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Therapeutic Efficacy of Quinapyramine Sulphate with Freund's Complete Adjuvant in Wistar Rats Infected with *Trypanosoma Congolense*

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Abstract: Therapeutic efficacy of QS (quinapyramine sulphate) and FCA (Freund's complete adjuvant) combination was studied. The aim of the study was to evaluate therapeutic efficacy of QS using FCA in *Trypanosoma congolense* infection. Groups treated with QS and FCA had parasite disappeared in peripheral circulation 2 days pi, relapse was observed one week later. Effect of treatment on rectal temperature shows no significance ($p < 0.05$), normalization of rectal temperature occurred in QS and FCA treated groups (34.1°C) than untreated (42.8 °C), QS (37.4 °C) and FCA (35.92 °C) treated groups. Mean body weight was significant ($p < 0.001$) in QS and FCA, QS, and FCA groups. Packed cell volume and hemoglobin concentration for untreated groups were lower, but increased in QS, FCA, QS and FCA treated groups, indicating anemia amelioration. White blood cell counted in untreated, QS and FCA treated groups showed no significance ($p < 0.05$), however, there was leukocytosis due to lymphocytosis in QS and FCA treated group (6.79×10^3/μl) compared with untreated and other groups. There was comparative decrease in serum liver enzymes in QS and FCA treated group than other groups. Therefore, QS at lower recommended dose with FCA may enhance efficacy of QS in trypanosomiasis.

Key words: Quinapyramine sulphate, Freund's complete adjuvant, *Trypanosoma congolense*, Wistar rats, clinical and hematological parameters.

1. Introduction

African animal trypanosomosis is an important constraint to livestock production and development in the tropics [1]. Tsetse transmitted trypanosomosis infects over 5 million people annually with sleeping sickness in 36 countries and an estimated annual livestock losses owing to the direct and indirect effects of the disease running into billions of dollars [1, 2]. This hampers development of sustainable and productive agricultural systems in most African states [3, 4], with some 46-62 million head of cattle and other animal species at risk of the disease. Thus, the disease causes great economic loss and food security problem where ever they are presenting [5, 6, 7]. In Nigeria, incidence of trypanosmiasis is increasing [8]. A wide range of biochemical changes occur in animals infected with trypansosmiasis, this alters the physiology of affected animals thereby resulting in haematological aberrations [9, 10]. In Africa, chemotherapy is the mainstay for the control of trypanosomiasis [11]. Efficient treatment and prophylaxis against the disease is beset with problems of drug resistance and toxicity, while search for vaccine against the disease remains elusive [12, 13]. This study explores the use of QS (quinapyramine sulphate) at a dose lower than recommended in combination with FCA (Freund's complete adjuvant) in the treatment of *T. congolense.* It is apparent that, this combination might have some merits with regards to reduction in length of treatments,

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reduction in dose rate, and total dose, hence toxicity [14, 15].

2. Materials and Methods

The materials used for this study were twenty five (25) male Wistar rats weighing 189 ± 5 grams, quinapyramine sulphate (Imperial Chemical Industries, Pharmaceutical Division, Alderly Park, Great Britain), Freund's complete adjuvant (Difco Laboratories, Detroit, USA) and Savanna strain of *Trypanosoma congolense*.

2.1 Experimental Animals

Twenty five (25) Wistar rats weighing 189 ± 5 grams were used in this study. The rats were obtained from the animal house of the National Institute for Trypanosomiasis and Onchocerchiasis Research (NITOR), Vom, Nigeria. They were transported and housed in cages under standard environmental conditions in the Animal house of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria, Nigeria. The Wistar rats were fed on a feed compounded using commercial growers' mash, maize bran and groundnut cake in the ratio of 4:2:1. The rats had access to tap water *ad libitum*. They were pre-conditioned for three weeks before the commencement of the experiment. Ethical considerations were observed as recommended by the Canadian Council on Animal Care.

2.1 Experimental Design

Twenty five (25) Wistar rats were randomly selected and divided into five (5) groups (I-V) of five rats each. 2 mL of rat blood containing approximately 1.2×10^6 *T. congolense* organisms were inoculated intraperitoneally into each rat in group I-IV as described by Herbert and Lumsden [16]. Infected rats were monitored daily as described by Murray et al. [17] and treatment on day nine (9) post infection (pi) after patency was administered. Consequently, group I was administered a single dose of QS at 2.5 mg/kg only, group II a combination of a single dose of QS at 2.5 mg/kg and FCA 0.1 mL, and group III 0.1 mL of FCA only, all subcutaneously. Group IV was infected but not treated, while group V served as uninfected control and were administered distilled water. The experiment was terminated after four (4) weeks post treatment.

