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Effects of crude leaf extract of *Terminalia catappa* on parasitaemia and haematology of *wistar* rats experimentally infected with *Trypanosoma brucei brucei*

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ABSTRACT

Aim: The study was aimed to evaluate the effects of crude leaf extract of *Terminalia catappa* on parasitaemia and haematology of *Wistar* rats experimentally infected with *Trypanosoma brucei brucei*.

Method and materials: Thirty adult *Wistar* rats were divided into 6 groups (I-VI), each comprising of 5 rats. Rats in groups I, II, III, IV and V were infected while VI were uninfected. At day 5 post-infection (pi), rats in groups I, II and III were administered crude leaf extract (CE) of *Terminalia catappa*; group IV administered diminazene aceturate (DA); and V untreated. Blood was collected and monitored for parasitaemia and haematology.

Results: Parasitaemia was detected by 2 days pi, persisted in I, II and III, and completely cleared in group IV at day 10 pi. There were significant ($P < 0.05$) decreases in packed cells volume, haemoglobin concentration, red and white blood cells, neutrophils and lymphocytes countsin infected groups; followed by significant increases($P < 0.05$) post-treatment. The significant increases in haematological parameters following treatment were highest in group IV followed by groups III at day 10 pi.

Conclusion: Crude extract of *Terminaliacatappa* leaves at dosage of 300 mg/kg did not completely cleared parasitaemia but mitigated changes in haematology induced by *T. brucei brucei* in *Wistar* rats following treatment.

Keywords: Crude extract, haematology, parasitaemia,*Terminalia catappa*, *Trypanosoma brucei brucei*.

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Introduction

Trypanosomosis, being a protozoan disease, has adversely affected human and animal health throughout sub-Saharan Africa and Latin America (Odeniran and Adeyemo, 2018). African trypanosomosis comprised of Human African Trypanosomosis (HAT) and Animal African Trypanosomosis (AAT), is caused by *Trypanosoma brucei* and transmitted by tsetse flies (Adams *et al*., 2010).*Trypanosoma cruzi*, transmitted by the *Triatoma*species (Reduviid bugs) is responsible for the American trypanosomiasis (also known as Chagas' disease) (Echeverria and Morillo, 2019).

In animals, *T. brucei brucei*, *T. vivax*, *T. congolense*, *T. evansi* and *T. equiperdum* have been implicated as causative agents of the disease (Giordani *et al*. 2016). Infections with *Trypanosoma* parasites have been associated with prolonged inflammatory host immune responses leading to excessive immune dysfunction and immunopathology (Vincendeau and Bouteille, 2006).Clinical signs of trypanosomosis include intermittent pyrexia, anaemia, chills, irritability, headache, anorexia, tiredness, myalgias, malaise, lymphadenopathy, and splenomegaly. However, clinical manifestation of the disease is dependent onhost and trypanosome species (Kennedy, 2012; Giordani *et al*. 2016). Due to the economic and social implications of trypanosomosis, priority has been placed on control of the disease (Coles, 2001; Bukachi *et al*. 2017).The absence of effective vaccination strategy against the parasite, has led to

control dependence on combination of active case diagnosis, treatment and vector control (Hargrove *et al*. 2012; Askoy *et al*. 2017). The varied clinical manifestations of AAT have made diagnosis based on clinical signs alone difficult thus necessitating parasites confirmation bylaboratory means (Giordani *et al*. 2016).

The treatment of trypanosomosis involved administration of trypanocidal drugs such as diminazene aceturate (Muhanguzi *et al*. 2015). These drugs have been reported to produce toxic effects in animals and there is need for safer effective alternative drugs (Ogunbanwo *et al*. 2001; Steverding, 2010; Jolayemi *et al*. 2021; Kolawole *et al*. 2021). These attempts involved utilization of herbs by local herdsmen to treat against the disease (Bala *et al*. 2009). Although, several plants have been demonstrated to possess medicinal properties including antitrypanosomal activity, there is need for studies on the active principles in them. *Terminalia catappa* being abundant in African has demonstrated antitrypanosomal activity *in vitro* and attempts toward its use *in vivo* to develop safer trypanocidal drugs will be appealing (Ojeleye *et al*. 2020). Hence, the antitrypanosomal effects of crude extract *Terminalia catappa* leaves on experimental *Trypanosoma brucei brucei* infection in *Wistar* rats were evaluated.

