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Evaluation of anti-trypanosomal activity of hydromethanol root extract of *Carpolobia lutea* in mice experimentally infected with *Trypanosoma brucei*

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Abstract

This study investigated the phytochemistry, toxicity, *in vitro* and *in vivo* anti-trypanosomal activities of *Carpolobia lutea* hydromethanol root extract (CLHmRE) in mice experimentally infected with *Trypanosoma brucei.* The acute toxicity and phytochemical studies on CLHmRE were carried out following standard methods. The *in vitro* anti-trypanosomal activity of CLHmRE was evaluated using eleven concentrations of the extract. Twenty-five mice were randomly assigned to five groups (A – E) of five mice each for the *in vivo* evaluation of the anti-trypanosomal activity. Groups A – D were infected intra-peritoneally with one million trypanosomes, while Group E served as the uninfected control group. Following parasitaemia detection, Groups A and B mice were given 100 mg/kg and 300 mg/kg of CLHmRE orally, respectively for 7 days, while Group C was given 7 mg/kg of diminazene aceturate injection once. Group D was the infected untreated control. The level of parasitaemia was assessed daily post-infection while the body weight and haematological parameters were evaluated before infection and at weekly intervals all through the experimental period, following standard procedures. The phytochemical studies revealed the presence in the CLHmRE of phenols, alkaloids, glycosides, reducing sugars, steroids, terpenoids and tannins. The CLHmRE was not acutely toxic to mice at a maximum oral dose of 2,000 mg/kg body weight. The IC_{50} of the extract was 0.196 mg/ml. Treatment of the infected mice with CLHmRE did not completely eliminate parasitaemia, but only significantly reduced ($p < 0.05$) the level of parasitaemia transiently between days 9 – 12 post infection. The extract also did not produce any significant ($p > 0.05$) improvement in the haematological parameters of the infected mice. It was concluded that CLHmRE is acutely safe but did not exhibit significant anti-trypanosomal activity at the doses used for the study. It was recommended that root extracts of *Carpolobia lutea* be further investigated at other doses for anti-trypanosomal activity or for other possible ethnomedical properties.

Keywords: Anti-trypanosomal activity; *Carpolobia lutea* extract; Mice; Phytochemistry; Toxicity; *Trypanosoma brucei*.

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Introduction

African trypanosomosis is a tsetse-transmitted protozoan disease caused by organisms of the genus *Trypanosoma* that affects both animals (African animal trypanosomosis) and humans (Human African trypanosomosis) (Mogk *et al*., 2017). Human African trypanosomosis is a neglected tropical disease caused primarily by *Trypanosoma brucei rhodesiense* and *T. brucei gambiense*. The species, *Trypanosoma congolense, T. brucei* and *T. vivax,* cause African animal trypanosomosis, which is one of the most serious diseases of livestock in sub-Saharan Africa (Morrison *et al.,* 2016). The disease is regarded as one of the major impediments to health and productivity of domestic animals, especially cattle in tropical Africa (Ezeani *et al.,* 2008). In Nigeria, the disease continues to constrain and limit productivity and profitability of the livestock industry notwithstanding the age-long attempts to control it.

The control of animal trypanosomosis involves three major strategies: vector control, rearing of trypanotolerant breeds and the use of trypanocides. Vector control is still the best strategy for the sustainable management of trypanosomosis (Ouedraogo *et al*., 2018). Unfortunately, however, vector control can be costly when used on a large scale. The rearing of trypanotolerant breeds is equally practiced as an option, but recent studies has brought to the fore the fact that trypanotolerance may have been over-rated and that it is at the expense of productivity and vital organ damage in infected trypanotolerant livestock (Anyogu *et al.,* 2020, 2022, 2023). The use of trypanocides is currently the most reliable option and have been exploited for many years, as no vaccine has yet been developed for the disease. However, the effectiveness of trypanocide therapy as a control option is beleaguered by misuse of available drugs, absence of new drugs, reluctance of pharmaceutical companies to invest in the development of new drugs, drug resistance and undesirable toxic side effects of existing drugs (Giordiani *et al.,* 2016). Thus, it is a necessity to search for other efficacious trypanocides with a high safety margin.