2.2 Methods

The evaluation of therapeutic efficacy was done by the following methods:

2.2.1 Clinical Parameters

Body weights of rats were determined using electronic a weighing balance. Rectal temperature was obtained through a digital thermometer from rectum and read in degree centigrade (^oC).

2.2.2 Haematological and Serum Parameters

Haematological parameters (PCV, Hb and WBC concentrations) were determined using methods described by Coles [18]. Serum biochemical parameters (ALT (alanine amino transferase), AST (aspartate amino tarnsferase), ALP (alkaline phosphatase) and gamma glutamyl transferase) were analyzed using an autoanalyzer (Bayer® Clinical Chemistry Analyzer, Germany). All these parameters were determined pre-infection, post-infection, and after treatment.

2.3 Statistical Analyses

Data obtained from this study were expressed as mean ± standard error of mean (± SEM) and subjected to ANOVA (analysis of variance) and Tukey's post-hoc test using GraphPad version 4.0 for windows (GraphPad Software, San Diego, California, USA). Values of $p < 0.05$ were considered significant.

3. Results

All the rats in QS with FCA treated group survived, while three mortalities were recorded in the QS treated group. However, no survivals were recorded in the FCA and infected untreated groups (Table 1).

After treatment, temperature in QS with FCA, QS

group decreased to normal while that of FCA and infected untreated group was higher than the normal range (Fig. 1).

There was a comparative increase in weight gain between the first and fourth week of the experiment (Fig. 2).

Table 1 Effect of treatment on survival rate of *Trypanosoma congolense* infected Wistar rats.

Treatment groups	Dose (mg/kg) and mL	Route of administration	Survival rate (%)
QS	2.5	SC	2/5 (40)
QS + FCA	2.5 and 0.1	SC	5/5 (100)
FCA	0.1	SC	0/5 (0)
IU	NT	NA	0/5 (0)
DW	2.5	SC	5/5 (100)

Notes. QS = quinapyramine sulphate; FCA = Freund's complete adjuvant; IU = Infected untreated; DW = Distilled water; SC = subcutaneous; NT = Not treated; NA = Not applicable.

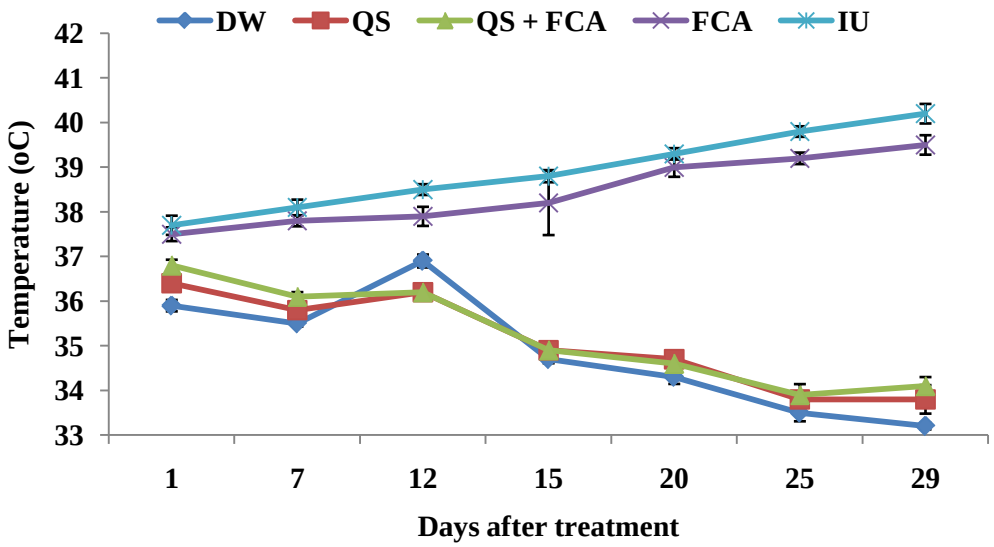

Fig. 1 Effect of treatments on daily rectal temperature in *Trypanosoma congolense* infected Wistar rats.

