

Influence of Lindane or Fenitrothion on the Response to Treatment of Trypanosomosis in Rats

*Abatan M. O. and Awonusi Elizabeth Oluwatoyin

Department of Veterinary Physiology and Pharmacology, University of Ibadan, Ibadan.

Target Audience:

Abstract

Studies were conducted on the influence of the insecticides, lindane or fenitrothion on the course of treatment of experimental trypanosomosis infection in rats by evaluating changes in hematological and biochemical parameters in groups of animals. Groups of rats were pretreated with either lindane or fenitrothion, infected with *Trypanosoma brucei*, before treatment with Berenil7 or quinapyramine.

It was observed that the group of animals pretreated with fenitrothion before administration of Berenil7 had significantly ($P < 0.05$) higher packed cell volume (PCV), hemoglobin concentration (Hb) total white blood cell count (WBC) and red blood cell count (RBC) when compared to the untreated control and those treated with berenil alone. The hematological parameters from the group of rats pretreated with fenitrothion before the use quinapyramine, were not significantly ($P > 0.05$) different from those treated with quinapyramine alone though the group given quinapyramine alone showed slightly higher serum biochemical parameters, Na^+ , Cl^- , HCO_3^{2-} , alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and serum total protein when compared to the pretreated group. This may suggest the influence of fenitrothion on the metabolism of quinapyramine.

Animals pretreated with lindane before treating with berenil had lower PCV, and Hb compared to the untreated control and may suggest that lindane had induced the metabolism of berenil. This observation is corroborated by the higher PCV, Hb, and RBC values of the group treated with berenil alone. Furthermore, the observation that the group pretreated with lindane before administering berenil had lower ALT, and AST compared to the untreated control group shows that the berenil had started action to reduce the parasitaemia and therefore tissue damage, before its metabolism.

The group pretreated with lindane before giving quinapyramine, had significantly ($P < 0.05$) lower AST, ALT, and serum protein levels compared with the untreated controls. This may suggest the non influence of lindane on quinapyramine metabolism.

Key words: Trypanosomosis, insecticides, berenil, quinapyramine, haematology, serum biochemistry.

Introduction

Trypanosomosis is tsetse borne blood protozoan disease in livestock in Africa. (1 ; 27;28) There are several regimes of treatments used in the management of the disease. (32; 33; 28; 25) Unfortunately due to the indiscriminate use of the various types of pesticides in the control of the

disease vectors, and other agricultural practices, surface and ground water sources are often contaminated by these chemicals (9; 25).

The occurrence of the different types of pesticides either directly when applied on the animal or indirectly as a result of environmental contamination, in the blood circulation of the

* Author for correspondence

animal has various biological and toxicological implications (2, 26 and 6). Occasionally, direct poisoning from the use of some of these chemicals do occur in animals and may results from excessive or unrecommended exposure. (26; 4) There are reported cases where insecticides are mis-identified and mixed with feed to cause poisoning. The cadavers of animals poisoned have been used as animal by product to feed other animals (26). Such poisoned animals are observed to have up 50-70 or greater level parts per million of the insecticides in their body fat (26). If the body fat contain a residue of organochlorine pesticide, sufficient pesticide may be released into the blood stream to cause clinical signs of poisoning.

Moreover some pesticides such as the organochlorine are associated with biomagnification and the induction of liver microsomal enzymes (2; 11 and 14). The process of enzyme induction may result in increased breakdown of both exogenous and endogenous substances (11; 15; 20 and 10). Such detoxication may have various biological implications which may or may not be beneficial. Organophosphorus compounds are reported to produce inhibition of liver drug metabolizing enzymes and thus also influencing the metabolism of other xenobiotics (8; and 5).

