

Effect of time of treatment on the efficacy of trypanocides in rats experimentally infected with *Trypanosoma brucei brucei*

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Abstract

The effect of treatment time on the efficacy of trypanocides in rats experimentally infected with *Trypanosoma brucei brucei* was assessed. The thirty albino rats utilized in this study were divided into six groups, each with five rats. Group 1 rats were uninfected while those in groups 2 - 6 were inoculated intraperitoneally with 10^6 trypanosomes. Group 2 rats were left untreated while groups 3 - 6 rats were treated respectively on days 5, 8, 11 and 14 post infection (PI) with 1.0 mg/kg Securidium® (brand of isometamidium chloride) intraperitoneally. Level of parasitaemia, rectal temperature, clinical signs, haematological indices (packed cell volume and total leucocyte count) and survivability were used to evaluate the treatment time on the efficacy of Securidium®. Pre-patent period was 4 - 5 days. On days 4, 5, and 11 following the treatments (PT), the parasites were eliminated in the treated groups 3 - 6 respectively. Also, two rats relapsed in group 3 from 37 days PT with 20% mortality, whereas groups 4 - 6 rats relapsed from days 19, 16 and 21 PT respectively. Groups 4 - 6 rats had 40%, 60% and 80% mortalities respectively. The mean PCV and TLC showed a striking drop ($p < 0.05$) PI, however, group 3 had a better result PT compared to other infected-treated groups. In conclusion, it is safe to say that treatment time is a significant determinant of therapeutic efficacy in trypanosomosis, as early treatment group had better overall indices of therapeutic efficacy than the late treatment groups.

Keywords: efficacy; isometamidium chloride; rats; time of treatment; trypanocides; *Trypanosoma brucei*

Introduction

African trypanosomosis is a serious progressively debilitating tsetse fly-borne protozoan disease that affects humans and animals (Okpala *et al.*, 2021). African trypanosomosis has been widely documented to be the major obstacle to sustainable livestock production and food security; also accountable for the underdevelopment in most tropical African countries (Oyewusi *et al.*, 2010). Economic losses from bovine trypanosomosis were estimated by reports from the Nigerian Institute for Trypanosomosis Research (NITR),

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Vom, to be US\$ 48.06 million, while losses from animal death and live weight were US\$ 2.46 million (Fadiga *et al.*, 2013). African trypanosomosis is primarily caused by protozoan parasite, *Trypanosoma*, and the prevalent species in domestic animals are *T. vivax*, *T. congolense*, and *T. brucei* (de Gier *et al.*, 2020). The acute and chronic diseases conditions in humans known as sleeping sickness are caused by *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* respectively.

Animal trypanosomosis control involves the exploitation of three strategies namely; vector control, breeding of trypanotolerant livestock and the use of trypanocides. Of all these strategies, chemotherapy using salts of diminazene, homidium and isomethamidium, is the most popular, successful, and widely acknowledged method of trypanosomosis control (Ezeokonkwo *et al.*, 2007; Yaro *et al.*, 2016). However, there are significant obstacles to using trypanocides including emergence of resistance, poor quality of drugs and abuse (Bengaly *et al.*, 2018).

In order to mitigate the drawbacks above, careful and effective use of the available trypanocides is advocated (Giordani *et al.*, 2016). This is necessary because of absence of new trypanocides and the unwillingness of most pharmaceutical companies to fund the development and patenting of new trypanocides. The absence of new trypanocides has continued to pose a great challenge to veterinary clinicians in the management, treatment and/or control of African trypanosomosis (Geerts *et al.*, 2001). Thus, the current way out is to devise ways to make the most use possible of the trypanocides that are already available. Information on the effect of time of intervention with trypanocidal agents during the course of trypanosomosis appears to be a potent resource in the hands of veterinarians in the treatment/management of trypanosomosis. It is often speculated that early treatment usually leads to prompt recovery of affected animals, while late treatment is usually associated with slow recovery and treatment failures, depending on the severity of symptoms and extent of organ damage. However, there is paucity of information to justify this claim.

This research was therefore intended to investigate the effect of treatment time on the efficacy of trypanocides, using a brand of isometamidium, Securidium[®], in experimental *Trypanosoma brucei brucei* infection in rats. It is therefore, hoped that the information obtained from this study will guide veterinarians and clinicians on the suitable use of antitrypanosomal agents to reduce the rate of treatment failures and the emergence of resistant trypanosome populations.

