Effect of stannous chloride combined with caffeine on fecundity of *Drosophila prosaltans*

Mary Massumi Itoyama and Hermione Elly Melara de Campos Bicudo

Abstract

The present study analyzed the number of progeny of stannous chloride- and/or caffeine-treated *Drosophila prosaltans*. A significant decreasing effect was observed in every case when compared to the control, except for the smallest stannous chloride dose used. Combinations of both substances using two different stannous chloride doses did not differ significantly from each other but number of progeny decreased 15% with the higher concentration combination when compared with caffeine-treated flies. The present results and data in the literature indicate that the effects of continuous ingestion of stannous chloride should be studied.

INTRODUCTION

Several studies have considered the sum effect of caffeine and other agents affecting DNA, such as X-rays (Mendelson and Sobels, 1974), gama-rays (Targa, 1983), UV radiation (Cremer et al., 1983), or substances such as the alkylation agent Thiotepa (Kilhman et al., 1982), tumorigenic drugs (Granberg-Ohman et al., 1980) and benzamide (Das, 1987). In some cases caffeine has shown a potentiating effect on mutagenic agents.

In this study we analyzed fecundity of *Drosophila prosaltans* treated with caffeine combined with stannous chloride (SnCl₂). In a previous study, in the same species, caffeine alone induced a dose-dependent fecundity decrease (Itoyama and Bicudo, 1992).

Mutagenic effects of caffeine were studied in prokaryotic as well as eukaryotic organisms with variable results, but in some cases it was considered a DNA repair inhibitor (Mendelson and Sobels, 1974; MacPhee and Leyden, 1985; Sasaki et al., 1989). Contradictory results were obtained for stannous chloride, in some cases showing genotoxic activity, causing changes in *Escherichia coli* DNA (Cunha et al., 1991) and decreasing SOS response in the same organism (Olivier and Marzin, 1987). In *Drosophila melanogaster* (Tripathy et al., 1990) and *Saccharomyces cerevisiae* (Singh, 1983), toxicity was observed but no mutagenic effect seemed to occur. Other studies on *E. coli* indicated induction of SOS response by treatment with SnCl₂ (Bernardo-Filho et al., 1994) and involvement of exonuclease III in repairing lesions caused by SnCl₂ (Cabral et al., 1998). Increasing doses of SnCl₂ were also observed to strongly reduce surviving of *E. coli* cells (Cabral et al., 1998). Thus, if SnCl₂ affects DNA and elicits DNA repair inhibited by caffeine, greater impairment might be expected from their ingestion together than separately.

Caffeine is largely consumed in coffee, teas and medicines (Benowitz, 1990) while stannous chloride, a powerful reducing agent, is used in radiopharmacy to synthesize scintigraphic tracers employed in nuclear medicine. The food industry uses it as a preservative (e.g., in soft drinks) and in some fluoride tooth pastes. Possible joint consumption of these drugs, therefore, makes relevant information on their effect, isolated or in interaction, even with other organisms used as indicators.

MATERIAL AND METHODS

*Drosophila prosaltans* (saltans group, saltans subgroup) is maintained in our laboratory at 20° ± 1°C, in banana-agar culture medium. The strain used is from Sangre Grande, Trinidad. Six-day-old virgin males and females were mass crossed (10 couples per vial) in bottles containing banana-agar culture medium (control experiments) or containing this culture medium with caffeine added (2,000 µg/ml; experiment named t₄) or with stannous chloride (500 µg/ml or 2,000 µg/ml; experiments named Sn₁ and Sn₂, respectively) or with both substances (t₄ + Sn₁ and t₄ + Sn₂). Six bottles were prepared for each type of medium. Flies were transferred once to new food of the same type, after five days. Parents were discarded five days later. Progeny were computed separately by sex.

Caffeine used was Cafeina Anidra, P.A. (1,3,7-trimethylxanthine), Reagen, Quimibrás, Indústrias Químicas S.A., Brazil. Stannous chloride (SnCl₂, H₂O) was Baker analyzed Reagent, Química Moura Brasil, S.A., Brazil.

For statistical purposes, exploratory data analysis, variance analysis for transformed data (square root) and lin-
ear model adjustment were used (Neter and Wasserman, 1974; Peres and Saldiva, 1983).

