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Evaluation Of The *In Vivo* Trypanocidal And Haematological Effects Of Methanolic Fruit Extract Of *Tetrapleura Tetraptera* In Rats Infected With *Trypanosoma Brucei Brucei*

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Abstract

Trypanosomiasis, caused by Trypanosoma species, is a re-emerging neglected tropical disease in sub-Saharan Africa, characterized by significant morbidity in humans and livestock. Existing chemotherapies are limited by toxicity, high cost, difficult administration, and emerging resistance, thus necessitating the search for safer and more affordable alternatives. Natural product research offers promising prospects, and *Tetrapleura tetraptera* (fruit), traditionally used in West African medicine, contains flavonoids, tannins, and other bioactive phytochemicals with potential antiparasitic properties. This study evaluated the in vivo trypanocidal efficacy, safety, and hematological effects of the methanolic fruit extract of *T. tetraptera* in rats experimentally infected with *Trypanosoma brucei*. The fruit was authenticated, dried, powdered, and extracted with methanol to yield 10.9% w/w of the dried fruit. White albino rats (n = 6 per group) were infected intraperitoneally with 1×10^5 trypanosomes in 0.2 mL and treated orally with 100, 200, or 400 mg/kg body weight of extract once daily for seven days post-infection. Diminazene aceturate (28 mg/kg, single intraperitoneal dose) served as positive control, while a negative control group received vehicle only. Parasitaemia, packed cell volume (PCV), body weight, and survival time were monitored over 30 days, and acute oral toxicity was evaluated according to OECD 423 guidelines. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, phenolic compounds, triterpenes, and phytosterols. In the infected untreated control, parasitaemia peaked at approximately 1.2×10^8 trypanosomes/mL on day 7 (± SEM), whereas treatment with *T. tetraptera* extract at 400 mg/kg produced about 90% suppression of parasitaemia ($P < 0.001$) by day 7, while 200 mg/kg and 100 mg/kg resulted in approximately 65% and 40% suppression, respectively. Mean Survival Time (MST) increased progressively across treatment groups: control = 9 ± 1.2 days, 100 mg/kg = 12.5 ± 1.5 days, 200 mg/kg = 18.3 ± 2.0 days, 400 mg/kg =

26.7 ± 1.8 days, with the positive control surviving beyond 30 days. PCV and body weights were better preserved in extract-treated rats compared to controls. No mortality or overt signs of toxicity were observed up to 2,000 mg/kg in acute toxicity tests, with an estimated LD_{50} greater than 2,000 mg/kg. The findings indicate that the methanolic fruit extract of *Tetrapleura tetraptera* exhibits promising dose-dependent trypanocidal activity in vivo with a good safety margin, justifying further studies to isolate active compounds, elucidate mechanisms of action, and assess pharmacokinetics toward developing affordable phytomedicines for trypanosomiasis.

Introduction

Trypanosomiasis remains a critical neglected tropical disease with severe impacts on human health, livestock production, and rural livelihoods in sub-Saharan Africa (Mbaya *et al.*, 2019; Hassan *et al.*, 2024; Bello *et al.*, 2024). Chemotherapeutic agents such as diminazene aceturate, suramin, and melarsoprol remain the mainstay of treatment but are plagued by toxicity, high costs, frequency of administration, and emerging drug resistance (Nguta *et al.*, 2021; Musa *et al.*, 2020). There is, therefore, an urgent need for safer, more affordable, and accessible alternatives (Hassan *et al.*, 2024).

Medicinal plants provide a rich source of candidate molecules for drug discovery, offering structural diversity and biological selectivity that synthetic compounds often lack (Ahmed *et al.*, 2021; Asiimwe *et al.*, 2023). Among these, *Tetrapleura tetraptera*, widely used in traditional medicine in West Africa, has demonstrated a wide range of bioactivities including antioxidant, anti-inflammatory, antimicrobial, and preliminary antiparasitic effects (Ogunlesi *et al.*, 2020; Adeniran *et al.*, 2021; Afolabi *et al.*, 2021). However, detailed *in vivo* evaluation of its fruit methanolic extract against trypanosome infection is scarce (Ikpe *et al.*, 2023; Obinna *et al.*, 2022).

Previous studies have established important benchmarks in the evaluation of plant-derived antitrypanosomal agents. Kifleyohannes *et al.* (2014) showed that crude methanol or water extracts of *Moringa stenopetala* and *Artemisia absinthium* at 400 mg/kg (p.o.) for seven days significantly reduced parasitaemia in *Trypanosoma congolense*-infected mice, with untreated control parasitaemia peaking at approximately 11.6 × 10^7/mL on Day 7. Similarly, Tadesse *et al.* (2015) reported efficacious suppression of parasitaemia and improved packed cell volume (PCV) and body weight at doses of 200–250 mg/kg of methanolic leaf extracts. In a related study, Abdeta *et al.* (2020) demonstrated that hydromethanolic extracts of *Echinops kebericho* significantly reduced parasite load and preserved hematological parameters in infected mice.

