**Full Length Research Article**

**Pathogenicity of *Trypanosoma congolense* Infection following Oral Calcium Chloride Administration in Rats**

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**Abstract**

Eighty healthy adult albino rats of both sexes weighing 180-200g were used in two experiments to study the effects of oral calcium chloride treatment on the pathogenicity of *Trypanosoma congolense* infection. Experiment 1 was terminated at the peak of parasitaemia while experiment II was allowed to run a full course. In each experiment, forty rats were divided into four groups of 10 rats each, namely: A, uninfected untreated control; B, treated uninfected control; C, infected untreated and D, treated infected. Aqueous solution (10%) of CaCl$_2$ was administered daily using stomach tube to each rat at 100mg/kg in groups B and D from 7 days before infection to the end of the study. Each rat in groups C and D was infected by intraperitoneal injection of $1 \times 10^6$ trypanosomes in phosphate buffered saline solution (pH 7.4) diluted donor rat blood. The infected untreated group exhibited higher ($P<0.05$) parasitaemia, more severe anaemia and hepatic and renal damage than the infected treated group. In conclusion, oral calcium chloride treatment in rats seemed to reduce the pathogenicity of *T. congolense* infection by delaying the onset of parasitaemia and reducing the levels of parasitaemia, accompanied anaemia as well as organ damage.

**Keywords:** Rats, *Trypanosoma congolense*, calcium chloride treatment, pathogenicity.

**INTRODUCTION**

Trypanosomosis is a disease of man and animals (Losos and Ikede, 1972). The disease in animals is produced by *Trypanosoma vivax*, *T. congolense*, *T. simiae*, *T. b. brucei*, *T. evansi* and *T. equiperdum*. The human trypanosomes are *T. b. rhodesiense*, *T. b. gambiense* and *T. cruzi* (Radostits et al, 1994). The pathology and pathogenesis of the disease have been described previously (Losos and Ikede, 1970; Losos, 1986; Radostits et al, 1994). *Trypanosoma congolense* has affinity for the microvasculature (Losos, 1986). Nutrition is known to modulate the severity of trypanosomal infection in animals (Otesile et al, 1991; Igbokwe, 1995; Egbe-Nwiyi, 2003). Calcium is an important cation in the animal's body and membrane function of both the trypanosomes and the hosts (Kaneko, 1989). Calcium plays a role in blood coagulation, cell membrane and capillary permeability (Guyton and Hall, 1996). Calcium is present in variable amount in almost all feed stuff (National Research Council, 1971). Milk and milk products are the best food sources of calcium in man and animals. Several non feed supplemented sources of calcium are used in animal diet, with the most common being ground limestone. Other common sources include Oyster shell, Calcium Sulphate, Calcium Chloride, Phosphate and bone meal (NRC, 1971). Many sources of calcium are
inexpensive and animals have a wide tolerance level for calcium.

This study was designed to determine the effect of oral calcium chloride treatment on the pathogenicity of *Trypanosoma congolense* infection in rats.

**MATERIALS AND METHODS**

**Experimental animals:** Eighty healthy adult albino rats of both sexes weighing 180-200g were obtained from the laboratory animals unit of the Department of Pharmacology, University of Jos, Nigeria. They were housed in clean cages at room temperature (30-35°C), fed standard commercial diet (ECWA Feeds Ltd) and provided clean water *ad libitum*. Before the commencement of the experiments, the animals were screened for the presence of blood parasites using standard techniques (Schalm *et al.*, 1975). Forty rats were used in each of the two experiments (I and II) each with four groups (A, B, C and D) of 10 rats. The groups were treated as follows; A, uninfected untreated control; B, treated uninfected control; C, infected untreated and D, treated infected. Experiment I was terminated at the peak of parasitaemia while experiment II was allowed to run a full course.