Materials and Methods

Laboratory animals

Thirty (30) female albino rats were acquired for this experiment. The rats were kept in hygienic cages in the Veterinary Parasitology and Entomology Department's animal house, University of Nigeria, Nsukka. They rats underwent three weeks of conditioning during which they were treated with albendazole. The rats had unrestricted access to water and commercial rat food.

Trypanosomes

The Federe strain of *Trypanosoma brucei brucei* utilized in this experiment was acquired from the Nigerian Institute for Trypanosomiasis Research, Vom, Nigeria. Subsequently, repeated passage in mice was used to maintain the parasite in the laboratory.

Experimental drug

Securidium[®] (Laprovat, France), a brand of isomethamidium chloride was used for this study.

Infection of the experimental rats

The veins at the median canthus of the donor rats' eyes were punctured to draw blood, and the infected blood was then serially diluted with phosphate buffered saline. The experimental rats were each inoculated intraperitoneally (IP) with 10^6 trypanosomes. The trypanosomes were quantified using Herbert and Lumsden's (1976) rapid matching method.

Experimental design

The rats were split into six groups (1 - 6) comprising five rats each at random. Group 1 rats were uninfected control while groups 2 - 6 rats were inoculated IP with 10^6 trypanosomes. From the third day post infection (PI), the rats were screened daily for parasitaemia. When all the infected rats developed parasitemia, treatment was undertaken. On days 5, 8, 11, and 14 PI, rats in groups 3 - 6 were administered a single dose of 1 mg/kg Securidium® IP respectively.

The level of parasitaemia (LOP), clinical signs, packed cell volume (PCV), total leucocyte count (TLC), rectal temperature and survivability were assessed to determine the effect of treatment time on the efficacy of Securidium in experimental *T. brucei brucei* rat infection. Daily LOP was evaluated utilizing the Herbert and Lumsden's (1976) rapid matching method. Weekly rectal temperature and body weight changes were assessed using a digital clinical thermometer and a standard weighing scale respectively. The weekly haematological parameters (PCV and TLC) were evaluated utilizing the protocols outlined in Coles (1986).

Statistical analysis

The data generated were tested for normality utilizing the Shapiro-Wilk test, and one-way analysis of variance (ANOVA) was employed to analyse them. The post-hoc separation of the variant means was performed using the least significant difference. Significant probabilities were defined as those with ($p < 0.05$). Kaplan-Meier survival analysis was employed to evaluate the rats' survival times. The equality of survivor function curves between the treatment groups was assessed using the log-rank test. Version 21 of SPSS was used for all statistical analysis.

Results

Parasitaemia was detected first on day 4 PI in groups 2 and 4 rats. All the rats in group 3 were parasitaemic by day 5 PI. By day 6 PI, all other infected groups of rats (groups 4 - 6) became parasitaemic. The LOP of the rats persistently increased until they all died (group 2) or treatments on days 5, 8, 11 and 14 for groups 3, 4, 5 and 6 respectively (Figure 1). The mean pre-patent period for the infected groups was approximately 5 days. There was a significant reduction in the level of parasitaemia following treatment of the infected groups with complete elimination of parasitaemia occurring on days 9 PI [day 4 post treatment (PT)], 12 PI (day 4 PT), 16 PI (day 5 PT) and 25 PI (day 11 PT) for groups 3 - 6 respectively (Figure 1). The parasite clearance time of the early treatment groups 3 and 4 were short ($p < 0.05$) compared to the late treatment group 6. Clinical signs noted in the infected rat groups were anaemia, depression, pyrexia, pale mucous membrane, ascites, oedema of the ventral neck region, loss of weight, emaciation, starry hair coat and death. However, these signs progressively disappeared after treatment and parasite clearance from the bloodstream of rats in the treated groups of rats, but reappeared following relapse infection.

Relapse of infection was observed following treatment of the infected rats. Two rats in group 3 relapsed (40% relapse) from day 42 PI (day 37 PT). 100% relapse of infection was observed in groups 4 - 6 with relapse occurring from days 27 PI (19 PT), 27 PI (16 PT) and 35 PI (21 PT) respectively (Figure 1). All the rats in the infected untreated group 2 died between days 14 and 20 PI.