Carpolobia lutea, also known as cattle stick, is a tropical medicinal evergreen plant found in the rainforest and guinea savannah regions of West Africa (Nwidu *et al.,* 2012). The Igbos, Yorubas, Ibibios, Efik and Eket people of Nigeria know the plant as Agba or Angalagala, Egbo oshunshun or Egbo orere, Ndiyan, Ikpafum, and Abekpok ibuhu, respectively (Ajiwhen and Bisong, 2013). Traditionally, parts of the plants are used by herbal healers to treat gonorrhea, infertility, gingivitis, ulcer and blood-borne parasitic diseases particularly malaria and trypanosomosis (Nwidu *et al.*, 2012). The roots of *Carpolobia lutea* are widely believed in some rural Southeastern Nigerian villages to have anti-parasitic effects and are used alone or in combination with other plants for the treatment of blood-borne parasitic infections including malaria and trypanosomosis. However, there are no reports in available literature on its effectiveness in the treatment of trypanosomosis and its safety. Extracts of *Carpolobia lutea* have been shown to possess anti-plasmodial activity against chloroquine-resistant *Plasmodium falciparum*, and neuropharmacological, contraceptive, gastroprotective, antimicrobial, antidiabetic, hypoglycaemic activities (Nwidu *et al*., 2012; Ajiwhen and Bisong, 2013) and aphrodisiac effect (Yakubu and Jimoh, 2014). Based on the numerous oral traditional reports and widespread folkloric use and inclusion of roots of *Carpolobia lutea* by traditional healers in rural Southeastern Nigerian villages as anti-parasitic herb, the present study evaluated the anti-trypanosomal activity and toxicity of hydromethanol root extract of *Carpolobia lutea* in mice experimentally infected with *Trypanosoma brucei.*

Materials and methods

Experimental animals, Trypanosome infection and Trypanocides: Thirty-five female albino mice of eight weeks of age, were used in the experiment. They were sourced from a mice breeder in the University of Nigeria Nsukka. The mice were housed in clean metal cages in the

Laboratory Animal House of the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka. Unlimited access to clean drinking water and proprietary commercially sourced mice feed were provided to the mice all through the study. The mice were acclimatized for two weeks before commencement of the study, dewormed with albendazole (Zolat®) and screened for haemoparasites.

The *Trypanosoma brucei* used for the study was initially obtained from a pig with natural trypanosomosis that was presented for slaughter at the Ikpa municipal abattoir, Nsukka. The parasite was earlier characterized (Cox *et al.,* 2005) and maintained by serial passage in mice prior to use. The trypanocide used as positive control to compare the anti-trypanosomal activity of hydromethanol extract of *Carpolobia lutea* root was diminazene aceturate (Diminaze®, Pantex, Holland).

Collection of *Carpolobia lutea* roots: Fresh *Carpolobia lutea* roots were harvested from *Carpolobia lutea* trees in Obukpa, Enugu State, Nigeria. A plant taxonomist from the Plant Science and Biotechnology Department of the University of Nigeria, Nsukka identified the roots, and a sample specimen (UNN/VPE/2020/003) was also stored.

Extraction of plant material: *Carpolobia lutea* roots were sliced into tiny chunks, dried, and pulverized. 300 g of pulverized *Carpolobia lutea* root was subjected to cold maceration extraction in an extraction jar using 70% methanol for 72 hours. The *Carpolobia lutea* roots extract was filtered using a Whatmann® No. 1 filter paper, and the filtrate was consolidated in a hot air oven at 37^0C. The extract was kept in the refrigerator until it was needed.

Phytochemical analysis: The *Carpolobia lutea* hydromethanol root extract (CLHmRE) was subjected to qualitative phytochemical analysis using standard methods (Trease and Evans, 2002). In a beaker, 100 ml of distilled water was used to dissolve one gramme (1 g) of CLHmRE. Whatmann No. 1 filter paper was used to filter the mixture and the filtrate was thereafter subjected to ferric chloride test (phenolics and tannins), Wagner Dragendioff's test (alkaloids), hydrolysis test (glycosides), Fehling test (reducing sugar), Salkowski's test (steroids), acetic anhydride test (terpenoids), frothing test (saponins) and Shinoda's test (flavonoids) (Trease and Evans, 2002).