Organophosphorus and organochlorine insecticides are used with antiparasitic agents for the purposes of getting rid of vectors of trypanosomiasis or vectors of other animal diseases. The fact that some of the insecticides used against vectors of animal diseases may influence the metabolism of other drugs used along with it (26, 28 and 6), means there is need to examine these interactions thoroughly. Fenitrothion an organophosphate is a 0, 0-dimethyl 0-4-nitro-M-tolyl phosphorothioate whereas lindane an organochlorine is a 1,2,3,4,5,6-hexachlorocyclohexane (22). This study therefore attempts to look at possible chemical interaction (31) that could take place with either the use of fenitrothion or lindane as acaricide if babesiosis is suspected, simultaneously with berenil or quinapyramine in the treatment of infection by *Trypanosoma brucei*.

Material Method

Experimental Animals

White albino rats of both sexes weighing between 180-250 grams were used. They were maintained on rat cubes (Ladokun Feeds Nig. Ltd) and provided clean water *ad libitum*. The rats were separated into seven groups with each group made up of six animals. The chemical compounds were administered orally using a cannula and include lindane (20% E.C. Luxadane (GLD) Netherland; fenitrothion 50% Hoechst Veterinary; berenil (diaminazene acetate Hoechst AG Frankfurt am Main); quinapyramine sulphate (Agropharm Ltd Perm U. K.)

Lindane (20Fg/kg bw) or fenitrothion (50Fg/kg bw) (17) were administered for two weeks at the end of which rats in each group were challenged with *Trypanosoma brucei*. (inoculum size 0.5ml containing 1.25×10^5 parasites intraperitoneally) The control group was challenged with *T. brucei* but were not pretreated with lindane or fenitrothion. Parasitaemia was monitored daily as described by 13 until the appearance of parasites when treatment with berenil (3.7mg/kg) quinapyramine (2.0mg/kg) was commenced. Parasitaemia was monitored for extra fourteen days before blood was obtained by cardiac puncture for hematological and serum biochemical analyses.

Serum Biochemistry and Haematologic Procedures.

RBC, and total WBC counts were made by the haemocytometer method, hemoglobin (Hb), concentration by the cyanmethemoglobin method, packed cell volume (PCV) by capillary tube method (18).

Serum enzymes were determined by procedures of 29, blood urea nitrogen by method of 7, total protein as done by 12, serum bilirubin by method of 21 as modified by Sigma Diagnostics.

Statistical Analysis

The results are presented as mean \pm s.e.m of values of parameters. Differences between means are determined using the Students' *t* test. Differences between means are stated to be significant at $P < 0.05$ (3).

Results**Fenitrothion pretreatment and the use of Berenil**

Animals in this group showed significantly ($P<0.05$) higher levels of the packed cell volume (PCV), hemoglobin concentration (Hb), white blood cell, (WBC) and red blood cells (RBC) when compared with untreated controls and the group

treated with berenil alone after infection with *Trypanosoma brucei*. (Table 1)

The group pretreated with fenitrothion before the use of berenil also showed increases in levels alkaline phosphatase (ALP) but significantly ($P<0.05$) lower levels of alanine aminotransferase (ALT), and aspartate aminotransferase (AST) as compared with the

Table 1: Effects of fenitrothion or lindane pretreatments on hematological parameters of *Trypanosoma brucei* infected rats

