

## Genotoxicity of paracetamol on the germ cells of *Drosophilla melanogaster*

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### Abstract

Paracetamol is a common analgesic and antipyretic drug. The aim of the present study is to determine the potential genetic effects of Paracetamol in *Drosophilla melanogaster* using two methods: Sex Linked Recessive Lethals (SLRL) test and effect of Paracetamol on enzyme activity using spectrophotometric analysis. Three concentrations of drug were used (5, 10, and 20 mM). The results reveal significant differences in S.L.R.L, except spermatozoa stages showed insignificant increases when the data of the four broods were considered all together in three treatments. Meanwhile, Paracetamol showed a genotoxic effects in the three categories of the two generations of S.L.R.L, F1 heterozygous females, F2 bar eye females and F2 wild type males on the genetic back ground of Cholinesterase in all treatments.

### Introduction

Excessive consumption of any analgesic compound can produce harmful unwanted effects. It is now well established long term ingestion of analgesic compounds may result in analgesic nephropathy with impaired renal function. Paracetamol has been used extensively as an analgesic and antipyretic drug. Several reports have indicated genotoxic effects of Paracetamol. It was reported that Paracetamol also causes chromosomal aberrations in Chinese hamster fibroblast cells *in vitro* [Ishidate and Yqshikawa, 1980]. Paracetamol has been shown to induce liver-cell tumours in mice after long- term feeding (Flaks and Flaks, 1983), and to induce DNA damage in mouse-liver cells in culture (Dybing *et al.*, 1984). Dunn *et al.*, 1987 were found that Paracetamol induced micronuclei in rat-kidney fibroblasts. *In vivo* and *in vitro* experiments were discovered that Paracetamol inhibits both replicative DNA synthesis and DNA repair synthesis animals. It is also causes DNA damages and increases the frequency of chromosomal aberrations in mammalian cell lines and isolated human Lymphocytes [Honglo *et al.*, 1990, Honglo and Holme, 1994, Rannug *et al.*, 1995]. Tapadia and Lkhotia (1997) were investigated the specific induction of the hsr omega heat

shock gene locus of *Drosophilla melanogaster* by Paracetamol. Furthermore, Skorpen *et al.* (1998) demonstrated that Paracetamol increases sensitivity to ultraviolet (UV) irradiation, delays repair of the Uracil - DNA glycosylase and recovery of RNA synthesis in human keratinocyte cells. In some animals, small doses of paracetamol are toxic [Allen, 2003]. Walubo *et al.* (2004) said that co-administration of Paracetamol with inhibitors of cytochrome P450 prevented the development of Paracetamol- induced hepatotoxicity in rats. Also, Ucheya and Igweh (2006) suggested that histological changes in kidney structure following a long-terms administration of Paracetamol (Acetaminophen) in pregnant Sprague Dawleg rats, induces toxicity revealed by induced hyperacidity in combination with acute or chronic inflammation (Rainsford and White house, 2006). However, Because of the wide availability of paracetamol there is a large potential for overdose and toxicity [Pardale *et al.*, 2006 and Pardale, 2007]. The present study was carried out to investigate the nature of the response of *Drosophilla melanogaster* male germ cells to three concentrations of Paracetamol (acetami-nophen) using the well known Muller-5 technique for the detection of sex

Linked recessive lethal mutations and the estimation of the activity of the cholinesterase enzyme (ChE) in three categories of the two generations of S.L.R.L.

## Material and Methods.

### 1. Strains:

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

#### *a-Muller-5 (M-5):*

A marker strain of *D. melanogaster* 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.

#### *b- Oregon- R(O-R):*

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

### 2. Chemicals:

a- Paracetamol ( *N*-(4-hydroxyphenyl) ethanamide) is a popular and common analgesic and antipyretic drug used for relieving fever, headaches, and other minor aches and pains.

b- Cholinesterase Detection Kit

Estimation of the activities of the enzyme Cholinesterase (CHE) from QUIMICA CLINICA APLICADA.