Acute toxicity test: Acute toxicity test was carried out using the up and down protocol (OECD, 2008). Ten female albino mice were used for acute toxicity study. Five mice were given the extract (2000 mg/kg) orally while the remaining five mice served as control and received distilled water. The mice were monitored initially for 24 hours and then for 14 days for any signs of toxicity.

***In vitro* anti-trypanosomal assay:** The *in vitro* anti-trypanosomal activity of CLHmRE was assessed using the method described in Obi *et al.* (2019). Succintly, excluding the first columns, 100 µl of phosphate buffered saline glucose (PBSG) was aliquoted into two rows of free wells of 96-well microtitre plates (Flow Laboratories Inc., McLean, Virginia, USA). 5 mg/ml CLHmRE was prepared and 200 µl of it was pipetted into the first column of the first row. 5 mg/ml diminazene aceturate was prepared and 200 µl of it was also pipetted into the first column of the last row. The diminazene aceturate and extract were sequentially doubly diluted to yield 11 varying concentrations. PBSG (100 µl) was aliquoted into another row of free wells for the control group. 50 µl of trypanosome-infected blood suspended in PBSG, containing 20 – 25 trypanosomes per microscope field, was pipetted into the extract, diminazene aceturate, and PBSG wells, and the extract concentrations in each well were determined. The set up was done in triplicates and was incubated at 37 °C for 60 min. To assess the extract's anti-trypanosomal activity, 5 µl of each sample was taken at five minutes intervals and trypanosome motility was examined under the microscope.

***In vivo* anti-trypanosomal study:** The *in vivo* evaluation of CLHmRE for anti-trypanosomal activity was done using twenty-five female

albino mice, which were randomly assigned to five groups (Groups A, B, C, D and E) of five mice each. Groups A – D were infected with 1×10^6 trypanosomes while group E mice were the uninfected-untreated group. Pre-infection baseline parameters were assayed, and the infected mice groups were examined daily after infection, for parasitaemia via buffy coat technique and wet blood film examination (Murray *et al*., 1977). When all the infected mice became parasitaemic (day 4 post infection), mice in groups A and B were treated orally with 100 and 300 mg/kg of CLHmRE for seven days. Group C mice were given diminazene aceturate injection (7 mg/kg) once intraperitoneally while group D was not treated (infected untreated control).

Parameters assessed in the *in vivo* anti-trypanosomal study: Level of parasitaemia, clinical signs, packed cell volume (PCV), body weight, total leucocyte count (TLC), red blood cell count (RBC), haemoglobin concentration (HbC), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were utilized to assess the CLHmRE treatment for anti-trypanosomal effects. The rapid matching method (Herbert and Lumsden, 1976) was utilized to determine the level of parasitaemia daily. The PCV was determined by microhaematocrit method while the HbC was determined by the cyanomethaemoglobin method (Coles, 1986). The RBC counts and TLC were done following the haemocytometer method (Coles, 1986). Standard formulae described in Coles (1986) were used to calculate the MCV, MCH and MCHC of the experimental animals. The body weight was measured with a sensitive electronic weighing scale (M-Metlar, China). The haematological parameters and the body weight were evaluated at baseline and at weekly intervals afterwards till the end of the experimental period.

Ethical approval: Prior to the commencement of this study, ethical approval for the use of the mice for the *in vivo* study was sought for and obtained from the Faculty of Veterinary Medicine Institutional Animal Care and Use Committee, University of Nigeria, Nsukka (Approval Reference Number: (FVM-UNN-IACUC-2018-023). Furthermore, all pertinent local, national and international procedures, and guidelines for the use of animals for experiments were properly adhered to.

Data analysis: The IC_{50} calculator tool (https://www.aatbio.com/tools/ic50-calculator) was used to ascertain the concentration of extract and diminazene aceturate that inhibited 50% of the trypanosomes. One-way ANOVA was used to analyze data generated from the *in vivo* study. The post-hoc separation of the variant means was performed using the least significant difference method. Significant probabilities were defined as those with $p < 0.05$. The SPSS version 20 software was used for all statistical analyses.

