The effects of oral manganese chloride supplementation on the severity of *Trypanosoma brucei* and *Trypanosoma congolense* infections in rats

Tobias Nnia Egbe-Nwiyi*, Anastasia Nwaosu, and Medugu Apagu Gazdama

Department of Veterinary Pathology, University of Maiduguri, Maiduguri, Nigeria


**ABSTRACT**

Eighty healthy adult albino rats of both sexes were used in two experiments to study the effects of manganese chloride supplementation on the severity of *Trypanosoma brucei* and *Trypanosoma congolense* infections. In each experiment, forty rats were divided into four groups of 10 each: A. Infected unsupplemented; B. Infected supplemented; C. Uninfected unsupplemented control; D. Uninfected supplemented control. Aqueous solution (5%) of MnCl₂ was administered daily using stomach tube to each rat at 50 mg/kg body weight in groups B and D from 10 days before infection to the end of the experiment. Each rat in groups A and B was infected by intraperitoneal injection of 1x10⁶ trypanosomes (*T. brucei* or *T. congolense*) in diluted donor blood. The prepatent periods were shorter (P<0.05) in *T. brucei* than *T. congolense* infections, and shorter (P<0.05) in infected unsupplemented than in infected supplemented rats. The infected unsupplemented groups had higher (P < 0.05) parasitaemia and more severe anaemia than the infected supplemented groups. Therefore, oral manganese chloride supplementation in rats appeared to reduce the severity of trypanosome infections by delaying the onset of parasitaemia, reducing the levels of parasitaemia and accompanying anaemia.

**Key words:** manganese chloride, *Trypanosoma brucei*, *Trypanosoma congolense*, rats

**Introduction**

Animal production in Nigeria may be hindered by many factors, such as economical, political, diseases and a host of others. The diseases may be bacterial, viral, parasitic or idiopathic (LOSOS, 1986). Parasitic diseases affect animal production substantially, some
of the diseases being anaplasmosis, babesiosis, trypanosomosis, haemobartonellosis, eperythrozoonosis, and others (LOSOS, 1986) Trypanosomosis is of great economic importance as it causes low milk yield, orchitis in males, infertility in females, abortion, anaemia and death in affected animals (LOSOS, 1986; ANOSA, 1988). The major pathogenic trypanosomes for domestic animals are *T. congolense*, *T. vivax* and *T. brucei* (ANOSA, 1988)

Manganese is a constituent component of plant and animal tissues and is an essential element in animal nutrition (MACDONALD et al., 1995). Large levels of manganese intake by calves can reduce growth rate and haemoglobin level and it is also known to interfere with the utilization of cobalt and zinc in ruminants (CUNNINGHAM et al., 1966). Dietary deficiency leading to infertility and skeletal deformities has been reported.

Animals placed on good nutrition are highly likely to withstand viral, bacterial and parasitic diseases better than those on a poor or low plane of nutrition (LOSOS, 1986; OTESILE et al., 1991). Various plant extracts have been reported to suppress trypanosome infections in animals (ASUZU and CHINEME, 1990; MADUBUNYI, 1995; RABO, 1998). Mineral supplementation has also been reported to ameliorate the severity of trypanosome infections in rats (EGBE-NWIYI et al., 2003). In view of the foregoing, this work was undertaken to determine whether manganese chloride supplementation enhances or reduces trypanosome infections in rats.

**Materials and methods**

**Experimental animals.** Eighty healthy adult albino rats of both sexes, weighing 150-200 grams, obtained from the laboratory animal unit of the Department of Veterinary Pathology, University of Maiduguri, Nigeria, were used. They were housed in clean cages at 30-35 °C, fed a standard commercial diet (ECWA Feeds Ltd, Jos, Nigeria) and clean water was provided ad libitum. Forty rats were used in each of the two experiments (1 and 11) in which there were four groups (A, B, C, D) of 10 rats each. The groups were, A: infected unsupplemented; B: infected supplemented; C: uninfected unsupplemented control, and D: uninfected supplemented control.