**Trypanosome Infection:** *Trypanosoma congolense* (Gboko strain) obtained from the Nigerian Institute for Trypanosomiasis Research (NITR), Vom, Nigeria were maintained in rats by serial passages. Phosphate buffered saline solution (pH 7.4) diluted infected blood from donor rats containing 1x10^6 trypanosomes was intraperitoneally injected into each rat in groups C and D in both experiments. The solution was administered from 7 days before trypanosome infection to the end of the study.

**Oral calcium chloride (CaCl₂) treatment:** Calcium chloride (May and Baker Laboratory Chemicals Ltd, Dagenham, England) solution (10% aqueous) was administered daily by stomach tube to each rat at 100mg/kg in groups B and D in both experiments. The solution was administered from 7 days before trypanosome infection to the end of the study.

**Haematological and biochemical analysis:**

Tail blood samples were collected from rats at intervals pre and post parasite inoculation and evaluated for parasitaemia and haematological changes. Parasitaemia was measured by haemocytometry (Schalm *et al.*, 1975) while packed cell volume (PCV) was used to assess the haematological response and determined by microhaemocrit method (Schalm *et al.*, 1975).

The rats in experiment I were decapitated under ether anaesthesia at 28 days post-infection (pi) and blood from the neck vessels collected, allowed to clot and serum harvested after centrifuging at 1000g for 5 minutes. Serum alanine (ALT) and aspartate (AST) aminotransferases activities, serum cholesterol and calcium levels were determined using commercial reagents kits (Randox, Laboratory LTD, UK). Serum urea and creatinine concentrations were estimated by methods of Tietz (1986).

**Statistical analysis:** The data obtained were summarized as means ± standard deviations and means were compared by analysis of variance (ANOVA) and students’s t-test (Chatfield, 1983).

**RESULTS**

**Parasitological findings:** In groups C and D, the mean prepatent periods in experiment I (Ex 1) were 10.1 ± 1.4 and 14.5 ± 2.5 days respectively while in experiment II (Ex II), they were 10.2 ± 1.3 and 13.3 ± 2.3 days respectively. The prepatent periods were significantly shorter (*P* < 0.05) in group C than group D in Ex I and Ex II. The level of parasitaemia was progressive in both infected groups however group C exhibited higher (*P* < 0.05) level of parasitaemia than group D (Table 1).

**Haematological findings:** The packed cell volume (PCV) decreased significantly (*P* < 0.05) in all the infected rats in groups C and D when compared with the values in the uninfected untreated or uninfected treated control rats in groups A and B respectively in both Ex I and Ex II. The level of decrease was more severe (*P* < 0.05) in group C than group D in both Ex I and Ex II.

**Biochemical findings:** The serum ALT, AST, Cholesterol, calcium, urea and creatinine values are presented in Table 3. The ALT, AST, urea and creatinine levels increased significantly (*P* < 0.05) in groups C and D with the level being higher (*P* < 0.05) in group C than group D. The serum cholesterol and calcium levels on the other hand, decreased significantly (*P* < 0.05) in both infected groups C and D with the level of decrease observed to be more (*P* < 0.05) in group C than group D.
Table 1: Mean (± SD), parasitaemia (x 10^3/μl) of rats orally treated or untreated with CaCl_2 and infected with *T. congolense* when experiment terminated at the peak of parasitaemia (PP) or allowed to run a full course (FC).

<table>
<thead>
<tr>
<th>Stage of infection</th>
<th>Days post - infection</th>
<th>T. congolense infected</th>
<th>T. congolense treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP</td>
<td>8</td>
<td>0.4±12.6^a</td>
<td>0.3^a</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>20±23.1^a</td>
<td>20±12.6^a</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>55±12.6^a</td>
<td>14±23.1^b</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>75±12.6^a</td>
<td>24±32.2^a</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>97±11.5^a</td>
<td>48±32.9^a</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>120±16.3^a</td>
<td>72±26.9^a</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>202±28.9^a</td>
<td>94±26.7^b</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>258±40.4^a</td>
<td>121±28.4^a</td>
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<tr>
<td></td>
<td>24</td>
<td>358±44.6^a</td>
<td>144±33.7^b</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>732±10.2^a</td>
<td>162±33.2^b</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>848±10.4^a</td>
<td>248±25.2^b</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>113±10.4^a</td>
<td>248±25.2^b</td>
</tr>
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</table>