RESULTS AND DISCUSSION

Fly fecundity in the six experiments is in Table I. Because the numbers of males and females did not differ significantly from each other in the progeny of every experiment, only the total progeny numbers are presented. ANOVA (analysis of variance) applied to the transformed data showed significant values (F = 58.36; P ≤ 0.05). Multiple comparisons of Tukey (Table II) showed significant differences in comparisons of control experiment with every other experiment except that in which 500 µg/ml of stannous chloride was used alone. Both mixtures of substances (t₄ + Sn₁ and t₄ + Sn₂) differed significantly from Sn₁ and Sn₂ but not from t₄. Combined treatments also did not differ. Besides, results using the two doses of stannous chloride did not differ significantly from each other.

Administered isolatedly, caffeine as well as stannous chloride in a 2,000 µg/ml concentration of culture medium decreased fly fecundity significantly. As mentioned, for caffeine this had already been shown by Itoyama and Bicudo (1992) and Itoyama et al. (1998). However, when the flies were fed stannous chloride and caffeine combined, fecundity decrease did not differ significantly from flies fed caffeine alone. Nor did results differ when, combined with caffeine, stannous chloride in the doses of 500 µg/ml and 2,000 µg/ml was used, indicating that its effects were not cumulative with those of caffeine in decreasing fecundity when jointly administered. However, considering progeny number, there was a decrease of about 15% between t₄ + Sn₁ or t₄ + Sn₂ and t₄ (caffeine alone), suggesting that some additional toxic effect of stannous chloride cannot be completely discarded. Between Sn₁ and Sn₂, there was a decrease of about 10%. Thus, the present results in Drosophila prosaltans and the other data in literature, previously mentioned, recommend for further study continuous ingestion of stannous chloride.

ACKNOWLEDGMENTS

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RESUMO

A fecundidade de Drosophila prosaltans foi analisada em indivíduos tratados com ion estanoso, cafeína ou ion estanoso, em duas concentrações, combinado com cafeína. Em todos os casos, a fecundidade foi significativamente menor quando comparada com o controle, exceto na menor concentração de ion estanoso. Nas combinações das duas substâncias, a redução não foi significativamente maior que a causada pela cafeína sozinha, podendo indicar que o efeito das duas substâncias juntas corresponde ao próprio efeito da cafeína separada. Mas a redução da fecundidade foi 15% maior no tratamento que utilizou ion estanoso em maior concentração, sugerindo que seu efeito tóxico nesse parâmetro, mesmo em combinação com a cafeína, não deve ser desconsiderado.

REFERENCES


Table I - Total fecundity in the experiments. Drugs and doses per ml of banana-agar medium: t₄ = 2,000 µg/ml caffeine; Sn₁ = 500 µg/ml of stannous chloride; Sn₂ = 2,000 µg/ml of stannous chloride; t₄ + Sn₁ and t₄ + Sn₂ = combined treatments.

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Control</th>
<th>t₄</th>
<th>Sn₁</th>
<th>Sn₂</th>
<th>t₄ + Sn₁</th>
<th>t₄ + Sn₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bottle 1</td>
<td>1177</td>
<td>747</td>
<td>883</td>
<td>895</td>
<td>578</td>
<td>586</td>
</tr>
<tr>
<td>Bottle 2</td>
<td>1159</td>
<td>692</td>
<td>1241</td>
<td>1033</td>
<td>656</td>
<td>634</td>
</tr>
<tr>
<td>Total</td>
<td>2336</td>
<td>1439</td>
<td>2124</td>
<td>1928</td>
<td>1234</td>
<td>1220</td>
</tr>
</tbody>
</table>

C, control.

Table II - Multiple comparisons of Tukey for fecundity in the control and treated flies.

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Control</th>
<th>t₄</th>
<th>Sn₁</th>
<th>Sn₂</th>
<th>t₄ + Sn₁</th>
<th>t₄ + Sn₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>t₄</td>
<td>3.30*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sn₁</td>
<td>0.73</td>
<td>2.57*</td>
<td></td>
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<td></td>
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<tr>
<td>Sn₂</td>
<td>1.42*</td>
<td>1.88*</td>
<td>0.69</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>t₄ + Sn₁</td>
<td>4.20*</td>
<td>0.90</td>
<td>3.47*</td>
<td>2.78*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t₄ + Sn₂</td>
<td>4.27*</td>
<td>0.97</td>
<td>3.54*</td>
<td>2.85*</td>
<td>0.07</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05.
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