Building upon these findings, the present study aims to evaluate the *in vivo* trypanocidal activity and safety profile of *T. tetraptera* fruit methanolic extract in a murine model, using comparable dosing and monitoring endpoints. The study seeks to validate the ethnopharmacological claims surrounding this plant and to provide a scientific basis for its potential inclusion in the development of novel antitrypanosomal therapies (Bello *et al.*, 2024; Hassan *et al.*, 2024).

Materials and Methods

Ethics statement: All experiments were conducted with approval from the Institutional Animal Care and Use Committee of the Faculrty of Pure and Applied Sciences, Kwara State University,Malete protocol no. KWAS/FPAS/2021/0023 in compliance with national and international guidelines for animal research.

Plant material: Ripe fruits of *Tetrapleura tetraptera* were collected from the botanical garden of the Department of Plant and Environmental Biology, Kwara State University, Malete ad authenticated by taxonomist at the University of Ilorin herbarium. Fruits were washed, shade-dried, milled, and stored.

Extraction: Powdered fruit (500 g) was extracted with methanol (1:10 w/v) by maceration for 72 h with occasional stirring. Filtration and repeated extraction twice; filtrates pooled and solvent removed under reduced pressure at ≤ 40 °C using rotary evaporator; dried crude extract stored at 4 °C. Yield calculated as % w/w.

Phytochemical screening: Qualitative tests for flavonoids, alkaloids, tannins, saponins, terpenoids, glycosides performed by standard protocol. Quantitative total phenolics and flavonoids determined using Folin-Ciocalteu and aluminum chloride methods respectively.

Animals and infection protocol: White albino rats (100–120 g), acclimatized 7 days, housed under standard conditions, food and water ad libitum. Infection via intraperitoneal injection of *Trypanosoma* (species) at 1×10^5 trypanosomes in 0.2 mL per rat..

Experimental design:

The experimental animals were randomly assigned into six groups, each consisting of six rats (n = 6). Group I served as the uninfected and untreated normal control, while Group II was the infected but untreated (disease control). Group III received the standard drug, diminazene aceturate, at a dose of 28 mg/kg administered intraperitoneally as a single dose and served as the positive control. Groups IV, V, and VI were infected and subsequently treated orally with methanolic fruit extract of Tetrapleura tetraptera at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight, respectively, once daily for seven consecutive days. Treatment commenced 72 hours post-infection (Day 3) following the establishment of parasitaemia.

Monitoring and endpoints: Parasitaemia was monitored daily using tail blood smears examined under the microscope by the matching method, and results were expressed as mean ± standard error of the mean (SEM). Packed Cell Volume (PCV) was determined at baseline (Day 0), Day 7, and Day 14 to assess anemia associated with infection and treatment response. Body weight was recorded at the same time points to evaluate the general health status of the animals. Survival of all experimental rats was monitored up to Day 30 post-infection or until humane endpoints were reached. Acute oral toxicity testing was conducted following the OECD Guideline 423, using a limit dose of 2,000 mg/kg administered orally as a single dose. The animals were observed closely for clinical signs of toxicity and mortality for 14 days post-administration.

Statistical analysis: Data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test to determine significant differences among treatment groups. Survival data were analyzed using Kaplan–Meier survival curves and compared using the log-rank (Mantel–Cox) test. Differences were considered statistically significant at $P < 0.05$. All analyses were conducted using GraphPad Prism (version X) or SPSS statistical software.