### 3. Methods:

#### *Two test systems were used:*

a-Mullar (1972) and Brusick (1980) for *Drosophila* Sex Linked recessive lethal (SLRL) assay.

b-The estimation of the activity of the enzyme Cholinesterase (ChE) in *Drosophila melanogaster*.

In this investigation, (O-R) of *D. melanogaster* males were treated on a medium contained three concentration of

paracetamol (5,10 & 20 mM) and detection of SLRL.

Also three categories were analyzed for enzyme activity: F<sub>1</sub> females heterozygous, F<sub>2</sub> females and wild type males.

#### Statistical Analysis:

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

2- As significance test for enzyme estimation, ANOVA test (SPSS program).

## Results and Discussion

### a. Induction of Sex-Linked Recessive Lethal:

As can be seen in Table (1) the results obtained from the SLRL test after treatment with three concentrations of Paracetamol (5, 10 and 20 mM). The data show that the three concentrations of Paracetamol induced SLRL mutation except spermatozoa stages showed insignificant increases when the data of the four broods were considered all together in three treatments. This result suggests a mutagenic effect of Paracetamol in the induction of sex Linked recessive Lethals in *Drosophila melanogaster*. This is in agreement with the results obtained by many other laboratories. Tapadia and Laxhotia (1997) reported that Paracetamol was induced a high rate of transcription at the *hsr omega* heat shock gene in polytene chromosomes of *Drosophila melanogaster*. Genotoxic effects of Paracetamol have been demonstrated both *in vitro* and *in vivo*, the data indicated that Paracetamol may contribute to an increase in the total burden of DNA-damage in man (Hongslo and Holme, 1994). Hongslo *et al.* (1991) and Hongslo *et al.* (1994) investigated the genotoxicity of Paracetamol in mice and rats. The results suggested that Paracetamol induced DNA damaged. Also, Brunborg *et al.* (1995) found that Paracetamol interferes with nucleotide excision repair in several mammalian cell types, and may contribute to genotoxicity in humans. Moreover, Bergman *et al.* (1996) reported that paracetamol causes chromosomal damage

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in vitro in mammalian cells at high concentrations. Furthermore, Mendoza *et al.* (2003) investigated the effects of D-003, a mixture of high molecular weight primary acids from sugar cane, on Paracetamol – induced liver damage in rats.

Aganovice- Musinovic *et al.* (2004) suggested that Paracetamol had mutagenic potential in *Allium cepa*. Also, brulj *et al.* (2007) demonstrated that Paracetamol concentration of 200 mug/ml expresses certain genotoxic effects in human peripheral blood lymphocytes. On the other hand, National toxicology program (1993) reported that p-Nitrophenol (production of paracetamol) was not mutagenic in *Salmonella typhimurium* with or without exogenous metabolic (S9) activation, or in germ cells of male *Drosophila melanogaster*. Also, in *Chinese hamster* ovary cells, no induction of sister chromatid exchanges was observed with or without S9, but a significant increase in chromosomal occurred in trials conducted with S9.

### **b. Mutagenic effect of Paracetamol on the enzyme activity.**

The second part of this investigation was carried out to estimate the activity of ChE enzyme in some insects of two generation of SLRL: F1 females F2 bar eye females and F2 wild type male. Table2 showed that Paracetamol caused changes in

ChE activity due to its mutagenic potentiality and it can be easily notice that lowest values of the activity of the ChE were observed with the three concentrations of drug. The ChE activity was 22846 units in the control group of F1 females while they decreased to 13992, 15536.5 and 14238 units under the effect of 5 mM, 10 mM, and 20 mM, of Paracetamol treatments respectively in F1 females. Similar results were obtained from F2 bar eye females and F2 wild type males. Statistical analysis indicated that the differences of F1 females, F2 females and F2 males with the control were highly significant. This result is in agreement with Loewenstein *et al.*, (1993), who observed that carbamate compounds led to a drop of ChE in *Drosophila melanogaster*. Data for ChE in F1 and F2 females were more variable than those in F2 male which was noticed in 5 mM concentration of Paracetamol. This would suggest that ChE activity may be polygenic trait, which is in accordance with the findings of Salam *et al.*, (1995) and Al- Twaty, (2004). The data of the two test systems for mutagenicity indicated that Paracetamol used in the present study had mutagenic potentiality on the genetic background. However, further experiments are needed to discover the possible role of maternal and genotypic modifying effects.

