Effect of trypanocidal drugs on some aspects of the reproductive biology of female Glossina palpalis palpalis (Diptera: Glossinidae)

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**Key words**

Glossina palpalis palpalis - Drugs - Trypanosomiasis - Reproductive performance - Fertility - Survival - Abortion - Nigeria.

**Summary**

Effects of isometamidium chloride (Samorin) and diminazene aceturate (Berenil) on some aspects of the reproductive biology of female Glossina palpalis palpalis (Gpp) were investigated. Samorin and Berenil were administered to the flies in vitro, through a silicone membrane at 0.14 mg/ml and 0.40 mg/ml blood respectively or in vivo on ears of rabbits treated at recommended prophylactic and therapeutic doses of 1 mg and 3.5 mg/kg body weight respectively. Flies were maintained at 24.5 ± 0.5°C, 80 ± 5% RH and 6 h photoperiod. Neither Berenil nor Samorin at the concentrations employed had any adverse effect on female survival rate, fecundity or mean puparial weights. Rather, females fed on Berenil at 0.40 mg/l blood through membrane had the best survival rate, fecundity and mean puparial weight amongst the groups, including control, also flies that emerged from pupae deposited by these flies were noted to be the most active (cage activities). The implication of these findings in relation to vector control is highlighted.
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**In vitro diet**

The diet consist of a mixture (50:50; v/v) of fresh frozen heparinized bovine and porcine blood. Sodium heparin was drawn from a concentration of 0.4 mg/10 ml of distilled water and added to blood at 10 ml and 12 ml per 25 l of blood respectively. Before storage at -20°C, each batch of blood was separately treated to an initial 50 krad of gamma-irradiation in a cobalt60 source. After mixing, and before the diet was used for feeding, the mixture was treated to an additional 100 krad of irradiation. Just before the flies were fed, a phagostimulant, adenosin-5-triphosphate (ATP), drawn from a concentration of 1 mg/4 ml of distilled water was added to the diet at the rate of 2 µl/ml of blood.

**Trypanocides**

Deminazene aceturate (Berenil) and isometamidium chloride (Samorin) were used. These were administered to the rabbits intramuscularly or directly dissolved into the *in vitro* diet.

**Experimental procedures**

A total of six experimental groups were investigated.

**Groups I and II**

Blood containing Berenil at 0.4 mg/ml and Samorin at 0.14 mg/ml was fed *in vitro* on two consecutive days to groups I and II flies respectively. Flies were kept for 55 days, by which time most females would have completed four reproductive cycles. Pupae collected were sorted out into the five weight classes, using a mechanical sorting device (20). Abortions were checked daily. All the dead flies were dissected and their uterine contents examined.

**Groups III and IV**

Rabbits treated with Berenil at 3.5 mg/kg body weight and Samorin at 1 mg/kg body weight were used on two consecutive days in the *in vivo* feeding of groups III and IV flies respectively. Otherwise, the procedure was the same as above.

**Group V**

Flies were fed *in vitro* on unmedicated blood throughout the period of experiment, again along the same procedure as above. The group served as control for groups I and II.

**Group VI**

Flies were maintained on untreated rabbits throughout the experimental period, otherwise the procedure was the same as from the 2nd sentence in “Groups I and II”. The group served as control for groups III and IV.

**RESULTS**

Table I shows a summary of survival and reproductive performance of flies in all the experimental groups. The results show the reproductive status from the dead and surviving females, including abortions recovered.

Surviving females at day 55 post emergence in all the groups did not show any significant difference ($x^2 = 7.84; df = 5; p > 0.05$), even though group II flies had the least survival rate (51.1%). Although group I flies recorded the highest number of pupae (161), there was no significant differences in fecundity (puparia/initial female) ($x^2 = 0.103; df = 5; p > 0.05$), nor in the puparia weight class frequency distribution ($f = 0.04, df = 5; p > 0.05$). However, group I flies had the highest frequency in the heaviest weight class, also pupae produced in this group were observed to be exceptionally darker, with flies that emerged from them noted to be most active (cage activities). There was no significant difference in the mean puparial weights ($x^2 = 0.14; df = 5; p > 0.05$). With the exception of group I flies that larviposited a day earlier, all the remaining groups larviposited on day 20 post emergence.

