Genotoxicity of paracetamol on the germ cells of
Drosophila 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 Drosophila 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
[Hongslo et al., 1990, Hongslo 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 Drosophila 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
Drosophila 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 acute (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:
   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: F1 females heterozygous, F2 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...
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 µg/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 trail, 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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Sperms B1</th>
<th>Spermatides B2</th>
<th>Spermatocytes B3</th>
<th>Spermatogonia B4</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>N. 982</td>
<td>L. 2</td>
<td>% 0.20</td>
<td>N. 1080</td>
<td>1</td>
</tr>
<tr>
<td>Paracetamol 5m M</td>
<td>N. 865</td>
<td>L. 6</td>
<td>% 0.7</td>
<td>N. 784</td>
<td>3</td>
</tr>
<tr>
<td>10 m M</td>
<td>N. 793</td>
<td>L. 6</td>
<td>% 0.76</td>
<td>N. 724</td>
<td>2</td>
</tr>
<tr>
<td>20 m M</td>
<td>N. 751</td>
<td>L. 4</td>
<td>% 0.53</td>
<td>N. 635</td>
<td>2</td>
</tr>
</tbody>
</table>

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*

<table>
<thead>
<tr>
<th>Category</th>
<th>ChE activity (units)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>F1♀</td>
<td>22827</td>
</tr>
<tr>
<td>B1</td>
<td>24637</td>
</tr>
<tr>
<td>B2</td>
<td>13767</td>
</tr>
<tr>
<td>B3</td>
<td>30153</td>
</tr>
<tr>
<td>Mean</td>
<td>22846</td>
</tr>
<tr>
<td>F2♀</td>
<td>37616</td>
</tr>
<tr>
<td>B1</td>
<td>26000</td>
</tr>
<tr>
<td>B2</td>
<td>52753</td>
</tr>
<tr>
<td>B3</td>
<td>31509</td>
</tr>
<tr>
<td>Mean</td>
<td>36969.5</td>
</tr>
<tr>
<td>F2♂</td>
<td>52363</td>
</tr>
<tr>
<td>B1</td>
<td>32633</td>
</tr>
<tr>
<td>B2</td>
<td>32200</td>
</tr>
<tr>
<td>Mean</td>
<td>34809</td>
</tr>
</tbody>
</table>

*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

References

السمية الوراثية لعقار الباراسيتامول على الخلايا الجرثومية لحشرة الدروسوفيلا ميلانوجاستر

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الباراسيتامول هو عقار شائع الاستعمال كخافض للحرارة ومسكن للألم.

الهدف من الدراسة الحالية هو إمكانية تحديد التأثيرات الوراثية للباراسيتامول في حشرة الدروسوفيلا ميلانوجاستر وتقييم النشاط SLRL للبلورات المحيطة المرتبطة بالجنس باستعمال التحليل الطيفي وقد تم استخدام الإنزيم ChE لإنزيم كولين استرائز ثلاثة تركيزات من الباراسيتامول (5, 10, and 20 Mm).

كشفت النتائج عن وجود فروق معنوية في الطفرات المحيطة المرتبطة بالجنس عالية في النشاط الإنزيمي ماعدا في مراحل الطنانه المبكرة حيث أظهرت فروق غير معنوية لكلا الجيلين وكانت متساوية في المعاملات الثلاثة.

وقد أظهر الباراسيتامول تأثيرات وراثية في جيلين من الطفرات المحيطة المنحية المرتبطة بالجنس لن ذات الجيل الأول الخليفية وإناث الجيل الثاني ذات العيون الحمراء العودية وذكور الجيل الثاني البرية على الخليفية الوراثية للإنزيم كولين استرائز في كل المعاملات.