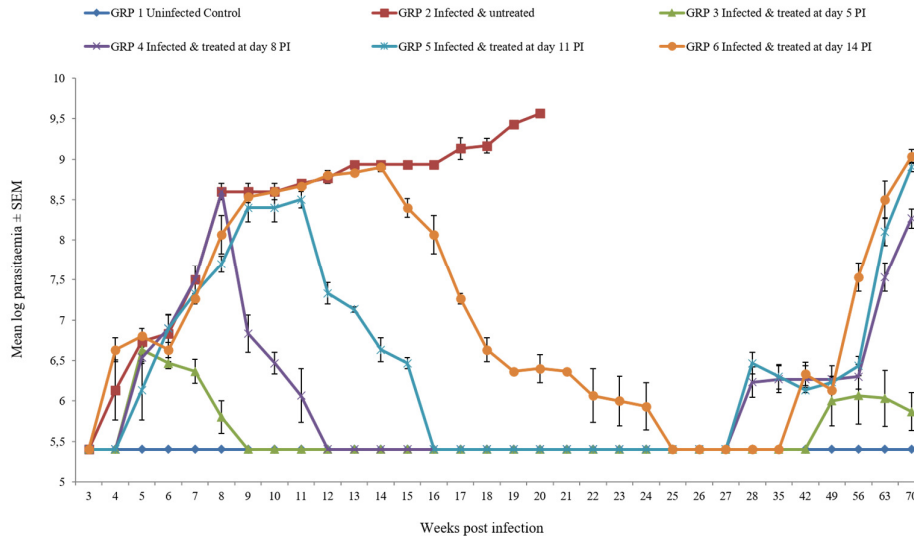


Figure 1. Mean log parasitaemia of rats infected with *Trypanosoma brucei* and treated with 1.0 mg/kg of Securidium at different time intervals
 Bars represent standard error of the mean. Group 1 was the uninfected control, Group 2 was infected but not treated, while Groups 3 - 6 were infected and treated with 1.0 mg/kg of Securidium on days 5, 8, 11 and 14 post infection respectively.

Therapeutic interventions extended the life span of the rats in groups 3 - 6. Four rats survived in the early treatment group 3 with only one death (20% mortality) occurring on day 49 PI (44 days PT) while three rats survived in group 4 with two deaths (40% mortalities) occurring on days 45 and 67 PI (37- and 59-days PT) (Figure 2). The late treatment group 5 had 60% mortality [days 18, 42 and 65 PI (7-, 31- and 54-days PT respectively)] while only one rat survived in group 6 with 80% mortality observed on days 13, 42, 64 and 66 PI (-1, 28-, 50- and 52-days PT). The mean post infection survival times for the groups of rats were 70 ± 0.0 , 17.2 ± 1.07 , 65.8 ± 4.20 , 64.4 ± 4.88 , 53 ± 10.17 and 51 ± 10.68 for groups 1 - 6 respectively. The early treatment groups 3 and 4 had longer ($p > 0.05$) survival times than the late treatment groups 5 and 6 (Figure 2).

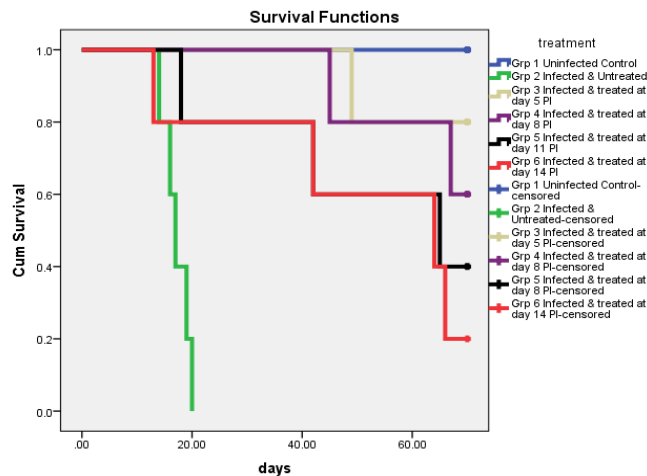


Figure 2. Kaplan-Meier survival estimates of rats infected with *Trypanosoma brucei* and treated with 1.0 mg/kg of Securidium at different time intervals
 Group 1 was the uninfected control, Group 2 was infected but not treated, while Groups 3 - 6 were infected and treated with 1.0 mg/kg of Securidium on days 5, 8, 11 and 14 post infection respectively.