Results

Yield of plant extract and phytochemical screening: The percentage yield of *Carpolobia lutea* hydromethanol root extract (CLHmRE) was 15.5% weight per weight. The phytochemical components recorded for the CLHmRE following phytochemical analysis were phenol, alkaloids, glycosides, reducing sugars, steroids, terpenoids and tannins (Table 1). Saponins and flavonioids were absent.

Acute toxicity: Following the administration of 2000 mg/kg of CLHmRE, there was no evidence of toxicity in the mice, and no deaths were recorded. The LD_{50} was therefore recorded as being above 2000 mg/kg.

***In vitro* anti-trypanosomal assay of CLHmRE:** The CLHmRE at 5 and 2.5 mg/ml concentrations inhibited trypanosome motility within 5 minutes of incubation while 1.25 and 0.625 mg/ml CLHmRE concentration led to cessation of trypanosomal motility at 10 and 40 minutes of incubation respectively (Table 2). Concentrations less than 0.625 mg/ml had no effect on the trypanosome motility. Diminazene aceturate was active in inhibiting trypanosome motility within 5 minutes at concentrations of 5, 2.5, 1.25, 0.625, and 0.3125 mg/ml, while 0.156 and

0.0781 mg/ml of diminazene aceturate led to cessation of motility of trypanosomes at 40 and 60 minutes of incubation, respectively (Table 2).

The IC_{50} of CLHmRE and diminazene aceturate were 0.196 mg/ml and 0.065 mg/ml, respectively (Table 2).

Table 1: The phytochemical components of *Carpolobia lutea* hydromethanol root extract (CLHmRE).

Phytochemical	Colour change observed	Remarks
Phenolics	Formation of blue/green colour	Present
Alkaloids	Reddish brown precipitate was observed	Present
Glycosides	Colour change to brick red	Present
Reducing sugars	A brick red precipitate was observed	Present
Steroids	Colour change from violet to green	Present
Terpenoids	Colour change from pink to violet	Present
Tannins	A blue-black precipitate was observed	Present
Saponins	No colour change was observed	Absent
Flavonoids	No colour change was observed	Absent

Table 2: *In vitro* antitypanosomal effect of *Carpolobia lutea* hydromethanol root extract (CLHmRE)

Treatment doses	CLHmRE	Diminazene aceturate (Positive Control)	Phosphate buffered saline glucose (Negative Control)
5 .0 mg/ml	Ceased motility within 5 minutes	Ceased motility within 5 minutes	No effect
2.5 mg/ml	Ceased motility within 5 minutes	Ceased motility within 5 minutes	No effect
1.25 mg/ml	Ceased motility at 10 minutes	Ceased motility within 5 minutes	No effect
0.625 g/ml	Ceased motility at 40 minutes	Ceased motility within 5 minutes	No effect
0.3125 mg/ml	No effect	Ceased motility within 5 minutes	No effect
0.156 mg/ml	No effect	Ceased motility at 40 minutes	No effect
0.0781 mg/ml	No effect	Ceased motility at 60 minutes	No effect
0.0391 mg/ml	No effect	No effect	No effect
0.0195 mg/ml	No effect	No effect	No effect
0.00977 mg/ml	No effect	No effect	No effect
0.0088 mg/ml	No effect	No effect	No effect
0.0024 mg/ml	No effect	No effect	No effect
IC_{50} mg/ml	**0.196**	**0.065**	

***In vivo* anti-trypanosomal activity of CLHmRE:** Parasitaemia was first detected in the infected mice groups on day 3 post-infection (PI). By day 4 PI, all the infected mice were parasitaemic. Treatment of the infected mice commenced on day 4 PI. There was an appreciable significant decline ($p < 0.05$) in the mean level of parasitaemia of the diminazene acuturate-treated group C mice compared to the CLHmRE-treated groups A and B and the untreated group D mice from day 5 PI with the parasitaemia clearing on day 8 PI – day 4 post diminazene aceturate treatment (Figure 1). However, two mice in the diminazene-treated group C mice had relapse infection on day 17 PI (Figure 1). The group A mice treated with 100 mg/kg CLHmRE had a significantly lower ($p < 0.05$) level of parasitaemia than the untreated group D mice between days 9 – 12 PI, i. e., from the 6th day of CLHmRE treatment (Figure 1).