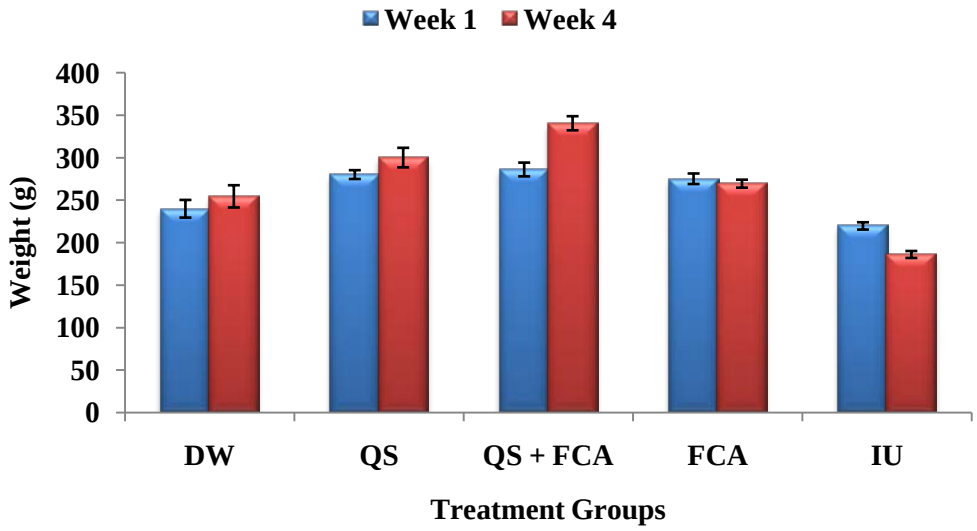

Fig. 2 Comparism of changes in weight gain from first week and fourth week Post-treatment in *Trypanosoma congolense* infected Wistar rats.

Anaemia due to decrease in PCV and Hb concentrations was observed in infected rats. However, anaemia was ameliorated after treatment. Amelioration was more in the QS with FCA treated group (Figs. 3-5).

There was leukocytosis due to lymphocytosis in the QS with FCA treated group when compared to other groups indicating ability of the combination to enhance immune response as FCA is known to be responsible for immune cell stimulation (Table 2).

There was a comparable increase in ALT, AST, ALP, and GGT in QS with FCA group than that of QS alone (Fig. 6).

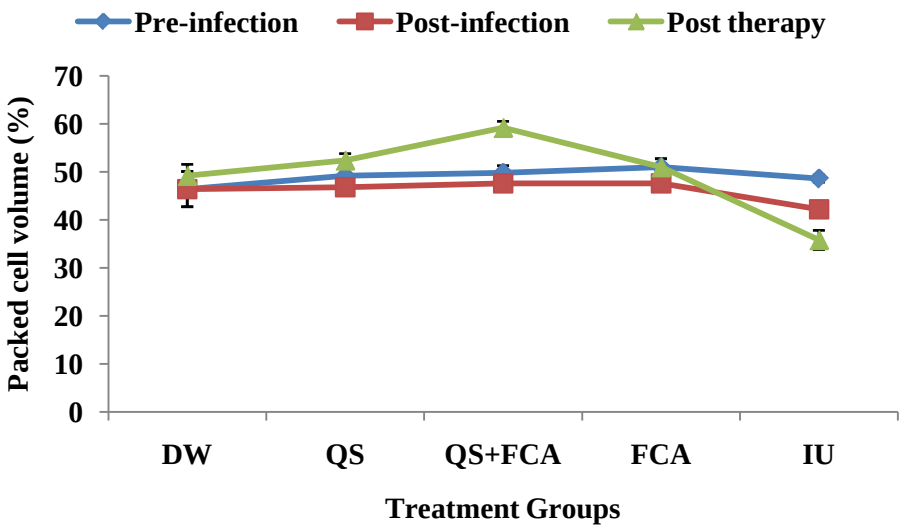

Fig. 3 Effect of treatments on packed cell volume in *Trypanosoma congolense* infected Wistar rats.