Materials and Methods

Ethical considerations

The use of rats in this study was reviewed and granted by the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC).

Collection of Plant Leaves and Preparation of Extract

Leaves of *Terminalia catappa* were collected from Ahmadu Bello University (ABU) Zaria Botanical Garden, identified at the herbarium unit, Department of Botany, ABU Zaria and voucher number (1556) was deposited.

The fresh leaves were dried for 3 weeks at room temperature, grounded using mortar and pestle, and macerated in ethanol. This was followed by washing and solvent evaporation using rotary evaporator. The LD_{50} of crude extract (CE) was determined to be 1000 mg/kg as described by Lorke (1983).

Trypanosome Parasite and Experimental Rats

Trypanosoma brucei brucei used in this study was provided by the Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna and the parasite was maintained in the laboratory by continuous passage in mice until required.

After7 days acclimatization, 30 adult rats were grouped into 6 (I-VI) each comprising of 5 rats. Rats in I, II, III, IV and V were infectedwith 10^6 trypanosomes/mL of bloodintraperitoneallywhile group VI was uninfected. At 3 days post-infection (pi), groups I, II and III were administered CE at dosages of 100 mg/kg, 200 mg/kg and 300 mg/kg respectively daily for 7 days; group 4 was treated with diminazeneaceturate; while group V was untreated.

Blood Collection and Analysis

Blood was collected daily from rats pre-infection (day 0) up to 10 days pi and the parasitaemia was determined. Also blood was collected into sample bottles containing anticoagulant (EDTA) and processed for haematology. Packed cells volume, haemoglobin concentration, red and total white blood cells, neutrophils and lymphocytes counts were determined using standard laboratory procedures (Schalm *et al*. 1975; Dacie and Lewis, 1991).

Data Analysis

Data were presented using charts, expressed as mean ± SEM and subjected to two-way analysis of variance (ANOVA); followed by *Bonferroni* multiple comparison post-hoc test. Graph Pad Prism version 5.0 for windows (Graph Pad Software, San Diego, California, USA) was used for the analyses. Values of $P \leq 0.05$ were considered significant.

Results and Discussion

Parasitaemia and clinical signs

The parasitaemia was detected 2 days pi and the presented clinical signs were pale mucous membranes, lethargy and rough hair coat. Mortality in groups V and I was 100% at days 6 pi and 9 pi respectively. Following treatment, parasitaemia persisted in groups II and III, and completely cleared in group IV at day 10 pi (Fig. 1).

Haematology

Pre-infection (day 0), the packed cells volume(PCV), haemoglobin concentration (Hb), red blood cells (RBC), total leukocyte (TLC), neutrophils and lymphocytes counts of all groups pre-showed no significant ($P > 0.05$)differences (Figures 2-7). At 3 days pi, there was significant ($P < 0.05$) decrease in these haematological parameters in infected groups (I-V).After treatment, there was significant ($P < 0.05$) increases in haematological parameters with the highest observed in groups IV (DA) followed by groups III (300 mg/kg CE) and II (200 mg/kg CE) at day 10 pi (Fig. 2-7).