TREATMENT GROUPS	HEMATOLOGICAL PARAMETERS							
	PCV %	Hb g/dl	WBC $\times 10^3$ /Fl	RBC $\times 10^6$ /Fl	Neutrophil %	Lymphocyte %	Eosinophil %	Monocyte %
CONTROL	32 \pm 4.5	10.8 \pm 1.5	2450 \pm 451	4.4 \pm 0.1	34.0 \pm 1.4	-	-	-
F & B	31.0 \pm 2.5	13.6 \pm 0.5	3466 \pm 546	7.0 \pm 0.3	28.6 \pm 13.8	71.3 \pm 1.4		
F & Q	33.3 \pm 1.5	10.6 \pm 0.9	5233 \pm 53.7	5.7 \pm 7.8	51.7 \pm 7.8	48.3 \pm 6.4		
L & Q	32.3 \pm 5.8	10.7 \pm 1.9	4400 \pm 104.5	4.8 \pm 0.8	34.3 \pm 15.5	64.6 \pm 1.6	1.0	2.0
L & B	27.3 \pm 3.2	9.0 \pm 1.2	4100 \pm 193.6	5.0 \pm 0.5	20.7 \pm 4.9	76.8 \pm 14.3	2 \pm 0.2	2 \pm 1.2
B	25.0 \pm 2.5	8.3 \pm 0.9	8400 \pm 160	3.7 \pm 0.8	62.0 \pm 2.0	34.7 \pm 2.9	-	5.0 \pm 0.8
Q	35.3 \pm 4.2	11.7 \pm 1.5	3633 \pm 904	5.3 \pm 1.1	25.7 \pm 1.0	73.3 \pm 10.7	-	0.3

F & B=Fenitrothion and Berenil; F & Q=Fenitrothion and Quinapyramine; L & Q=Lindane and Quinapyramine; L & B=Lindane and Berenil; B=Berenil; Q=Quinapyramine

Table 2: Effects of fenitrothion or lindane pretreatments on the serum biochemical parameters from *Trypanosoma brucei* infected rats.

PARAMETERS	TREATMENT GROUPS						
	Control	Q	B	F & Q	F & B	L & Q	L & B
Ca ²⁺ mg/100ml	8.5 \pm 0.1	8.5 \pm 0.1	8.3 \pm 0.1	8.4 \pm 0.2	8.6 \pm 0.1	8.6 \pm 0.1	8.4 \pm 0.2
Po ₄ ²⁻ mg/100ml	4.6 \pm 0.4	4.4 \pm 0.2	4.1 \pm 0.1	4.0 \pm 0.03	4.1 \pm 0.03	4.2 \pm 0.1	4.6 \pm 0.4
Urea mg/100ml	14.3 \pm 1.4	17.7 \pm 0.9	18.6 \pm 0.7	13.7 \pm 2.2	13.0 \pm 1.5	13.3 \pm 1.9	16.3 \pm 2.7
Creatinine mg/100ml	0.7 \pm 0.1	0.53 \pm 0.03	0.8 \pm 0.1	0.6 \pm 0.2	0.6 \pm 0.3	0.7 \pm 0.1	0.7 \pm 0.13
ALP(U/L)	101.3 \pm 7.6	122 \pm 10.7	115.3 \pm 3.2	118.6 \pm 1.3	129 \pm 10.2	122 \pm 5.1	113.3 \pm 3.7
ALT(U/L)	39 \pm 1.6	24.3 \pm 2.3	18.7 \pm 0.7	22.3 \pm 1.2	22.3 \pm 2.6	18.3 \pm 3.3	24 \pm 3.1
AST(U/L)	29 \pm 3.9	26.6 \pm 0.9	11.7 \pm 0.3	11.6 \pm 0.9	12 \pm 1.50	11.0 \pm 1.7	15.6 \pm 3.5
Total Protein(g/dl)	6.0 \pm 0.2	8.7 \pm 1.2	6.9 \pm 0.1	8.0 \pm 0.1	8.3 \pm 0.2	7.1 \pm 0.2	7.9 \pm 3.6
Albumin (g/dl)	2.8 \pm 0.1	4.0 \pm 0.5	3.2 \pm 0.1	3 \pm 0.2	4.0 \pm 0.3	3.3 \pm 0.1	3.5 \pm 0.2
Globulin (g/dl)	3.12 \pm 0.2	4.6 \pm 0.7	3.7 \pm 0.2	4.3 \pm 0.2	4.3 \pm 0.1	3.8 \pm 0.3	4.4 \pm 0.4

F & B=Fenitrothion and Berenil; F & Q=Fenitrothion and Quinapyramine; L & Q=Lindane and Quinapyramine; L & B=Lindane and Berenil; B=Berenil; Q=Quinapyramine

untreated group and berenil treated group. (Table 2).