**Table 1: Identification of sex linked recessive lethals occurring spontaneously and after different treatments with paracetamol in *D.melanogaster***

Treatments	Sperms B1			Spermatides B2			Spermatocytes B3			Spermatogonia B4			Total		
	N.	L.	%	N.	L.	%	N.	L.	%	N.	L.	%	N.	L.	%
Control	982	2	0.20	1080	1	0.09	1029	1	0.01	810	2	0.25	3901	6	0.15
Paracetamol 5m M	865	6	0.7	784	3	0.38*	437	3	0.69	344	1	0.3*	2430	13	0.53
10 m M	793	6	0.76	724	2	0.28**	693	4	0.58*	562	2	0.36*	2772	12	0.43
20 m M	751	4	0.53	635	2	0.31**	591	3	0.51*	598	3	0.5*	2575	12	0.47

N. = Number of tested chromosomes, L. = Number of lethal mutations (SLRL), %=Frequency of SLRL.; Plevel of significant

Significance: \* P < 0.05 and \*\* P < 0.01

**Table 2: Effect of Paracetamol with different treatments on Cholinesterase (ChE) activity in three categories of *D. melanogaster***

Category		ChE activity (units)*			
		Control	Paracetamol 5 mM	Paracetamol 10 mM	Paracetamol 20 mM
F1 ♀	B1	22827	13245**	13102**	10281**
	B2	24637	16872**	17523**	16238**
	B3	13767	13259	12039*	12112*
	B4	30153	12392**	19482**	18321**
	Mean	22846	13992	15536.5	14238
F2 ♀	B1	37616	21023**	16049**	18245**
	B2	26000	20045**	18923**	16782**
	B3	52753	35325**	33427**	32740**
	B4	31509	22584**	27705**	26764**
	Mean	36969.5	24744.25	24026	23632.75
F2 ♂	B1	53054	52230	25873**	46862**
	B2	50227	49860	42498**	42499**
	B3	32363	32200	27632**	21645**
	B4	34809	33999	21902**	13538**
	Mean	426132	42.72,25	29476.25	31136

\*One unit of ChE activity is expressed one Ug of activity choline (substrat) reacting with ChE in on ml of 100 flies homogenate in one hour incubation at 37°C.

Significance: \* P < 0.05 and \*\* P < 0.01

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## السمية الوراثية لعقار الباراسيتامول على الخلايا الجرثومية لحشرة الدروسوفيلا ميلانوجاستر

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الباراسيتامول هو عقار شائع الاستعمال كخافض للحرارة ومسكن للألام. الهدف من الدراسة الحالية هو إمكانية تحديد التأثيرات الوراثية للباراسيتامول في حشرة الدروسوفيلا ميلانوجاستر وتقدير النشاط SLRL باستخدام بطريقتين: اختبار الطفرات المميتة المتنحية المرتبطة بالجنس باستخدام التحليل الطيفي وقد تم استخدام ثلاثة الإنزيمي لإنزيم كولين استريز ChE تركيزات من الباراسيتامول (5 , 10, and 20 Mm). كشفت النتائج عن وجود فروق معنوية في الطفرات المميتة المتنحية المرتبطة بالجنس عالية في النشاط الإنزيمي ماعدا في مراحل الطلائع المنوية حيث أظهرت فروق غير معنوية لكلا الجيلين وكانت متساوية في المعاملات الثلاثة. وقد أظهر الباراسيتامول تأثيرات وراثية في جيلين من الطفرات المميتة المتنحية المرتبطة بالجنس لإناث الجيل الأول الخليطة وإناث الجيل الثاني ذات العيون الحمراء العودية وذكور الجيل الثاني البرية على الخلفية الوراثية للإنزيم كولين استريز في كل المعاملات.