In this study, parasitaemia was detected in

infected groups of rats 2 days pi and the clinical signs presented were pale mucous membranes, lethargy, rough fur coat and mortality. Parasitaemia at 4 days pi with similar clinical signs were reported following *T. brucei brucei* infection in *Wistar* rats (Umar *et al*. 2000; 2007; Kobo *et al*. 2014; Ibrahim *et al*. 2016; Erin *et al*. 2019). The discrepancy in the time parasitaemia was detected might be due to variation in the ages and weights of rats, feeds, season and virulence of the *T. brucei brucei* used.The presence of parasitaemia and clinical signs following infection indicated initial replication and high virulence of the parasite. These were accompanied by significant decrease in PCV, Hb, RBC, TLC, neutrophils and lymphocytes counts indicating anaemia and leucopaenia. The anaemia might have resulted from disruptions of erythrocyte membrane components via the actions of reactive oxygen species, lipid peroxidation, lashing by trypanosomes flagella, toxins and metabolites from parasites and increased erythrophagocytosis (Anosa *et al*. 1988; Ngure *et al*. 2009; Mbaya *et al*. 2012). The leucopaenia was suggested to be due to coating of leucocytes by trypanosomal antigen and depression of leucocytes production (Kagira *et al*. 2006).

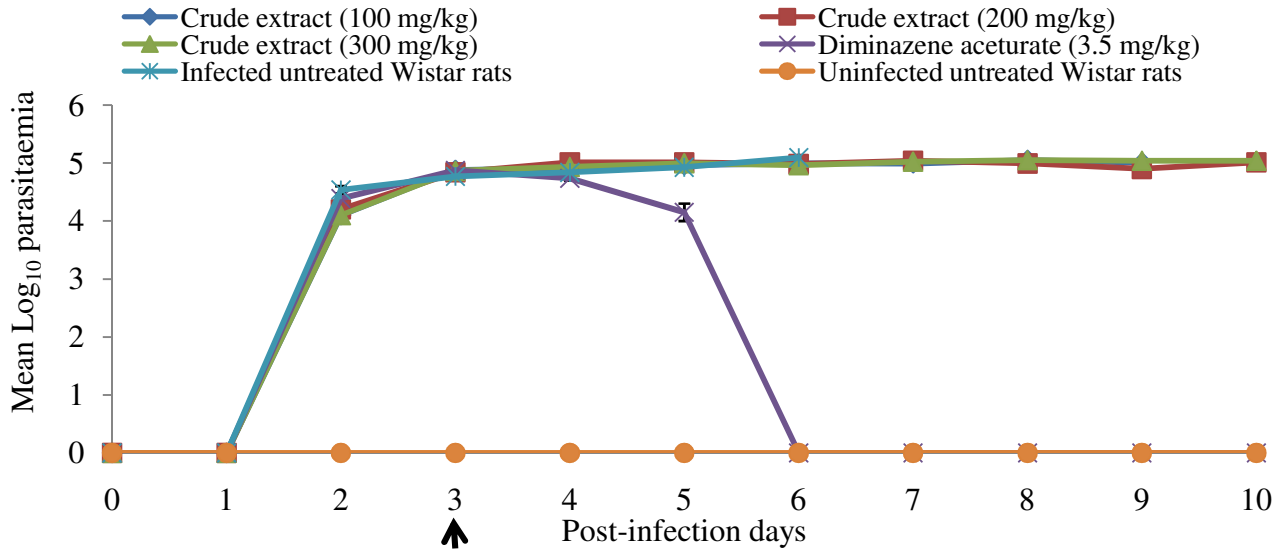

Fig. 1: Mean Log_{10} parasitaemia in *Wistar* rats experimentally infected with *T. b. brucei* and administered crude methanol leaf extract of *Terminalia catappa*.

