

# 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  $24.5 \pm 0.5^\circ\text{C}$ ,  $80 \pm 5\%$  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.

## INTRODUCTION

Chemotherapy and chemoprophylaxis will remain among the most reliable and widely used methods in the control of animal trypanosomiasis, especially where other methods of control have not been successful. Isometamidium chloride (Samorin<sup>®</sup>, May & Baker) is a widely accepted trypanocide due to its curative properties and currently the only drug available for prophylaxis against animal trypanosomiasis. Its remarkable economic value in reducing livestock mortality and increasing productivity has been demonstrated in the field (7, 9, 10). Whitelaw *et al.* (19) have observed a prophylactic action of Samorin in the field between 14-36 weeks when administered at a recommended dose of 0.5-1 mg/kg body weight. Diminazene aceturate (Berenil<sup>®</sup>, Hoechst) is widely used as a curative trypanocide in animal trypanosomiasis. The recommended dose of 3.5 mg/kg body weight has been used intensively in the field (6, 8, 10).

Until the late 70s, the above trypanocides were being used in the fields without any assessment of their possible effect on survival and reproductive biology of *Glossina* spp. The pioneering work of

Oladunmade and Balogun (16), later Moloo and Kutuza (13) and Abbeele *et al.* (1) have tried to assess the possible effect of *in vivo* administered Samorin on survival and reproductive performance of tsetse species. Results obtained by these investigators were unreconcilable. The present investigation is aimed at assessing the effect of *in vitro* and *in vivo* administered Berenil and Samorin, in an effort to add to our understanding of their effects on survival and reproductive biology of *Glossina* spp.

## MATERIALS AND METHODS

### Rabbits

Rabbits purchased for feeding were screened for trypanosomes before they were put into uses. They were maintained on standard pellets, with cabbage supplement. All *in vivo* feedings were carried out on ears of rabbits.

### Tsetse species

A total of 292 teneral females *Glossina palpalis palpalis* (*Gpp*) were used. These were obtained from a laboratory reared colony at the Biological Control of Tsetse Project (BICOT). Flies were held in single size oblong polyvinyl chloride (PVC) cages (18 cm x 8 cm

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x 4 cm), each containing 20 females and were maintained throughout the period of experiment at  $24.5 \pm 0.5^\circ\text{C}$ ,  $80 \pm 5\%$  RH and 6 h photoperiod. They were fed *in vivo* on the ears of rabbits or *in vitro* through a silicone rubber membrane (3). At least 7 days old males were mated to 2 days old females at ration 1:1. Mating lasted 3 days before separation.

### 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  $-20^\circ\text{C}$ , each batch of blood was separately treated to an initial 50 krad of gamma-irradiation in a cobalt<sup>60</sup> 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

Diminazene 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. Table II is the dissection result showing 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 ( $\chi^2 = 7.84$ ;  $df = 5$ ;  $p > 0.05$ ), 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) ( $\chi^2 = 0.103$ ;  $df = 5$ ;  $p > 0.05$ ), 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 ( $\chi^2 = 0.14$ ;  $df = 5$ ;  $p > 0.05$ ). With the exception of group I flies that larviposited a day earlier, all the remaining groups larviposited on day 20 post emergence.

Table I

Reproductive performance of female *Glossina palpalis palpalis* fed *in vivo* and *in vitro* on Berenil and Samorin, then maintained for 53 days on untreated blood meal

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

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* ( $f = 1.81$ ;  $df = 4, 11$ ;  $p > 0.05$ ) 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 *Gpp* 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 against *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

Table II

Reproductive status and abortions recovered from female *Glossina palpalis palpalis* fed *in vivo* and *in vitro* on Berenil and Samorin, then maintained for 53 days on untreated blood meal

Exp. Group	Treatment	% female survivors at day 55	Pupae/initial female (ppf)	Egg	Abortions recovered larvae (I, II, III)	Dissection results																		
						Dead						Surviving												
						Ms <sup>+</sup>	Sp <sup>+</sup>	E	I	II	III	IUP	Ab	B1	P1	Ms <sup>+</sup>	Sp <sup>+</sup>	E	I	II	III	Ab	B1	P1
I	Berenil (M)	68.6	3.21	3	3	15	13	2	2	1	3	2	1	-	5	34	32	-	1	7	25	-	1	2
II	Samorin (M)	51.1	2.60	5	6	21	20	1	4	2	2	-	4	3	3	24	22	-	2	7	14	-	1	1
III	Control (M)	71.5	3.12	4	8	15	15	-	-	1	1	1	1	1	3	35	35	-	1	11	21	1	1	2
IV	Berenil (R)	60.4	3.04	4	10	18	18	3	2	2	2	-	3	2	4	28	28	-	2	10	17	1	2	2
V	Samorin (R)	68.8	3.17	3	4	13	12	2	3	-	-	-	4	1	3	33	33	-	1	13	19	-	1	1
VI	Control (R)	80.9	3.32	5	4	8	7	1	-	-	-	1	2	-	6	37	36	1	3	13	20	-	1	3

Ms<sup>+</sup>: positive mating scar; Sp<sup>+</sup>: positive spermathecae; E: egg;

I: 1st instar larva; II: 2nd instar larva; III: 3rd instar larva; IUP: in utero pupation; Ab: abortion; B1: blockage; P1: post larviposition

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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## 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'isoméamidium (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  $24,5 \pm 0,5$  °C, à  $80 \pm 5$  % 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 des pupes de ce groupe étaient plus actives en cage. L'implication de ces découvertes en relation avec le contrôle de vecteur est étudiée.

**Mots-clés :** *Glossina palpalis palpalis* - Trypanosomose - Médicament - Performance de reproduction - Fertilité - Survie - Avortement - Nigeria.

## 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  $24,5 \pm 0,5$  °C, a  $80 \pm 5$  % 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.