Animals pretreated with fenitrothion before using berenil and those treated with berenil alone had significant increases ($P < 0.05$) in electrolyte levels Na^+ , K^+ , Cl^- , and HCO_3^{2-} when compared with untreated controls (Fig. 1).

Fenitrothion pretreatment and the use of Quinapyramine

The group pretreated with fenitrothion before use of quinapyramine had significantly lower levels of Na^+ , ALT, AST but increased levels of Cl^- and HCO_3^{2-} when compared with the untreated infected control and quinapyramine alone treated groups. There were no significant ($P > 0.05$) changes in the hematological parameters between the group pretreated with fenitrothion, before use of quinapyramine and those treated with quinapyramine alone, except the total WBC and neutrophil counts which were significantly ($P < 0.05$) higher with the group pretreated with fenitrothion before administering quinapyramine.

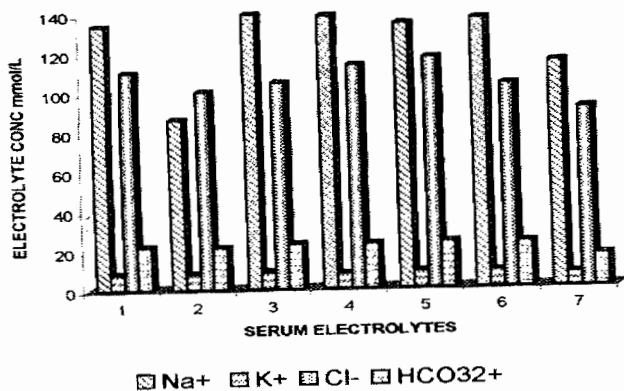
Lindane pretreatment and the use of Berenil

The group pretreated with lindane before administering berenil, had lower PCV, Hb, and neutrophil levels compared to the untreated infected controls. There were no significant ($P > 0.05$) changes in RBC levels. However a comparison with the group treated with berenil alone shows significantly ($P < 0.05$) higher PCV, Hb, RBC, and lymphocyte levels. The pretreated group also showed higher levels of Na^+ , K^+ , Cl^- , HCO_3^{2-} (Figure 1) urea, and ALP but significantly ($P < 0.05$) lower ALT and AST when compared with untreated controls. The group treated with berenil alone also had lower levels of AST and ALT when compared with those pretreated with lindane.

Lindane pretreatment and administration of Quinapyramine

The group pretreated with lindane before administering quinapyramine had significantly ($P < 0.05$) higher levels of Na^+ and urea but lower levels of ALT, AST and total protein when compared with untreated control and those treated with quinapyramine alone. (Table 2)

Figure 1: SERUM ELECTROLYTE LEVELS IN TREATED TRYPANOSOMA INFECTED RATS



- 1=Untreated Control. 2= Quinapyramine treated group
 3= Berenil group 4= Fenitrothion pretreated before quinapyramine
 5= Fenitrothion pretreatment before berenil
 6= Lindane pretreatment before quinapyramine
 7= Lindane pretreatment before berenil

Table 3. Changes in *Trypanosoma brucei* level in rats pretreated with lindane or fenitrothion before use of berenil or quinapyramine

TREATMENT	AVERAGE PARASITAEMIA POST TREATMENT/DAY							
	1	2	3	4	5	6	7	14
Control	48x10 ⁷	55x10 ⁷	55x10 ⁸	55x10 ⁷	56x10 ⁶	56x10 ⁴	Dead	-
Q	49x10 ⁷	52x10 ⁷	52x10 ³	52x10 ²	43x10 ⁴	38x10 ⁶	29x10 ⁸	3x10 ⁵
B	56x10 ⁷	68x10 ⁷	62x10 ⁴	54x10 ⁴	32x10 ³	13x10 ⁵	0	-
F & Q	60x10 ⁷	65x10 ⁷	66x10 ²	64x10 ⁵	58x10 ²	46x10 ⁸	34x10 ⁷	2x10 ⁴
F & B	50x10 ⁷	57x10 ⁷	44x10 ²	28x10 ⁶	15x10 ⁵	0		
L & Q	68x10 ⁷	70x10 ⁷	72x10 ³	66x10 ⁷	55x10 ⁴	56x10 ⁵	57x10 ⁴	0
L & B	60x10 ⁷	62x10 ⁷	63x10 ⁸	59x10 ⁶	47x10 ⁸	38x10 ⁵	31x10 ⁷	8x10 ²