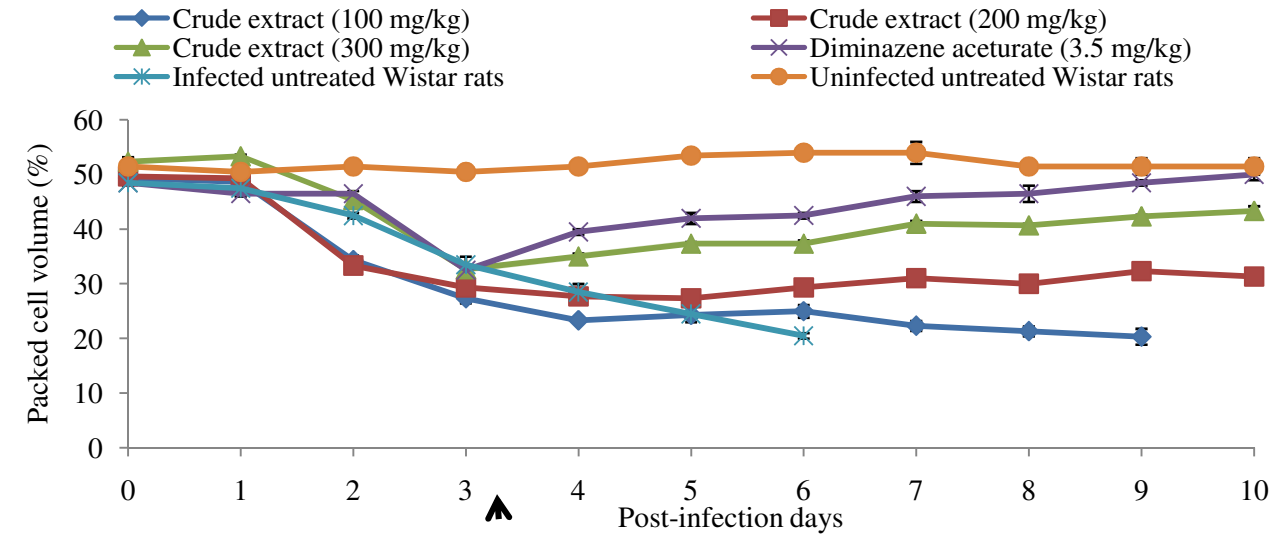

Fig. 2: Packed cell volumeof *Wistar* rats experimentally infected with *T. b. brucei* and administered crude methanol leaf extract of *Terminaliacatappa*.

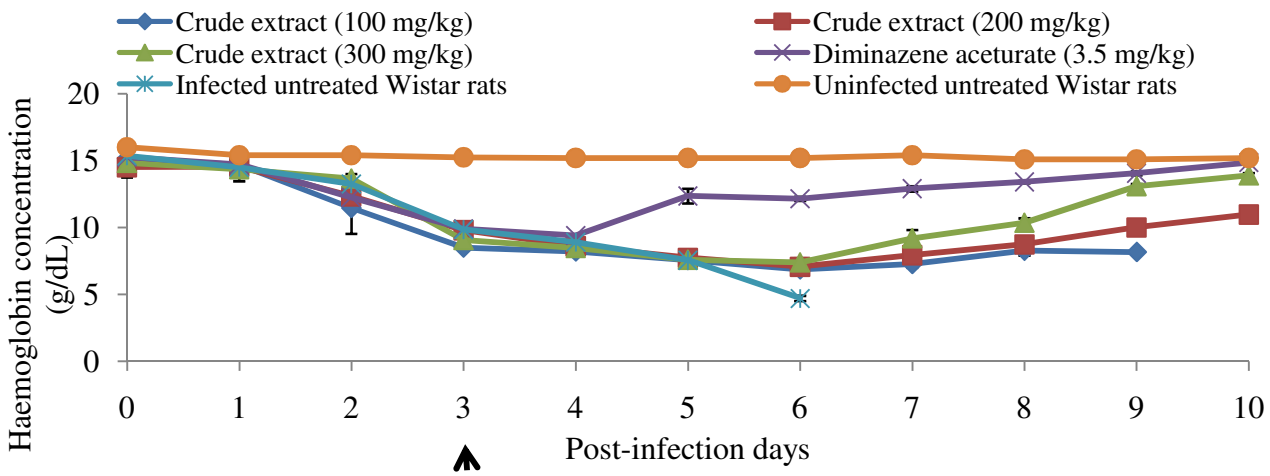

Fig. 3: Haemoglobin concentration of *Wistar* rats experimentally infected with *T. b. brucei* and administered crude methanol leaf extract of *Terminalia catappa*.