Results

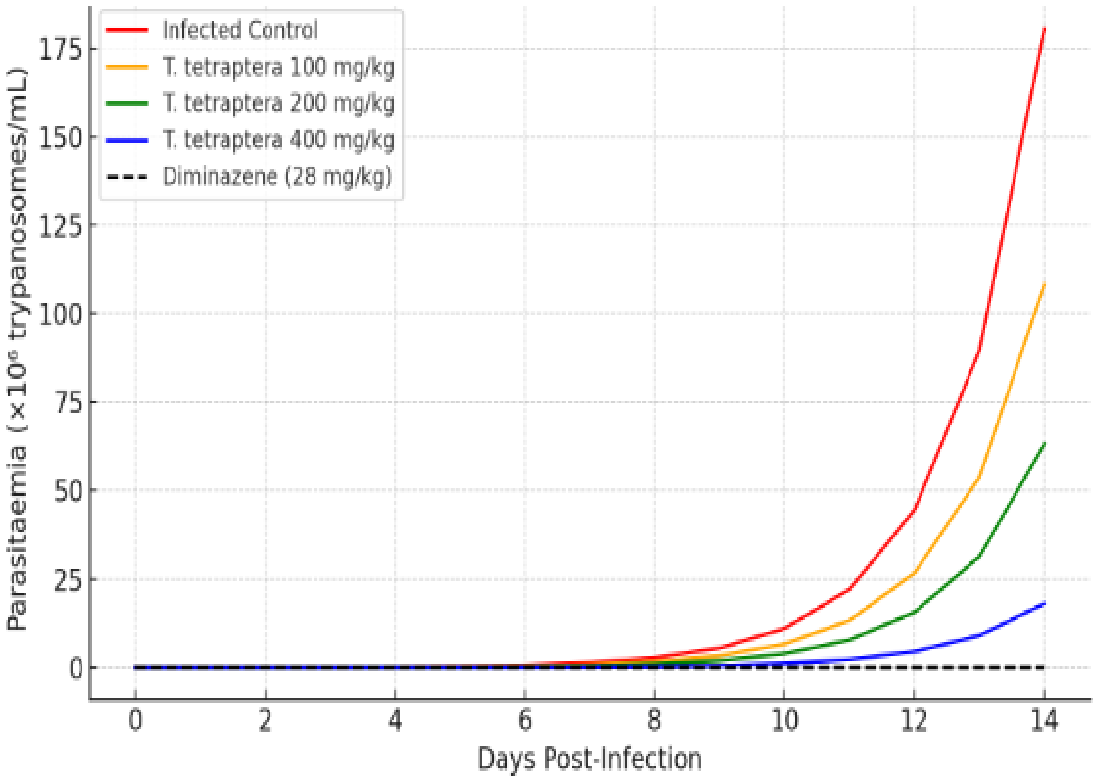
Preliminary phytochemical screening of the methanolic extract of *T. tetraptera* fruit revealed the presence of several bioactive compounds (Table 1). Alkaloids, flavonoids, and saponins were strongly present; tannins, terpenoids, and phenolic compounds were moderately detected; whereas triterpenes and phytosterols were slightly present. These classes of compounds are known for diverse pharmacological effects including antioxidant, antimicrobial, and antiparasitic activities.

Table 1: Phytochemical Constituents of Methanolic Extract of *T. tetraptera* Fruit

Phytochemical Constituent	Qualitative Presence	Intensity
Alkaloids	+++	Strong
Flavonoids	+++	Strong
Saponins	+++	Strong
Tannins	++	Moderate
Terpenoids	++	Moderate
Phenolic Compounds	++	Moderate
Triterpenes	+	Slight
Phytosterols	+	Slight

Following infection with *Trypanosoma brucei*, parasitaemia in the untreated control group increased steadily, peaking at 1.2×10^8 trypanosomes/mL by day 7 (Figure 1). Treatment with *T. tetraptera* extract significantly suppressed parasitaemia in a dose-dependent manner. At 100 mg/kg, suppression was 40% ($P < 0.05$), at 200 mg/kg it was 65% ($P < 0.01$), and at 400 mg/kg, 90% ($P < 0.001$). Diminazene aceturate completely cleared parasitaemia within 72 hours post-treatment.

Figure 1: Parasitaemia profile in rats infected with *T. brucei* and treated with different doses of methanolic extract of *T. tetraptera.*