| FC                 | 8                     | 4.0±12.6^a             | 0.0^a                 |
|                    | 10                    | 30±27.0^a              | 4.0±12.6^a            |
|                    | 12                    | 59±19.1^a              | 18±23.9^b             |
|                    | 14                    | 75±15.0^a              | 38±25.9^a             |
|                    | 16                    | 96±15.0^a              | 61±29.1^b             |
|                    | 18                    | 122±19.8^a             | 76±21.1^a             |
|                    | 20                    | 163±23.1^a             | 87±19.4^b             |
|                    | 22                    | 185±25.4^a             | 99±21.8^b             |
|                    | 24                    | 224±42.9^a             | 115±23.6^b            |
|                    | 26                    | 282±56.1^a             | 134±25.0^b            |
|                    | 28                    | 593±14.0^a             | 156±24.5^c            |
|                    | 30                    | 613±48.1^a             | 340±82.7^b            |
|                    | 32                    | -                      | 382±51.8              |

^a, b values in rows with different superscript differ significantly (P < 0.05); - = death

The serum calcium level in group B (CaCl_2 treated and uninfected control) was slightly higher (P > 0.05) than that in group A (uninfected and untreated control) while the level was significantly lower (P < 0.05) in group C (infected and untreated rats) when compared with group D (infected and treated rats). The mean survival times in groups C and D rats were 26.5 ± 0.5 and 32.0 ± 1.6 days respectively, with the value being shorter (P < 0.05) in group C than group D.

**DISCUSSION**

In the present study, calcium chloride treatment appeared to have ameliorated the pathogenicity of *Trypanosoma congolense* infection in rats. This is based on the premise that the observed prepatent period and survival time were shorter, level of parasitaemia higher and anaemia more severe in *T. congolense* infected and calcium chloride untreated (group C) than in the infected and treated (group D) rats. Calcium chloride (CaCl_2) treatment can be considered as the only exogenous factor that could have accounted for the differences in observations of the parameters assessed. Infective dose of trypanosome species has been reported to influence variations in some parameters in trypanosomosis (Losos, 1986; Murray and Dexter, 1988) but such phenomenon is not applicable in this study, since all the infected rats got similar dose.

Table 2: Packed cell volume (%) of control rats and rats infected with *T. congolense* (TC) with or without oral CaCl_2 treatment when the experiment was terminated at the peak of parasitaemia (PP) or allowed to run a full course (FC).

<table>
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<tr>
<th>Stage of infection</th>
<th>Days post – infection</th>
<th>Uninfected untreated control</th>
<th>Treated infected control</th>
<th>TC infected untreated</th>
<th>TC infected treated</th>
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<tr>
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<tr>
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<td>4</td>
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<td>50.6±0.9^a</td>
<td>50.7±0.5^a</td>
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<td></td>
<td>8</td>
<td>50.2±1.0^a</td>
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<td>50.1±0.7^a</td>
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<td>50.4±0.7^a</td>
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<td>49.6±0.2^a</td>
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<td>44.3±0.8^b</td>
<td>48.0±2.2^c</td>
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<td>46.0±1.6^c</td>
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<td>50.0±0.0^a</td>
<td>50.0±0.0^a</td>
</tr>
<tr>
<td></td>
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<tr>
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<td>50.3±0.5^a</td>
<td>50.0±0.0^a</td>
<td>49.8±0.1^a</td>
<td>50.0±0.0^a</td>
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<td>50.3±0.5^a</td>
<td>50.0±0.0^a</td>
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<td>49.1±1.1^a</td>
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<td>32</td>
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<td>50.2±0.7^a</td>
<td>-</td>
<td>32.4±2.1^c</td>
</tr>
</tbody>
</table>

^a = death; ^ab Values in rows with different superscripts differ significantly (p<0.05)
Anaemia which was more severe in the infected and untreated rats generally determines the severity of trypanosome infection in animals (Lisos, 1986; Murray and Dexter, 1988). The anaemia coincided with the onset of parasitaemia and progressed with the height of the latter.