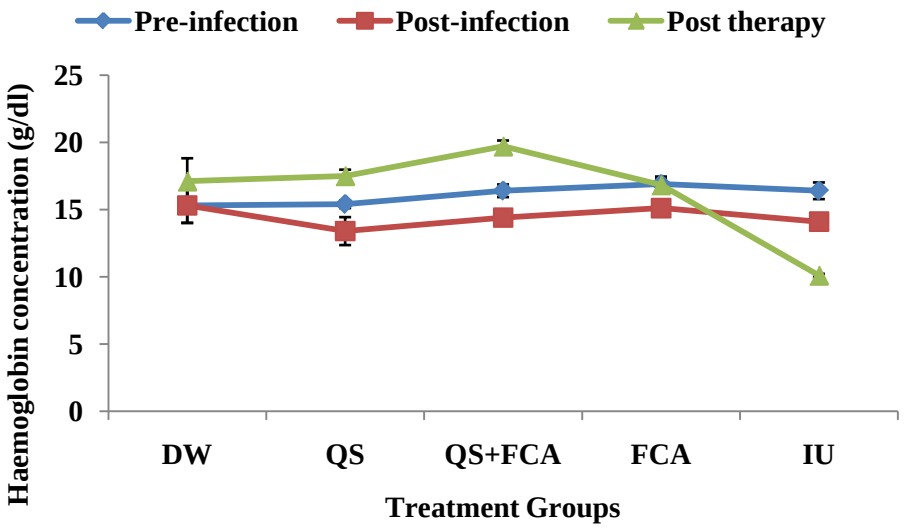

Fig. 4 Effect of treatments on haemoglobin concentration in *Trypanosoma congolense* infected Wistar rats.

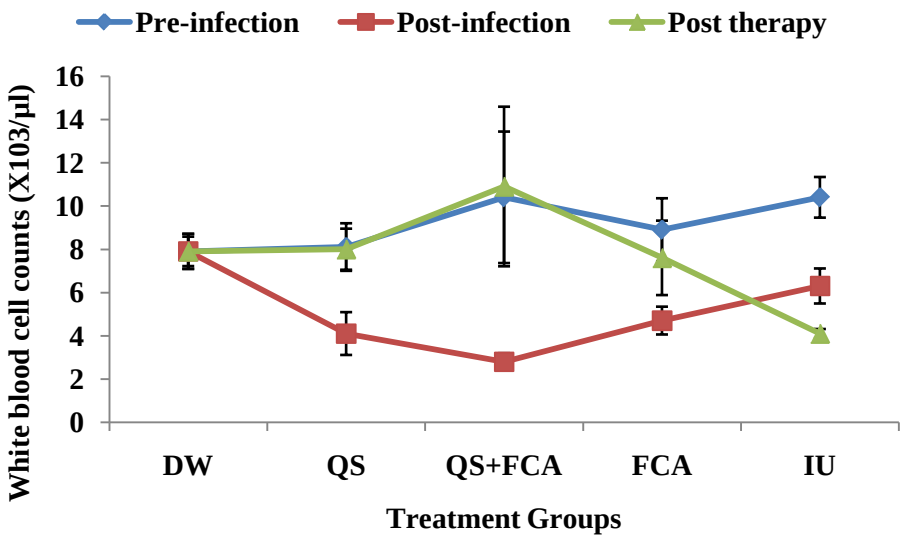

Fig. 5 Effect of treatments on white blood cell counts in *Trypanosoma congolense* infected Wistar rats.

Table 2 Effect of treatments on differential leukocyte counts in *Trypanosoma congolense* infected Wistar rats.

	Treatment groups				
Parameters ($\times 10^3$/µL)	DW	QS	QS + FCA	FCA	IU
WBC	6.9 ± 0.68	8.0 ± 0.95	8.9 ± 3.69	7.2 ± 0.43	4.2 ± 0.53
Neutrophils	1.58 ± 4.80	1.66 ± 4.08	2.18 ± 2.38	8.5 ± 2.98	0 ± 0
Lymphocytes	5.46 ± 3.68	6.20 ± 2.94[a]	6.79 ± 2.91[b]	5.90 ± 2.75	0 ± 0
Monocytes	0 ± 0	0.4 ± 0.4	0.3 ± 0.4	0.4 ± 0.6	0 ± 0
Eosinophils	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Basophils	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Band cells	1 ± 0.77	0 ± 0	0.6 ± 0.4	0 ± 0	0 ± 0

Notes. QS = Quinapyramine sulphate; FCA = Freund's complete adjuvant; IU = Infected untreated; DW = Distilled water; superscript (a, b) indicate significant difference ($p < 0.05$) between the groups.