There was a substantial decline ($p < 0.05$) in the PCV values of the infected-untreated group 2 rats compared to the uninfected group 1 rats at weeks 1 and 2 PI (Figure 3). The PCV values of group 3 rats did not vary statistically ($p > 0.05$) from those of the uninfected group 1 throughout the duration of the experiment except at weeks 7 and 8 PI. Amongst the treated groups of rats, the mean PCV of group 3 rats were higher ($p < 0.05$) than those of groups IV and VI at week 1 PI; group 5 at week 4 PI; groups 4, 5 and 6 at weeks 5, 6 and 10 PI; and group 6 at weeks 7 - 9 PI.

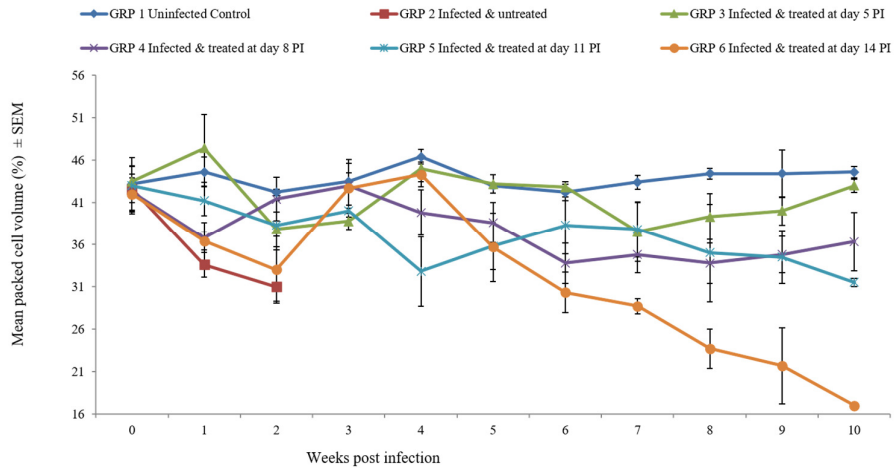


Figure 3. Mean Packed cell volume (%) of rats infected with *Trypanosoma brucei* and treated with 1.0 mg/kg of Securidium at different time intervals
 Bars represent standard error of the mean. Group 1 was the uninfected control, Group 2 was infected but not treated, while Groups 3 - 6 were infected and treated with 1.0 mg/kg of Securidium on days 5, 8, 11 and 14 post infection respectively.

The TLC of the uninfected group 1 rats were significantly elevated ($p < 0.05$) compared to those of the infected groups at week 3 PI (Figure 4). Group 1 also had higher ($p < 0.05$) TLC than group 4 rats at week 6 PI, group 5 rats at week 4 PI and group 6 rats at weeks 4 - 10 PI. Rats in the infected and treated groups showed no discernible differences ($p > 0.05$) in TLC except at week 7 PI where group 3 rats had elevated ($p < 0.05$) TLC compared to group 6 rats.

