of ativan 5% and ativan 10% on AliE activity in three categories of *Drosophila melanogaster*.

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دراسة السمية الوراثية لعقار اللورزبام (الأتيفان) باستخدام حشرة *الدروسوفيلا ميلانوجاستر*

ندى حسن التواتي

قسم علوم الأحياء ، كلية العلوم ، جامعة الملك عبد العزيز

جـــدة – المملكة العربية السعودية

المستخلص. استهدفت هذه الدراسة تقدير السمية الوراثية لأحد العقاقير المهدئة وهو اللورزبام (الأتيفان) وقد تم تقدير المقدرة الطفرية لهذا العقار بقياس الطفرات المميتة المنتحية المرتبطة بالجنس باستخدام الحشرات البالغة لحشرة *الدروسوفيلا ميلانوجاستر* من سلالة M5 مع استعمال تركيزين من العقار وهما ١٠,٥ ملجم لكل ١٠٠مل من البيئة المستخدمة لتغذية الذكور .

كما تم تقدير النشاط الإنزيمي لإثنين من الإنزيمات التي لها دور في النظام العصبي وهما إنزيم كولين استريز (Ch E) وآلي استريز (Ali E) لبعض الحشرات الناتجة من التجارب المميتة المنتحية المرتبطة بالجنس .

وكانت النتائج المتحصل عليها تفيد بأن للعقار القدرة على استحداث طفرات مميتة منتحية مرتبطة بالجنس في حشرة *الدروسوفيلا ميلانوجاستر* وأيضًا له القدرة على تغير مستوى النشاط الإنزيمي لكل من أنزيم الكولين استريز والآلي استريز .