**Table I**

<table>
<thead>
<tr>
<th>Exp. group</th>
<th>Treatment</th>
<th>Initial No. of females</th>
<th>No. of puparia deposited</th>
<th>Puparia/puparina female (ppf)</th>
<th>Puparia freq. distribution into the five weight classes</th>
<th>Mean puparia weights (mg ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Berenil (M)</td>
<td>51</td>
<td>163</td>
<td>3.21</td>
<td>3 12 53 63 25</td>
<td>30.58 ± 0.54</td>
</tr>
<tr>
<td>II</td>
<td>Samorin (M)</td>
<td>47</td>
<td>122</td>
<td>2.60</td>
<td>11 23 47 32 9</td>
<td>28.10 ± 1.01</td>
</tr>
<tr>
<td>III</td>
<td>Control (M)</td>
<td>51</td>
<td>159</td>
<td>3.12</td>
<td>5 12 58 67 17</td>
<td>30.12 ± 0.76</td>
</tr>
<tr>
<td>IV</td>
<td>Berenil (R)</td>
<td>48</td>
<td>146</td>
<td>3.04</td>
<td>4 10 66 56 10</td>
<td>29.77 ± 0.87</td>
</tr>
<tr>
<td>V</td>
<td>Samorin (R)</td>
<td>48</td>
<td>152</td>
<td>3.17</td>
<td>5 11 54 66 15</td>
<td>29.94 ± 1.21</td>
</tr>
<tr>
<td>VI</td>
<td>Control (R)</td>
<td>47</td>
<td>156</td>
<td>3.32</td>
<td>2 11 53 71 19</td>
<td>30.58 ± 0.96</td>
</tr>
</tbody>
</table>

M: membrane (drug was administered to the flies through membrane feeding)
R: rabbit (drug was administered to the flies through feeding on rabbits)
Results of dissection from the female daily mortality did not differ in their uterine contents in groups fed in vitro (t = 1.81; df = 4, 11; p > 0.05) and those fed on rabbits (F = 0.97; df = 4, 11; p > 0.05). The number of empty uteri due to aborted pre-mature larval stages and blockages did not differ between all the groups. Also there were few recorded cases of empty uterus due to aborted pre-mature stages and blockages in female survivors, with the majority of them having 2nd or 3rd instar larvae in utero.

**DISCUSSION**

Attempts made in the past are assessing the effect of trypanocides on survival and reproductive performance of *Glossina* spp. have yielded unreconcilable results. Whereas Oladunmade and Balogun (16) claimed that Samorin administered to flies *in vivo* through rabbits at a prophylactic dose of 0.5 mg/kg body weight adversely affected survival and reproductive performance of *G. morsitans*, Moloo and Kutuza (13) and Abbeele et al. (1) on the contrary did not observe any adverse effect of Samorin on survival, reproductive performance and gut endosymbionts in *G. m. centralis* and *G. p. respectively*, when they fed flies on animal host treated at a prophylactic dose of 1 mg/kg body weight.

Results from this investigation have shown that neither Berenil nor Samorin, at the concentrations employed and the forms (metabolite or unchanged) administered to the insect vector had any negative effect on survival and reproductive performance of the flies. This is evident from the high fecundity values and the dissection results of both dead and surviving flies, that revealed the majority of these females with 2nd and 3rd instar larval stages in utero, thus indicating their high reproductive potentials. Besides, the low number of cases of abortions in the premature larval stages and the few ovarian blockages also serve as evidence against any possible negative effect of these drugs on the gut endosymbiotic bacteroids. These microorganisms are known to play a significant role in furnishing the host fly with essential amino acids, vitamins of the B-complex and other substances for embryonic development (4, 5, 12). Although antibacterial properties of Berenil have been observed with *Brucella* spp and streptococci species in livestock—Taylor et al., 1956, quoted in Mulligan (14)—factors responsible for the enhancement of productivity in the group I flies are not understood. Berenil administered at 0.4 mg/ml blood and fed to the flies through membrane may have had some antibacterial activity against species of bacteria that have been identified and found to cause heavy mortality and poor reproductive performance in tsetse colonies fed *in vitro* (2). Parenteral administration of Samorin in livestock and laboratory animals did not give rise to any putative metabolite (11, 17), thus ruling out the possibility of *in vivo* fed flies of picking up any metabolite. However, Taylor (18) and Phillip et al. (17) have shown that Samorin orally administered to cattle, rats and mice breaks down into homidium (curative trypanocide) due to the acidic environment in the stomach. It therefore implies that both *in vitro* and *in vivo* fed flies ingested Samorin in unchanged form, but due to acidic environment in the stomach of the insect vector, it breaks down into homidium. The optimal performance put up by flies in this investigation clearly rules out the possibility of any negative effect of homidium on survival and reproductive performance of the flies.

Also since flies in this investigation were fed on ears of rabbits, it is obvious that they could not have picked up high concentrations of the drug from the injection site, contrary to observations made by Oladunmade and Balogun (16).