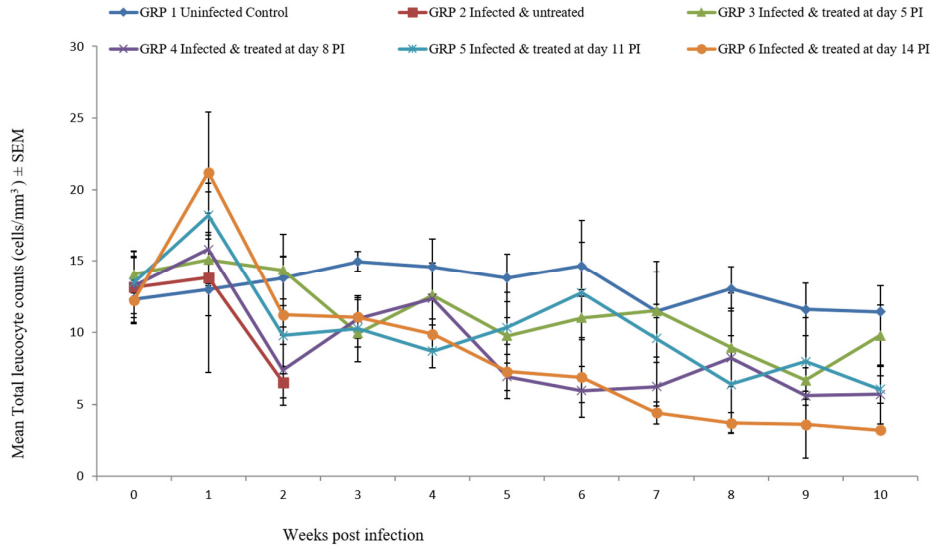


Figure 4. Mean total leucocyte counts (cells/mm³) of rats infected with *Trypanosoma brucei* and treated with 1.0 mg/kg of Securidium⁺ at different time intervals. Bars represent standard error of the mean. Group 1 was the uninfected control, Group 2 was infected but not treated, while Groups 3 - 6 were infected and treated with 1.0 mg/kg of Securidium⁺ on days 5, 8, 11 and 14 post infection respectively.

The rectal temperature of groups 2, 4 and 5 rats were significantly elevated compared to the uninfected group at week 1 PI (Figure 5). Also, the infected groups of rats had substantially elevated rectal temperature compared to the uninfected group 1 at weeks 2, 7 and 8 PI. Amongst the treated groups of rats, the early treatment groups 3 and 4 had significantly lower mean rectal temperature at weeks 7 and 8 PI than the late treatment group 6.

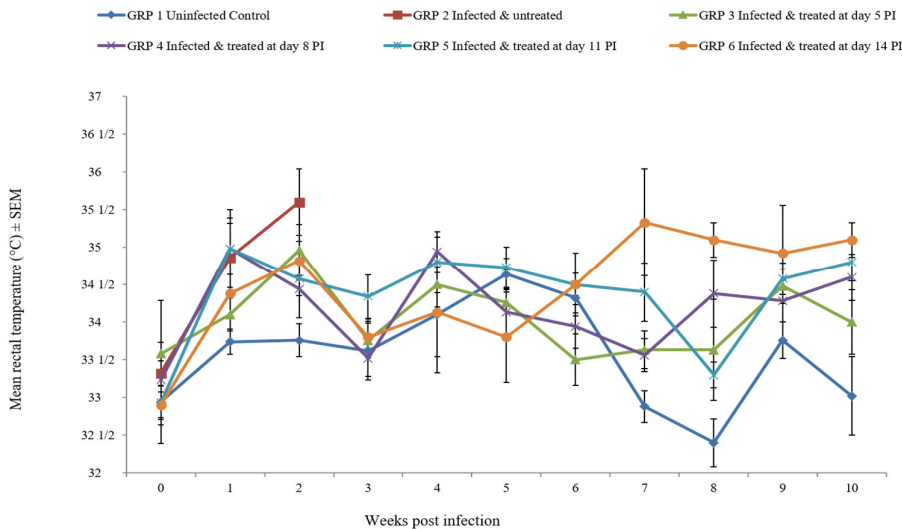


Figure 5. Mean rectal temperature (°C) of rats infected with *Trypanosoma brucei* and treated with 1.0 mg/kg of Securidium⁺ at different time intervals. Bars represent standard error of the mean. Group 1 was the uninfected control, Group 2 was infected but not treated, while Groups 3 - 6 were infected and treated with 1.0 mg/kg of Securidium⁺ on days 5, 8, 11 and 14 post infection respectively.

Discussion

The pre-patent period (PPP), which was 4 - 5 days in our study, is consistent with previous reports (Okpala *et al.*, 2019; Amaechi *et al.*, 2016). However, this PPP differed with reports of Okpala *et al.* (2021) who recorded a pre-patent period of 8-14 days. This variation may be due to the differences in the trypanosome isolates/strains used in the studies. The age, immune response of the rats and quantity of trypanosome parasites inoculated may also have contributed to this disparity (Taylor and Authie, 2004).