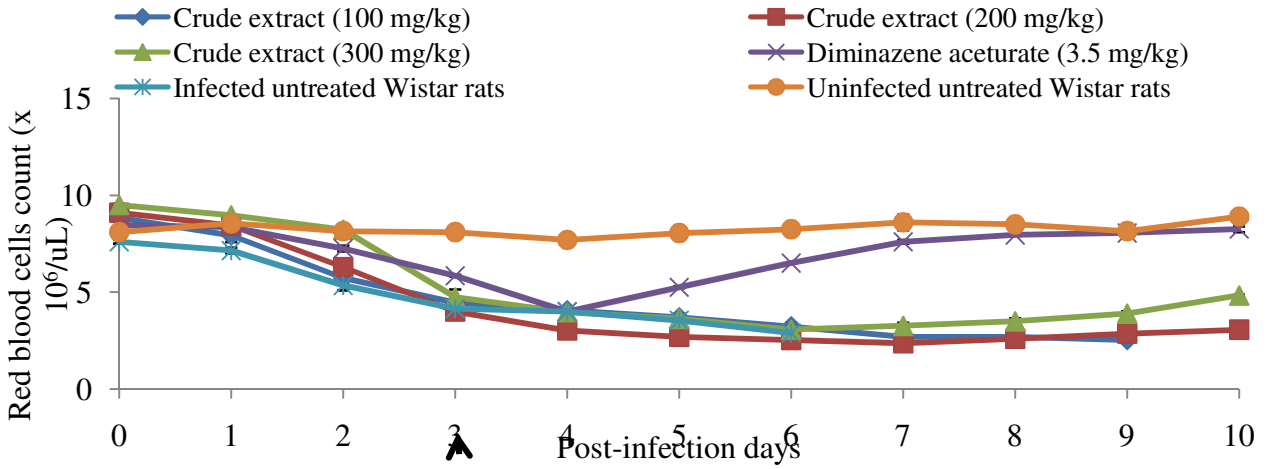

Fig. 4: Red blood cells count of *Wistar* rats experimentally infected with *T. b. brucei* and administered crude methanol leaf extract of *Terminalia catappa*.