The biochemical parameters indicated liver and kidney damage. The increase in serum ALT and AST and reduced cholesterol levels may be associated with liver damage (White et al., 1980; Baron, 1982; Kaggwa et al., 1984), while the elevated serum urea and creatinine levels may probably be due to renal damage (Anosa, 1988). The increase in the serum ALT, AST, urea and creatinine levels was less in the T. congolense infected and CaCl$_2$ treated rats and this also buttresses the positive effect of the treatment on trypanosome infections. Furthermore, the serum calcium levels were low in T. congolense infected untreated rats when compared with the level being much lower in the T. congolense infected untreated rats. The hypocalcaemia reported in the present study did not differ from earlier reports (Fiennes et al., 1946; Zia-Ur-Rahman et al., 1996) and may be associated with hypoproteinaemia (Losos, 1986; Anosa, 1988). It is also important to note that in the control groups, the calcium level was slightly higher but not significant in the CaCl$_2$ treated uninfected rat groups when compared with the values of the uninfected untreated rats. The slight increase in the treated uninfected rats may be associated with high intestinal absorption potential of calcium chloride. Calcium chloride treatment did not manifest any negative effect on the PCV value of the treated uninfected control rats. Calcium compounds ingested orally usually are not considered toxic. But high intake of calcium in animals can have a depressing effect on utilization of other nutrients like phosphorous, zinc, magnesium and iron (Guthrie et al., 1975).

In conclusion, the oral CaCl$_2$ treatment in rats appeared to delay the onset of parasitaemia and reduce the levels of parasitaemia, anaemia, liver and kidney damages in Trypanosoma congolense infection.

Acknowledgement:
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REFERENCES


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<th>Parameters</th>
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<th>Treated uninfected control</th>
<th>T. congolense infected untreated</th>
<th>T. congolense infected treated</th>
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<tr>
<td>ALT (i.u/L)</td>
<td>9.2±1.0$^a$</td>
<td>9.4±1.1$^a$</td>
<td>60.2±3.5$^b$</td>
<td>30.2±1.9$^a$</td>
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<tr>
<td>AST (i.u/L)</td>
<td>10.3±1.8$^a$</td>
<td>10.1±1.8$^a$</td>
<td>60.1±3.4$^b$</td>
<td>30.1±1.8$^c$</td>
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<td>Cholesterol (mg/dl)</td>
<td>140.6±2.9$^a$</td>
<td>140.7±2.1$^a$</td>
<td>50.5±3.9$^b$</td>
<td>93.0±3.2$^c$</td>
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<tr>
<td>Calcium (mg/dl)</td>
<td>9.1±0.4$^a$</td>
<td>9.9±0.5$^a$</td>
<td>2.7±0.4$^b$</td>
<td>5.0±0.4$^c$</td>
</tr>
<tr>
<td>Urea (mmol/dl)</td>
<td>4.8±0.1$^a$</td>
<td>4.8±0.1$^a$</td>
<td>13.2±0.3$^b$</td>
<td>8.4±0.2$^c$</td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>63.3±1.7$^a$</td>
<td>63.5±1.7$^a$</td>
<td>125.7±3.9$^b$</td>
<td>90.5±4.3$^c$</td>
</tr>
</tbody>
</table>

$^a$,$^b$,$^c$ Values in rows with different superscripts differ significantly (p<0.05)