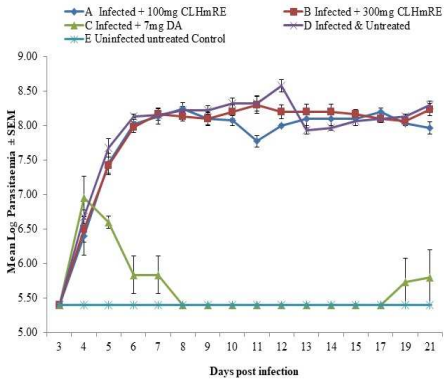

Figure 1: The mean log parasitaemia level of mice infected with *T. brucei* and treated with *Carpolobia lutea* hydromethanol root extract (CLHmRE) or diminazene aceturate (DA).

The clinical findings in the infected groups of mice were rough hair coat, dullness, dyspnoea, respiratory rales, emaciation, oedema, recumbency and death. Following treatment, the clinical signs were no more observed in the diminazene treated group C, while they appeared to be slightly reduced in the group A mice treated with 100 mg/kg CLHmRE.

On day 7 PI, group B mice had a lower mean body weight ($p < 0.05$) than groups A and E mice (Figure 2). On day 14 PI, group A mice had significantly higher ($p < 0.05$) mean body weight compared to the rest of the groups (Figure 2). The body weights of groups B, C, D, and E mice did not significantly vary ($p > 0.05$) on day 14 PI, but by day 21 PI, groups A, C, and E mice had significantly higher ($p < 0.05$) mean body weight than groups B and D mice (Figure 2). The mean body weights of groups A, C and E mice did not significantly vary ($p > 0.05$) on day 21 PI (Figure 2).

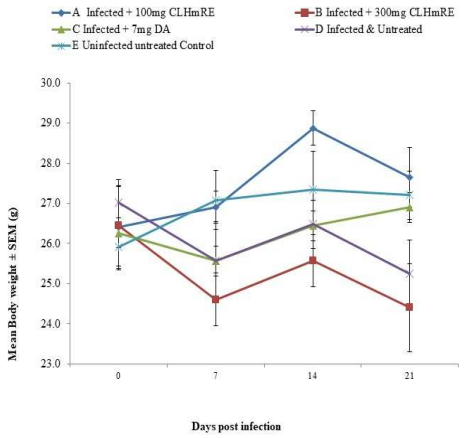

Figure 2: The mean body weights of mice infected with *T. brucei* and treated with *Carpolobia lutea* hydromethanol root extract (CLHmRE) or diminazene aceturate (DA).

A significant decline ($p < 0.05$) was recorded in the mean PCV and RBC counts of the infected mice groups compared with the uninfected group E mice from day 7 PI (Table 3). Throughout the course of the post-infection treatment, the mean PCV and RBC counts of the CLHmRE treated groups did not vary ($p > 0.05$), but the PCV was significantly lower ($p < 0.05$) compared to the diminazene aceturate treated group C mice on days 14 and 21 PI (Table 3). The CLHmRE-treated mice groups had comparable mean PCV ($p > 0.05$) and significantly lower ($p < 0.05$) mean RBC counts than the infected-untreated group D mice on day 21 PI (Table 3). The TLC of group C mice were significantly higher ($p < 0.05$) than the mean TLC of the other groups on day 7 PI, while that of group A mice was significantly lower ($p < 0.05$) on day 7 PI

(Table 3). The mean TLC of groups C and E mice were significantly higher ($p < 0.05$) than those of other groups on day 14 PI, but on day 21 PI the mean TLC of the all the infected groups were significantly ($p < 0.05$) lower than that of the uninfected untreated Group E mice (Table 3).

Table 3: Means of packed cell volume, red blood cell and total leucocyte counts of mice experimentally infected with *T. brucei* and treated with *Carpolobia lutea* hydromethanol root extract (CLHmRE) or diminazene aceturate.