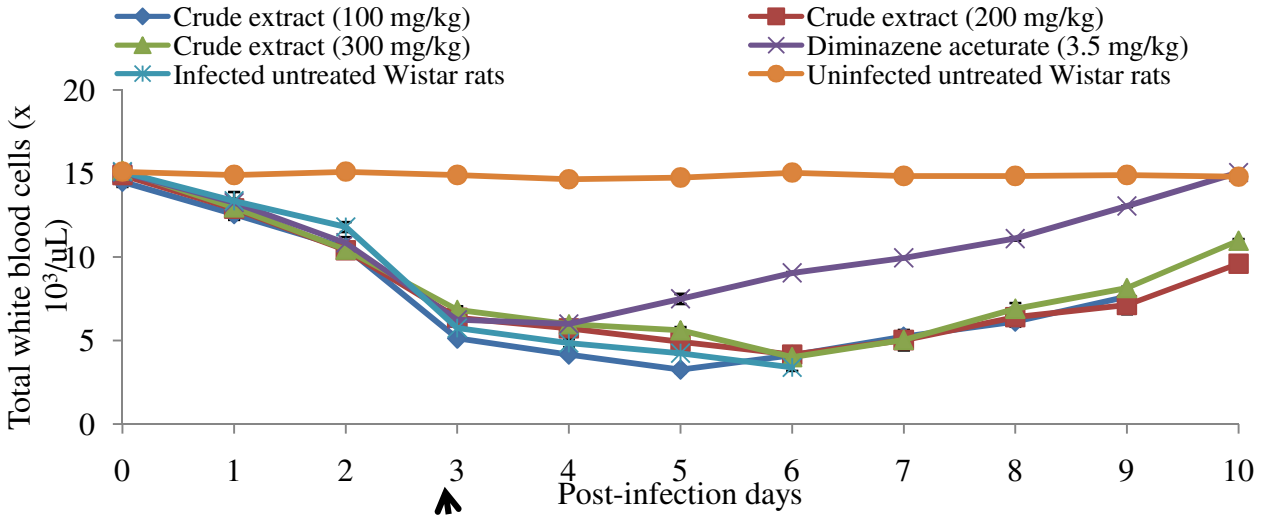

Fig. 5: Total leukocyte counts of *Wistar* rats experimentally infected with *T. b. brucei* and administered crude methanol leaf extract of *Terminalia catappa*.

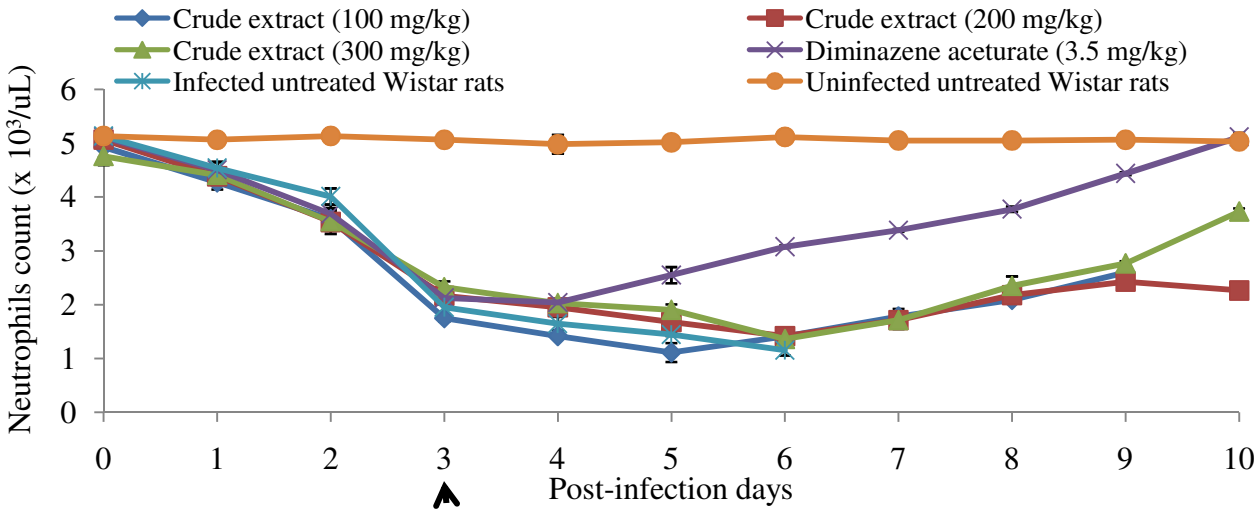

Fig. 6: Neutrophils count of *Wistar* rats experimentally infected with *T. b. brucei* and administered crude methanol leaf extract of *Terminalia catappa*.