**Trypanosome infection.** *Trypanosoma brucei* (Lafia Strain) and *Trypanosoma congolense* (Gboko Strain) obtained from the Nigerian Institute for Trypanosomosis Research (NITR), Vom, Nigeria, were used. Each species of the parasite was maintained by serial passages in donor rats. Diluted infected blood from the donor rats containing $1 \times 10^6$ trypanosomes was intraperitoneally injected into each rat in groups A and B in experiments 1 and 11 using *T. brucei* and *T. congolense*, respectively. Tail blood from the infected rats was examined daily until parasitaemia established, and the level of parasitaemia was estimated using the haemocytometer method (COLES, 1980).
Oral manganese chloride supplementation. Manganese chloride \((\text{MnCl}_2)\) (BDH Chemicals Ltd, Poole, England) solution (5% aqueous) was administered daily by stomach tube to each rat at 50 mg/kg in groups B and D in both experiments. The solution was administered 10 days before trypanosome infection to the end of the experiment.

Determination of packed cell volume (PCV). The PCV of the tail blood of the rats was determined every 4 days by the microhaematocrit method (COLES, 1980).

Statistics. The data obtained were summarized as means ± standard deviations and means were compared by analysis of variance (ANOVA) and Student’s \(t\)-test (CHATFIELD, 1983).

Results

Prepatent periods were 3.0 ± 0.8 and 5.7 ± 1.2 days in \(T. \text{brucei}\) infected unsupplemented and supplemented rats, respectively, while in \(T. \text{congolense}\) infected unsupplemented and supplemented rats prepatent periods were 5.1 ± 0.6 and 7.2 ± 1.0 days, respectively. The prepatent periods were shorter (P<0.05) in \(T. \text{brucei}\) than \(T. \text{congolense}\) infections, and shorter (P<0.05) in infected unsupplemented than infected supplemented rats in both experiments.

Table 1. Mean parasitaemia (x10³/µl) of rats infected with \(T. \text{brucei}\) with or without oral manganese chloride supplementation, \((n = \text{number of rats})\)

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Days post-infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>(T. \text{brucei}) infected unsupplemented ((n = 10))</td>
<td>Oa</td>
</tr>
<tr>
<td>(T. \text{brucei}) infected supplemented ((n = 10))</td>
<td>Ob</td>
</tr>
</tbody>
</table>

Values in columns with different superscripts differ significantly (P < 0.05).

Parasitaemia in \(T. \text{brucei}\) and \(T. \text{congolense}\) infected unsupplemented and supplemented rats are presented in Tables 1 and 2. The infected unsupplemented groups had higher (P<0.05) parasitaemia than the infected supplemented groups in both experiments (Tables 1 and 2).
Table 2. Mean parasitaemia (x10³/µl) of rats infected with *T. congolense* with or without oral manganese chloride supplementation (n = number of rats)

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Days Post-infection</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>16</th>
<th>18</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. congolense</em> infected unsupplemented (n = 10)</td>
<td>±</td>
<td>15.0 ± 5.2a</td>
<td>29.0 ± 3.1a</td>
<td>32.0 ± 4.2a</td>
<td>39.0 ± 7.3a</td>
<td>45.0 ± 8.4a</td>
<td>52.0 ± 6.3a</td>
<td>89.0 ± 5.6a</td>
<td>104.0 ± 11.7a</td>
</tr>
<tr>
<td><em>T. congolense</em> infected supplemented (n = 10)</td>
<td>0b</td>
<td>15.0 ± 5.6b</td>
<td>19.0 ± 5.2b</td>
<td>25.0 ± 5.2b</td>
<td>29.0 ± 3.7b</td>
<td>37.0 ± 8.2b</td>
<td>52.0 ± 4.2b</td>
<td>56.0 ± 5.1b</td>
<td></td>
</tr>
</tbody>
</table>

Values in columns with different superscripts differ significantly (P < 0.05).