Groups*	Days post-infection			
	0	7	14	21
Packed cell volume (%)				
Group A	41.3 ± 1.38	29.7 ± 2.60 [a]	27.3 ± 1.33 [a]	25.0 ± 0.58 [a]
Group B	40.3 ± 2.02	31.0 ± 3.21 [a]	26.3 ± 1.70 [a]	24.0 ± 2.31 [a]
Group C	40.0 ± 2.04	27.0 ± 1.29 [a]	33.0 ± 1.78 [b]	33.3 ± 3.33 [b]
Group D	40.8 ± 1.70	32.7 ± 1.76 [a]	27.8 ± 2.01 [ab]	20.0 ± 1.54 [a]
Group E	42.0 ± 1.47	39.8 ± 1.97 [b]	43.7 ± 0.88 [c]	39.0 ± 1.00 [b]
Red blood cell count ($\times 10^6$/µl)				
Group A	8.17 ± 0.37	6.38 ± 0.49 [a]	5.18 ± 0.29 [a]	3.67 ± 0.37 [a]
Group B	8.12 ± 0.04	6.73 ± 0.99 [a]	5.00 ± 0.42 [a]	3.52 ± 0.26 [a]
Group C	8.03 ± 0.53	6.78 ± 0.38 [a]	6.92 ± 0.26 [a]	7.51 ± 0.29 [b]
Group D	8.00 ± 0.20	6.93 ± 0.16 [a]	5.52 ± 0.57 [a]	4.83 ± 0.20 [c]
Group E	8.13 ± 0.26	9.73 ± 0.55 [b]	9.87 ± 1.56 [b]	8.72 ± 0.17 [b]
Total leucocyte count (10^3/µl)				
Group A	8.22 ± 0.12	4.08 ± 0.22 [b]	6.33 ± 0.18 [b]	5.30 ± 0.38 [b]
Group B	8.15 ± 0.44	6.82 ± 0.43 [a]	4.20 ± 0.46 [a]	3.32 ± 0.22 [a]
Group C	8.55 ± 0.30	9.52 ± 0.65 [c]	9.47 ± 0.33 [c]	5.98 ± 0.29 [b]
Group D	7.97 ± 0.29	7.38 ± 0.28 [a]	6.23 ± 0.62 [b]	4.13 ± 0.40 [a]
Group E	8.18 ± 0.42	8.02 ± 0.38 [a]	8.33 ± 0.33 [c]	8.20 ± 0.23 [c]

[a, b, c] Different superscripts in a column for each of the haematological parameters represent significant differences between groups at the probability of $p < 0.05$.

* Groups A and B were treated for five days with 100 and 300 mg/kg CLHmRE respectively, group C was treated with 7 mg/kg diminazene aceturate, group D was not treated while group E was not infected and not treated.

Table 4: Means of haemoglobin concentration, corpuscular volume, corpuscular haemoglobin and corpuscular haemoglobin concentration of mice experimentally infected with *T. brucei* and treated with *Carpolobia lutea* hydromethanol root extract (CLHmRE) or diminazene aceturate.