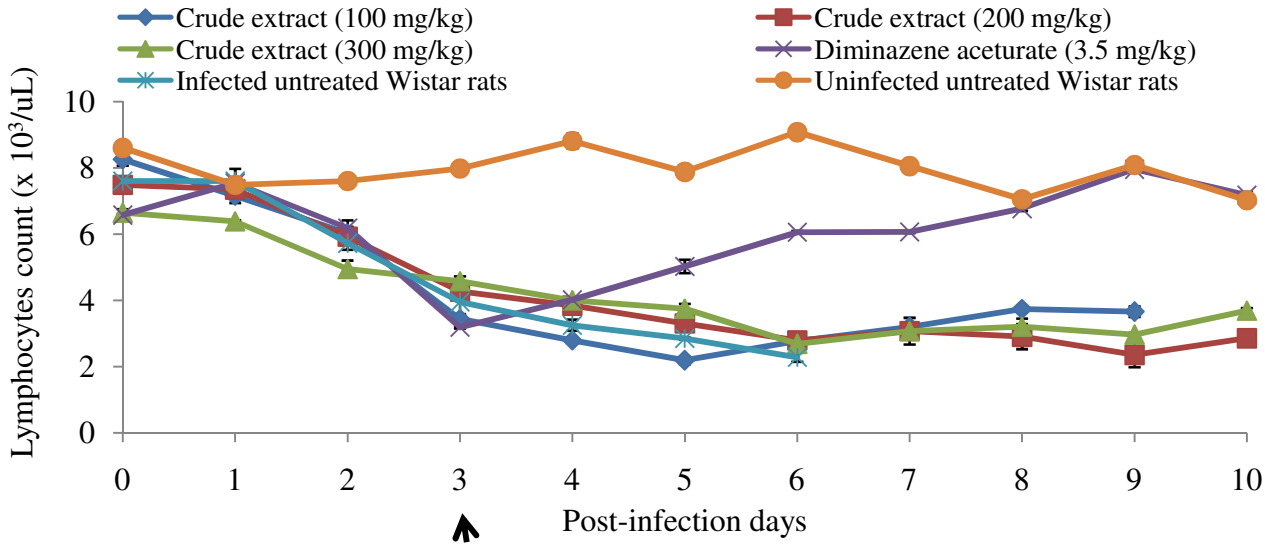

Fig. 7: Lymphocytes count of *Wistar* rats experimentally infected with *T. b. brucei* and administered crude methanol leaf extract of *Terminalia catappa*.

Following treatment, parasitaemia was completely cleared with diminazene aceturate (DA) but persisted with the crude extract (CE) of *Terminalia catappa* leaves. Also, significant return of haematological parameters to pre-infection values was observed with DA but slightly with CE (300 mg/kg). The onset of recovery from anaemia and leucopaenia in the face of persistent parasitaemia in this study suggests that crude extract of *Terminalia catappa* leaves has ameliorative effects on the haematological changes due to *T. brucei brucei*. There was 100 % mortality in rats administered 100 mg/kg of CE suggesting that at this lowest dose, damages already caused by the infection could not be restored. This might possibly be due torapid metabolism of the extracts leading to inactivation of the active components *in vivo*. Also, it could be that at this lowest dose, the therapeutic level against the parasite was not attained, hence, the persistence of infection and consequential damages leading to mortality (Adamu*et al*. 2008; Antia *et al*. 2008; Dell'Agli *et al*. 2009; Inabo *et al*. 2011; Ojeleye *et al*. 2020).

The onset of repair of damages observed by CE at 300 mg/kg suggests that complete clearance of parasitaemia and full restoration could be achieved if this highest dose of extract used is increased or administered beyond 10 days. This was evidenced

by the return of haematological parameters towards their pre-infection values. The return of haematological parameters towards pre-infection values caused by *Terminalia catappa* leaves could have resulted from antioxidant property by scavenging of generated reactive oxygen species to protect erythrocytes from haemolysis (Umar *et al*. 2007; Ajakaiye *et al*. 2013; Ojeleye *et al*. 2021). Also, the amelioration observed might be due toneutralization of harmful metabolites, scavenging of free radicals induced by the trypanosome or immunomodulation (Mpiana *et al.* 2007; Ekanem *et al.* 2008; Ogoti *et al.* 2009; Mergia *et al*. 2014).

Conclusion

The crude extract of *Terminalia catappa* leaves at 300 mg/kg has been demonstrated to ameliorate the haematological changes induced by *T. brucei brucei* in the face of persistent parasitaemia. Hence, further studies involving much higher doses over more prolonged period of time should be carried out to establish the *in vivo* antitrypanosomal effects of *Terminalia catappa* leaves.

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