F & B=Fenitrothion and Berenil; F & Q=Fenitrothion and Quinapyramine; L & Q=Lindane and Quinapyramine; L & B=Lindane and Berenil; B=Berenil; Q=Quinapyramine

Discussion

The simultaneous exposure to drugs or chemicals from environmental pollution predisposes the body to drug or chemical interaction (31 and 16). Such chemical pollution as it occurs with organochlorines such lindane or organophosphorus compounds such as fenitrothion may upset some biological balance in endogenous or exogenous substances which results in various biological changes in the body of the animal (26 and 14). Lindane is reported to induce the liver drug metabolizing enzymes and therefore has the tendency of increasing the metabolism of many xenobiotics and endogenous substances which are metabolized by the same group of enzymes which are induced (6, 24 and 14). This occurrence has the effect of influencing the manner therapy of diseases are effective (26). In this study, tissue damage in rats arising from infection with *Trypanosoma brucei* (27 and 28). and the effect of treatment with antitypanosomal drugs, such berenil or quinapyramine with concurrent exposure to lindane or fenitrothion have been examined. The hematological parameters such as PCV, Hb, WBC, and RBC from the group given fenitrothion before berenil were higher than the untreated infected controls and the group given only berenil and this may suggests that fenitrothion has not enhanced the metabolism of

berenil. Therefore the level of berenil has been effective in treating infection by *T. brucei* when compared with the untreated control group. The is reflected in the parasitaemia levels observed in these group of animals (Table 3).

The hematological parameters from the group pretreated with fenitrothion before using quinapyramine were not significantly different ($P>0.05$) from the group treated with quinapyramine alone. Though the serum biochemical result shows that the group treated with quinapyramine alone has slightly higher Na⁺, Cl⁻, HCO₃²⁻, ALP, AST, ALT, and total protein levels than the group pretreated with fenitrothion before administering quinapyramine. These differences were not significant ($P>0.05$) and thus may not suggest an effect of fenitrothion on the metabolism of quinapyramine.

The group exposed to lindane before administering berenil, had lower PCV, and Hb which are signs of loss of the circulating red blood cell (18 and 27) when compared with the untreated control group. This may suggest that berenil which has the antitypanosomal action, (4 and 28) may have been metabolized faster than it should normally be, as a result of the induction of enzymes responsible for its metabolism by lindane(26 and 14). This observation is further supported by the higher PCV, Hb and RBC values of the group

treated with berenil alone when compared with the group pretreated with lindane before giving berenil. This is also observed in the level of parasitaemia observed daily in the animals (Table 3). However, the fact that the group exposed to lindane before treating with berenil, had significantly ($P < 0.05$) lower ALT, and AST, values compared with the untreated group suggest that berenil may have acted on the parasites before commencement of metabolism.

The group that were pretreated with lindane before administering quinapyramine showed significantly ($P < 0.05$) lower AST, ALT and serum total protein, compared with untreated control, which is indicative of less tissue damage (19). This may mean that lindane induced the enzyme for metabolism of quinapyramine (30) into an intermediate product which may be responsible for the antitrypanosomal activity and thus the reduced tissue damage produced with the group pretreated with lindane.

The study therefore shows that the simultaneous use of some antitrypanosomal drugs and some insecticides in the treatment of vector borne diseases has some associated damaging effects to the animal.

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