Clearance time, relapse and survivability are acknowledged as principal pointers of trypanocide efficacy (Osondu *et al.*, 2016; Aregawi *et al.*, 2021). Thus, the shorter clearance time (4 days PT) observed in groups 3 and 4 rats against the group 6 rats (14 days PT) may be attributed to the fact that treatments were instituted at different periods and the level of parasitaemia varied in each infected group at the time of treatment, with group 6 which was treated at day 14 PI having the highest parasitaemia level. Also, the peak plasma concentrations of isometamidium occurs within an hour post administration and then drop quickly during the first week post treatment, and thereafter more slowly (Kinabo, 1993; Eisler *et al.*, 1994).

Relapse of infection recorded in the treated groups may be due to the fact that isometamidium chloride possesses large molecular size, which could not pass the blood brain, thereby hampering the ability of this agent to clear parasites already sequestered in the brain (Barret, 2001; Geert *et al.*, 2001). However, it is pertinent to note that 60% of rats in group 3 did not relapse throughout the study period while 100% of treated rats in groups 4 - 6 relapsed. Also, relapse time was shorter in the late treatment groups (*i.e.* groups 5 - 6 rats) than the early treatment group 3. Trypanosome relapse infections post treatment, has been previously reported (Anene *et al.*, 2006; Ezech *et al.*, 2011; Okpala *et al.*, 2021).

The observed low mortality, high survival rates, low relapse incidence, long relapse time, short clearance time and improved haematological indices in group 3 compared to the group 6 indicates that therapeutic failure is minimal with early treatments during trypanosomosis compared to late treatments. This might be possible as the infecting trypanosome species may not have invaded sites inaccessible to the drug or that the infection may not have overwhelmed the host immune system when treatments were carried out early in the course of infection. Late treatment of trypanosomosis has been associated with treatment failures and is a risk factor for the development of drug-resistant trypanosome isolates (Geerts and Holmes, 1998). However, it is expected that the drug used (Securidium) should prevent relapse infection especially in the early treatment group 3. The inability to prevent relapse infection could insinuate that the *Trypanosoma brucei brucei* utilized in this experiment could possess some levels of resistance to isometamidium chloride though the sensitivity of the *Trypanosoma brucei brucei* isolate utilized in this study to trypanocides was not determined.

The symptoms/clinical signs observed during this study were indicative of trypanosomosis and have been documented by a number of authors (Obi *et al.*, 2020; Okpala *et al.*, 2021). Reduction in the PCV of the infected groups of rats signifies anaemia. Previous studies have documented similar findings (Obi *et al.*, 2020; Okpala *et al.*, 2021). Anaemia observed could be due to phagocytosis of erythrocytes, depression of erythropoiesis, haemodilution and increased plasma volume, dyshaemopoiesis, etc. (Taylor and Authie, 2004). Transient leucocytosis was noted in all infected groups of rats at week 1 PI in this study and this may have been caused by a heightened immune response to the invasive trypanosomes (Taylor and Authie, 2004). Thereafter, leucopaenia was recorded in the infected groups from week 2 PI, and could be credited to trypanosome-induced immunosuppression. The initial rise and subsequent decline in the rectal temperatures are typical of trypanosomosis, according to published studies (Taylor and Authie, 2004). Pyrexia may have been noticed as a consequence of tryptophan being converted by parasites to tryptophol, which may have altered the hypothalamic thermoregulatory center and raised the body's thermostatic level (Taylor and Authie, 2004; Ezeokonkwo *et al.*, 2007).

Conclusions

This study has shown that time of treatment is critical to therapeutic outcomes in trypanosomosis, as early treatment group had better indices of therapeutic efficacy than the late treatment groups. Therefore, there is a need for close infection monitoring, diagnosis and prompt treatment of trypanosomosis in animals.

Authors' Contributions

Conceptualization: DNO, RCE, IOE; Formal analysis: IOE, CFO; Investigation: COO, CNI, CFO, MIO, GEA; Methodology: IOE, CNI; Writing - original draft: MIO, CFO; Writing - review and editing: IOE, CFO; Supervision: DNO, RCE, IOE. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Prior to the start of this study, ethical approval (FVM/UNN/IACUC/2019/0918) was obtained from the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Also, all pertinent local, national and international procedures, guides and rules were fully observed.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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