In conclusion, Berenil and Samorin administered *in vivo* to rabbits at recommended therapeutic and prophylactic doses of 3.5 mg/kg

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**Table II**

<table>
<thead>
<tr>
<th>Exp Group</th>
<th>Treatment</th>
<th>% female survivors at day 55</th>
<th>Eggs</th>
<th>Egg larvae recovered</th>
<th>Dissection results</th>
<th>% female pupae/Abortions</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Berenil (M)</td>
<td>68.6</td>
<td>3.21</td>
<td>3</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>II</td>
<td>Samorin (M)</td>
<td>51.1</td>
<td>2.60</td>
<td>5</td>
<td>6</td>
<td>21</td>
</tr>
<tr>
<td>III</td>
<td>Control (M)</td>
<td>71.5</td>
<td>3.12</td>
<td>4</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>IV</td>
<td>Berenil (R)</td>
<td>60.4</td>
<td>3.04</td>
<td>4</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>V</td>
<td>Samorin (R)</td>
<td>68.8</td>
<td>3.17</td>
<td>4</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>VI</td>
<td>Control (R)</td>
<td>60.9</td>
<td>3.32</td>
<td>5</td>
<td>6</td>
<td>8</td>
</tr>
</tbody>
</table>

Ms+ : positive mating scar; Sp+ : positive spermathecae; E : egg; IUP : IUP, castration; Ab : abortion; Bl : blockage; P : post larviposition
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and 1 mg/kg body weight respectively, did not have any adverse effect on survival and reproductive performance of female Gpp fed on their ears. Hence, the use of these drugs in the field may not have potentials in the control of Glossina spp., thus contradicting earlier claims by Oladunmade and Balogun (16), who used Samorin at a lower dose.

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REFERENCES


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Résumé

Dede P.M. Effets des médicaments trypanocides sur certains aspects de la biologie reproductive de Glossina palpalis palpalis femelle (Diptera : Glossinidae)

Les effets du chlorure d’isométamidium (Samorin) et de l’acétate de diminazène (Bérénil) sur certains aspects de la biologie reproductive de la Glossina palpalis palpalis (Gpp) femelle ont été examinés. Le Samorin et le Bérénil ont été administrés aux mouches in vitro à travers une membrane de silicone à raison de 0,14 mg/ml et 0,40 mg/ml de sang respectivement ou in vivo sur les oreilles de lapins traités aux doses prophylactiques et thérapeutiques recommandées de 1 mg et 3,5 mg/kg respectivement. Les mouches étaient maintenues à 24,5 ± 0,5 °C, à 80 ± 5 % d’humidité relative et à une photopériodicité de 6 h. Ni le Bérénil, ni le Samorin, aux concentrations employées, n’ont eu un effet défavorable sur le taux de survie de la femelle, sa fécondité et le poids moyen des pupes. Bien au contraire, les femelles nourries sur Bérénil à 0,40 mg/ml de sang à travers la membrane avaient une meilleure survie, une fécondité et un poids moyen des pupes plus élevés que les autres groupes, y compris les témoins. De même, les mouches qui émergeaient de la pupa de ce groupe étaient plus actives en cage. L’implication de ces découvertes en relation avec le contrôle de vecteur est étudiée.


Resumen

Dede P.M. Efectos de los medicamentos tripanocidas sobre ciertos aspectos de la biología reproductiva de Glossina palpalis palpalis hembra (Diptera: Glossinidae).

Se estudiaron los efectos del cloridio de isometamidio (Samorin) y del acetato de diminaceno (Berenil) sobre ciertos aspectos de la biología reproductiva de Glossina palpalis palpalis (Gpp) hembra. El Samorin y el Berenil fueron administrados a las moscas in vitro a través de una membrana de silicona, a razón de 0,14 mg/ml y de 0,40 mg/ml de sangre respectivamente, o in vivo sobre las orejas de conejos tratados con dosis profilácticas y terapéuticas recomendadas de 1 mg y de 3,5 mg/kg respectivamente. Las moscas se mantuvieron a 24,5 ± 0,5 °C, a 80 ± 5% de humedad relativa y a una fotoperiodicidad de 6 horas. Ni el Berenil ni el Samorin, a las concentraciones utilizadas, tuvieron efecto desfavorable sobre la tasa de supervivencia de la hembra, su fecundidad y el peso medio de las pupas. Al contrario, las hembras alimentadas sobre Berenil a 0,40 mg/ml de sangre a través de la membrana tuvieron una mejor supervivencia, una fecundidad y un peso medio de las pupas más elevados con respecto a otros grupos, incluyendo los testigos. De igual manera, las moscas que emergieron de las pupas de este grupo fueron más activas en las jaulas. Se estudia la implicación de estos descubrimientos en relación con el control del vector.

Palabras clave: Glossina palpalis palpalis - Trypanosomiasis - Medicamentos - Reproductividad - Fertilidad - Supervivencia - Aborto - Nigeria.