Table 3. Mean packed cell volume (PCV %) of control rats and *T. brucei* or *T. congolense* infected rats with or without oral MnCl₂ supplementation (n = number of rats)

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Days Post-infection</th>
<th>0</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected unsupplemented controls (n = 10)</td>
<td>±</td>
<td>46.8 ± 0.7a (49.1 ± 0.6a)</td>
<td>46.8 ± 0.8a (49.2 ± 0.4a)</td>
<td>47.0 ± 0.9a (49.6 ± 0.8a)</td>
<td>47.0 ± 0.9a (49.4 ± 0.4a)</td>
<td>ND (49.6 ± 0.1a)</td>
<td>ND (49.4 ± 0.2a)</td>
</tr>
<tr>
<td><em>T. brucei</em> or <em>T. congolense</em> infected unsupplemented (n = 10)</td>
<td>±</td>
<td>46.9 ± 0.9a (48.2 ± 0.6a)</td>
<td>46.9 ± 0.9a (48.6 ± 0.9a)</td>
<td>24.2 ± 1.9b (48.6 ± 0.4a)</td>
<td>17.0 ± 2.2b (42.1 ± 0.4a)</td>
<td>ND (35.0 ± 0.8a)</td>
<td>ND (30.1 ± 0.2a)</td>
</tr>
<tr>
<td><em>T. brucei</em> or <em>T. congolense</em> infected supplemented (n = 10)</td>
<td>±</td>
<td>46.8 ± 0.8a (49.4 ± 0.6a)</td>
<td>46.8 ± 0.8a (49.3 ± 0.6a)</td>
<td>40.8 ± 3.5b (49.6 ± 0.4a)</td>
<td>31.3 ± 1.9b (48.1 ± 0.6a)</td>
<td>ND (43.8 ± 0.4a)</td>
<td>ND (33.1 ± 0.2a)</td>
</tr>
<tr>
<td>Uninfected supplemented controls (n = 10)</td>
<td>±</td>
<td>46.8 ± 0.1a (49.1 ± 0.6a)</td>
<td>46.8 ± 0.1a (49.2 ± 0.4a)</td>
<td>46.8 ± 0.1a (49.6 ± 0.8a)</td>
<td>46.8 ± 0.1a (49.4 ± 0.4a)</td>
<td>ND (49.6 ± 0.1a)</td>
<td>ND (49.4 ± 0.2a)</td>
</tr>
</tbody>
</table>

ND = no data, ( ) = value of *T. congolense* group, Values in rows with different superscripts differ significantly (P < 0.05).
PCV decreased progressively in all the infected groups in both experiments (Table 3). There was no significant (P>0.05) variation in the PCV of the uninfected unsupplemented and supplemented control rats, although there was such a variation in the infected groups. Mean PCV values in the infected unsupplemented groups were significantly (P<0.05) lower than those of the infected supplemented rats in both experiments between days 8-12 post-infection for *T. brucei* and 16-20 post-infection for *T. congolense* (Table 3).

**Discussion**

The *T. brucei* and *T. congolense* infected unsupplemented rats exhibited shorter prepatent periods and higher level of parasitaemia than the corresponding infected supplemented rats. The variations in parameters in the *T. brucei* and *T. congolense* infected unsupplemented and supplemented groups might not be related to the dose of trypanosomes used as a similar infective dose was applied in all the infected groups. Although MURRAY and DEXTER (1988) observed that the level of parasitaemia and prepatent period could be influenced significantly by the number of trypanosomes used in any given infection. The onset of parasitaemia was earlier in *T. brucei* than *T. congolense* infection, and in both *T. brucei* and *T. congolense* infected unsupplemented than infected supplemented rats. This is in agreement with the findings of other investigators (BROWN and LOSOS, 1977; IGBOKWE and NWOSU, 1997; EGBE-NWIYI et al., 2003). The level of parasitaemia was persistently higher in *T. brucei* infected unsupplemented from days 4-12 post-infection and 6-20 post-infection in *T. congolense* infected unsupplemented rats. These findings did not differ from the observations of EGBE-NWIYI et al. (2003) in *T. brucei* and *T. congolense* infected supplemented and unsupplemented rats. It is pertinent to suggest, therefore, that the MnCl₂ supplementation might have modulated the course of the infection in the animals, as evidenced by lower prepatent periods and parasitaemia levels in the infected supplemented groups. It was established in the present study that the only key factor that can account for variations in the mentioned parameters was MnCl₂. AWOLAJA et al. (1997) reported higher plasma/serum and erythrocytes manganese levels in trypanotolerant keteku than in trypanosusceptible white fulani breeds of cattle.