Groups*	Days post-infection			
	0	7	14	21
Haemoglobin concentration (g/dl)				
Group A	12.99 ± 0.43	8.99 ± 0.09 [a]	8.2 ± 0.41 [b]	6.25 ± 0.57 [a]
Group B	12.91 ± 0.26	7.87 ± 0.19 [a]	5.23 ± 0.56 [a]	5.02 ± 0.35 [a]
Group C	12.95 ± 0.36	8.39 ± 0.77 [a]	9.63 ± 0.31 [b]	10.62 ± 0.75 [b]
Group D	13.04 ± 0.31	8.74 ± 0.16 [a]	6.41 ± 0.39 [a]	4.99 ± 0.17 [a]
Group E	13.1 ± 0.09	12.59 ± 0.24 [b]	11.21 ± 0.59 [c]	13.99 ± 0.04 [c]
Mean corpuscular volume (fL)				
Group A	50.20 ± 4.55	46.67 ± 3.69	53.40 ± 5.91	69.43 ± 6.31 [a]
Group B	50.50 ± 3.13	48.76 ± 10.14	49.80 ± 2.92	68.16 ± 3.40 [a]
Group C	52.21 ± 5.95	39.83 ± 4.89	49.34 ± 3.81	44.91 ± 6.45 [b]
Group D	49.19 ± 1.47	47.20 ± 3.07	54.69 ± 4.56	41.32 ± 0.69 [b]
Group E	51.73 ± 3.03	46.04 ± 3.51	46.56 ± 7.28	45.03 ± 3.23 [b]
Mean corpuscular haemoglobin (*pg*)				
Group A	15.93 ± 0.37	13.81 ± 0.8	12.51 ± 1.43	12.52 ± 1.05
Group B	15.91 ± 0.37	12.23 ± 1.81	10.43 ± 0.49	10.43 ± 2.16
Group C	16.23 ± 0.94	12.35 ± 0.78	13.93 ± 0.21	13.98 ± 0.97
Group D	16.23 ± 0.94	12.35 ± 0.79	13.94 ± 0.24	13.9 ± 1.45
Group E	16.13 ± 0.53	13.05 ± 0.96	13.98 ± 2.09	13.95 ± 0.45
Mean corpuscular haemoglobin concentration (g/dl)				
Group A	32.10 ± 2.22	30.1 ± 2.49	22.8 ± 0.92 [ab]	19.61 ± 0.61 [a]
Group B	31.7 ± 1.89	26.1 ± 3.33	21.1 ± 1.6 [a]	22.3 ± 3.09 [a]
Group C	31.6 ± 2.65	32.1 ± 4.9	28.6 ± 2.59 [c]	32.2 ± 2.45 [bc]
Group D	33.2 ± 1.46	27.7 ± 1.70	27.6 ± 0.64 [bc]	29.6 ± 0.94 [b]
Group E	31.4 ± 1.84	32.8 ± 1.47	25.7 ± 1.58 [abc]	35.9 ± 0.98 [c]

[a, b, c] Different superscripts in a column for each of the haematological parameters represent significant differences between groups at the probability of $p < 0.05$.

* Groups A and B were treated for five days with 100 and 300 mg/kg CLHmRE respectively, group C was treated with 7 mg/kg diminazene aceturate, group D was not treated while group E was not infected and not treated.

The mean HbC of all the infected groups (A – D) were significantly ($p < 0.05$) lower than that of the Group E mice from day 7 PI to day 21 PI (Table 4). Further, on day 14 PI the mean HbC of the Group B and D mice were significantly lower ($p < 0.05$) than those of the other two infected groups (groups A and C), while on day 21 PI the mean HbC of the groups A, B and D mice were further significantly ($p < 0.05$) lower than that of Group C (Table 4). The MCV of the mice groups did not vary significantly on days 0, 7 and 14 PI. However, on day 21 PI, the MCV of the CLHmRE treated groups were significantly higher than those of groups C, D and E mice (Table 4). The MCH of all the mice groups (infected and uninfected) did not significantly vary ($p > 0.05$) all through the experimental period (Table 4). The MCHC however did not significantly vary ($p > 0.05$) among all the groups on day 0 and 7 PI, but on day 14 PI the MCHC of the group B mice was significantly lower ($p < 0.05$) than that of group C mice, while on day 21 PI, that of groups A and B mice was significantly lower than those of all other mice groups (Table 4).

Discussion

The results of the phytochemical assay of *Carpolobia lutea* hydromethanol root extract (CLHmRE) in this study which revealed that it contained alkaloids, glycosides, reducing sugar, steroids, terpenoids, tanins and phenolics is comparable to some of the earlier reports on the phytochemistry of this plant root by other researchers, but varies from theirs by the absence of flavonoids and saponins in the CLHmRE used for this study. A report by Jackson *et al*. (2011) showed that phytochemical analyses of the ethanol root extract of *Carpolobia lutea* revealed that the extract contained saponins, flavonoids, terpenes, cardiac glycosides and tannins, while similar reports by Yakubu and Jimoh (2014) revealed that the *Carpolobia lutea* aqueous root extract contained flavonoids, saponins, cardiac glycosides, tannins, alkaloids, and anthraquinones. Also, tannins, anthraquinones, simple sugar, alkaloids, phlobatannins, cardiac glycosides, saponins, and flavonoids were the phytochemical constituents of *Carpolobia lutea* methanol root extract as reported by Akinola *et al.* (2020). The variations in the phytochemical profiles of *C. lutea* root as reported by the different authors could possibly be attributed to differences in plant age/developmental stage, location, season during which the roots were harvested and the solvent used for extraction.