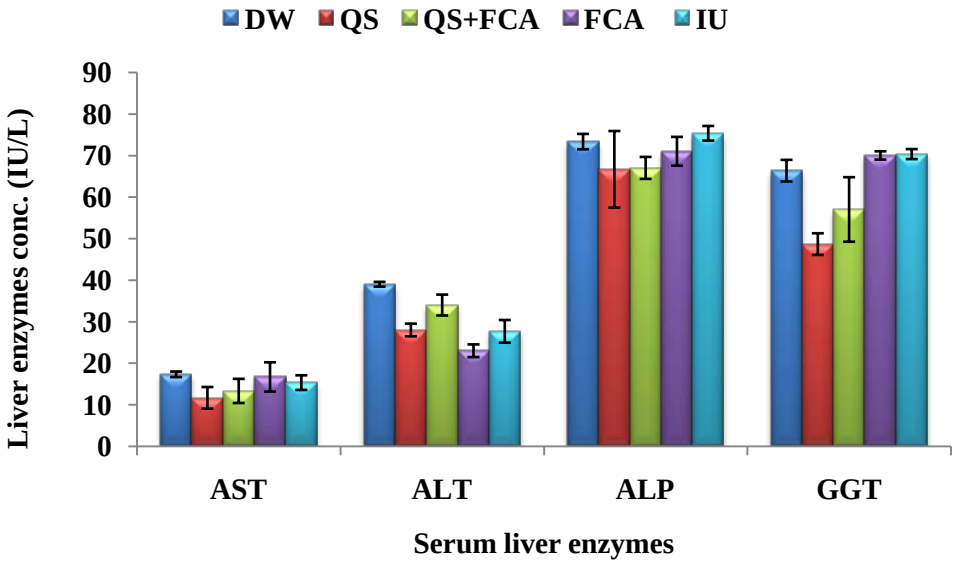

Fig. 6 Effect of treatments on serum liver enzymes in *Trypanosoma congolense* infected Wistar rats.

4. Discussion

In this study, the parasite clearance and survival period were more in QS with FCA treatment. Indicating that combination could have synergistic effect and may be an effective treatment regimen in *T. congolense* infection, further buttresses the fact that FCA helps to potentiate immunostimulatory response against pathogens reported by Petrovsky and Aguilar [19] and Ehizibolo et al. [20], and may offer a better treatment regimen alternative for eliminating *T. congolense* infection without toxicity and perhaps resistance. Increase temperature in this study pi, agrees with previous report by Uza et al. [21]. Temperature in this study decreased after treatment. However, the temperature observed in QS with FCA treated group was within the normal range. Increase in body weight of rats administered QS with FCA and FCA compared with QS treated group may be indicative of the potential ability of FCA to prevent muscle wasting normally associated with trypanosomiasis. Amelioration of anaemia in this study in QS with FCA group could be linked to the immunostimulatory effects of FCA [14, 22], thus reflecting the ability of FCA to reverse anaemia. The exact mechanism by which haemolysis of RBCs (red blood cells) were prevented cannot be readily explained. However, it may be reasonable to assume that it is due to its stimulatory effects on haemopoietic organs which possibly resulted in increase RBC production as they are destroyed with the concomitant destructive effects on the trypanosomes. This study observed leucocytosis which agrees with previous reports [23]. Increase in WBC counted in the QS with FCA group portrays the role of FCA in preventing cytodestruction of T lymphocytes and natural killer lymphocytes [14, 19, 24] thereby suggestive of the ability of FCA to enhance immune response via activation of immune cells to combat the effect of the trypanosome organism.

Changes observed in ALT, AST, ALP and GGT were within normal range in all groups [25], which agrees with the reports of Chaudhary and Iqbal [26] and Guiterrierez et al. [27], who argued that, organs (liver and kidney) may not have been seriously affected to cause the expected damage that results in leakage of these enzymes into the plasma.

5. Conclusions

In conclusion, this study has shown that, there was total parasite clearance with no evidence of relapse, increased weight gain, normalization of rectal temperature, improved haematological parameters which resulted in amelioration of anaemia and improved serum biochemistry in QS with FCA treated group when compared to infected untreated, QS only and FCA treated groups. Thereby establishing QS at a lower recommended dose in combination with FCA may enhance the therapeutic efficacy of the drug in *T. congolense* infected Wistar rats.

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