The anaemia exhibited by all infected groups was more severe in *T. brucei* and *T. congolense* infected unsupplemented groups, and on species comparison the anaemia was more severe in *T. brucei* infected unsupplemented than *T. congolense* infected unsupplemented rats. In some animal species, *T. brucei* is known to cause more severe anaemia than *T. congolense*, while the reverse might be the case depending on certain variables, such as virulence and strain of the species of the trypanosome, age and immune status of the host, and some other factors (LOSOS, 1986; MURRAY and DEXTER, 1988). The anaemia seen in the present study coincided with the onset and height of parasitaemia. The latter was higher in all the infected unsupplemented rats. Therefore, the more severe anaemia
in the infected unsupplemented groups may be directly related to the level of parasitaemia. Virulence is related to the height of parasitaemia, and anaemia is usually used as a measure of degree of severity of trypanosome infections (ANOSA, 1988; MURRAY and DEXTER, 1988). Trypanosomes which divide very rapidly produce a high level of parasitaemia and tend to kill the host quickly (LOSOS, 1986; MURRAY and DEXTER, 1988).

There is wide range of safety between the toxic dose of Mn and normal level in food, and most animals tolerate an intake of high levels of Mn (MACDONALD et al., 1995). The MnCl₂ supplementation appeared not to have any detectable effect on the PCV of the uninfected supplemented control rats, which showed no Mn toxicity in this experiment.

In conclusion, oral manganese chloride supplementation in rats appeared to delay the onset of parasitaemia and reduced the parasitaemia and anaemia. Therefore, MnCl₂ supplementation may assist in boosting trypanotolerance in animals.

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References
T. N. Egbe-Nwiyi et al.: The effects of oral manganese chloride supplementation on the severity of Trypanosoma brucei and Trypanosoma congolense infections in rats


SAŽETAK
Osamdeset zdravih odraslih albino štakora oba spola rabljeni su za istraživanje učinka dodatka manganova klorida na težinu invazije vrstama Trypanosoma brucei i Trypanosoma congolense. Istraživanje je provedeno u dva pokusa. Za svaki pokus uzeto je 40 štakora podijeljenih u četiri skupine po deset. Štakori skupine A bili su invadirani, ali nisu dobivali manganov klorid. Štakori skupine B bili su invadirani i dobivali su manganov klorid. Štakori skupine C nisu bili invadirani niti su dobivali manganov klorid, dok su oni skupine D ostali neinvadirani s dodatkom manganova klorida i služili su kao kontrola. Vodena otopina (5%) MnCl₂ davana je peroralno u dnevnoj dozi od 50 mg/kg tjelesne mase štakorima skupine B i D od 10. dana prije invadiranja do kraja pokusa. Svaki štakor skupina A i B bio je invadiran razrijeđenom krvi donora intraperitonealno s 10⁶ tripanosoma (T. brucei ili T. congolense). Prepatentni period bio je kraći (P<0,05) u štakora invadiranih vrstom T. brucei nego u štakora invadiranih vrstom T. congolense. Također je bio kraći (P<0,05) u invadiranim koji nisu dobivali manganov klorid nego u onih invadiranima koji su ga dobivali. Invadirane skupine koje nisu dobivale manganov klorid imale su duže razdoblje parazitemije (P<0,05) i mnogo težu anemiju nego koje su dobivale MnCl₂. Čini se da oralno davanje manganova klorida smanjuje težinu invazije tripanosomama odgađanjem pojave parazitemije, smanjenjem razine parazitemije i popratne anemije.

Ključne riječi: manganov klorid, Trypanosoma brucei, Trypanosoma congolense, štakor

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