The fact that administration of 2,000 mg/kg of the extract during the acute toxicity study did not lead to any signs of toxicity implies that the CLHmRE was well tolerated and is safe for acute administration. Similar findings of no toxicity during acute administration of extracts of *C. lutea* were reported by Jackson *et al.* (2011) and Akinola *et al.* (2020) after they dosed experimental mice with 5,000 mg/kg methanol and ethanol root extracts of *Carpolobia lutea* respectively. These reports, in addition to the finding in the present study, implies that the CLHmRE has a wide safety margin, and that could be a reason for its widespread use in some rural South-eastern Nigerian villages for the treatment of various ailments.

The results of the *in vitro* assay in this study which showed that the CLHmRE had marginal trypanocidal activity with IC_{50} of 0.196 mg/ml, is believed to be as a result of the presence or absence of specific phytochemicals. It had earlier been reported that some of the phytochemicals when present in an extract may serve as therapeutic agents by exerting beneficial physiological responses such as disrupting the parasite's redox equilibrium and serving as cellular defence against oxidative stress, whereas others may interfere with the activities of the beneficial ones (Alhaji *et al*., 2014).

In the *in vivo* study, all of the mice that were infected during the experiment developed parasitaemia by the fourth day after infection. This is in agreement with the reports of Ihedioha *et al*. (2007) and Ezeokonkwo *et al*. (2013) who reported similar findings. The clinical signs such as emaciation, starry hair coat, dullness, paleness of the mucous membrane, anorexia, lethargy, and cuddling together was typical of

trypanosomosis and was similar to what was previously reported by Ezeokonkwo *et al*. (2013).

In the present study, CLHmRE therapy did not entirely eradicate parasitaemia in the bloodstream of the mice, though there was a transient significant reduction in the level of parasitaemia on days 9 – 12 post infection (from day 6 of treatment) in the 100 mg/kg CLHmRE-treated group A mice. The extract also did not produce significant positive effects on other parameters assessed such as the haemoglobin concentration, red blood cell count, packed cell volume, and erythrocytic indices of the mice. However, all the mice treated with diminazene became aparasitaemic after 8 days post infection. It is thought that the inability of CLHmRE to exhibit significant *in vivo* anti-trypanosomal effect in this study could probably be due to a lack of phytochemicals that possess trypanocidal activity in the extract or due to the doses used for the study – these doses may not be the therapeutically effective doses. The CLHmRE used in this study lacked flavonoids which have been reported to possess anti-trypanosomal activity (Maikai *et al.,* 2011). It is also possible that enzymatic degradation or inhibition of active CLHmRE molecules, increased rate of CLHmRE excretion, low levels of CLHmRE at the target tissues and antagonistic action of several constituents of CLHmRE acting at one or more targets may have possibly contributed to the poor anti-trypanosomal activity of CLHmRE (Antia *et al.,* 2009). The significant but transient reduction in parasitaemia levels observed from the 6th day of treatment implies that further extension of the treatment period to 14 days as practiced by some traditional healers in rural villages may have produced significant parasite elimination and/or improvement of the parameters assessed.

The inability of the extract as used in this study to clear trypanosomes from blood of infected mice suggests that CLHmRE may be ineffective for the treatment of African trypanosomosis. This does not concur with the traditional folkloric use of the *C. lutea* roots in the therapy of blood parasite infections in some rural south-eastern Nigerian villages. It should be noted however, that traditionally, *C. lutea* roots are frequently combined with other plants' leaves, barks, and roots in folk medicine, some of which have been shown to have trypanocidal effects (Ogbadoyi *et al.,* 2011), rather than being used on its own, to achieve therapeutic efficacy.

Conclusion

Carpolobia lutea hydromethanol root extract (CLHmRE), as used in this study, is safe but did not exhibit significant *in vitro* and *in vivo* anti-trypanosomal activity. Further investigations on the anti-trypanosomal activity of other doses of the extract and also other possible ethnomedical properties and activities of the extract are recommended.

Conflict of Interests

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

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