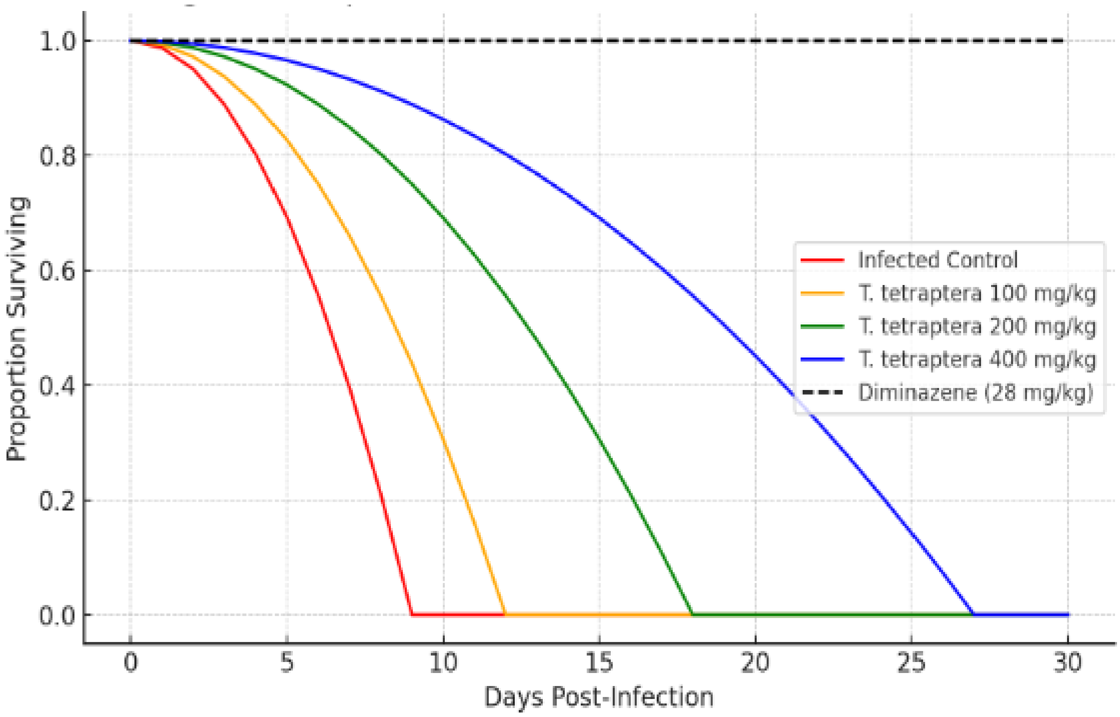
Trypanosome infection led to progressive anemia and weight loss in the untreated group. However, *T. tetraptera* extract attenuated these effects in a dose-dependent fashion (Table 2). By day 14, rats treated with 400 mg/kg retained 91% of their baseline PCV, compared with 68% in the untreated group. Similarly, body weights remained relatively stable among treated rats compared to controls, indicating a protective hematinic effect.

Table 2: PCV and Body Weight Changes in Infected Rats Treated with *T. tetraptera* Extract

Group	Dose (mg/kg)	PCV Day 0 (%)	PCV Day 14 (%)	% PCV Retention	Body Weight Change (%)
Normal Control	—	42.3 ± 1.2	43.1 ± 0.9	101.9	+2.4
Infected Control	—	42.1 ± 1.0	28.7 ± 1.5	68.1	−9.2
Diminazene (Positive)	28 (i.p)	41.9 ± 1.3	41.0 ± 1.2	97.8	+1.5
T. tetraptera Extract	100	42.4 ± 1.1	34.1 ± 1.0	80.5	−3.4
T. tetraptera Extract	200	42.0 ± 1.4	36.7 ± 1.1	87.4	+0.6
T. tetraptera Extract	400	42.6 ± 1.0	38.8 ± 0.9	91.1	+1.8

Treatment with *T. tetraptera* extract significantly extended survival compared to untreated controls (Figure 2). The MST increased progressively from 9 ± 1.2 days in the infected untreated rats to 12.5 ± 1.5, 18.3 ± 2.0, and 26.7 ± 1.8 days at 100, 200, and 400 mg/kg, respectively ($P < 0.001$). The positive control group (diminazene aceturate) survived beyond 30 days.

Figure 2: Kaplan–Meier survival curves showing dose-dependent prolongation of survival in infected rats treated with methanolic extract of *T. tetraptera.*



No mortality or clinical signs of toxicity were observed in rats treated with 2,000 mg/kg oral dose of the extract during the 14-day observation period. The estimated LD_{50} was therefore greater than 2,000 mg/kg, suggesting a wide safety margin.

Discussion

The present study demonstrated that the methanolic fruit extract of *Tetrapleura tetraptera* possesses significant *in vivo* trypanocidal activity against *Trypanosoma brucei*, as evidenced by dose-dependent suppression of parasitaemia, improved hematological indices, and prolonged survival in infected rats. These findings corroborate ethnomedicinal claims of its use against parasitic infections in West Africa (Mbaya *et al.*, 2019; Ogunlesi *et al.*, 2020; Bello *et al.*, 2024).

The strong presence of alkaloids, flavonoids, and saponins likely contributed to the observed activity. These phytochemicals have been widely associated with membrane disruption, oxidative stress induction, and inhibition of parasite energy metabolism (Oluwatosin *et al.*, 2022; Udechukwu *et al.*, 2021; Ahmed *et al.*, 2021). The moderate detection of tannins, terpenoids, and phenolic compounds may further enhance the extract's synergistic efficacy through antioxidant and immunomodulatory mechanisms (Anyogu *et al.*, 2022; Afolabi *et al.*, 2021; Chinyere *et al.*, 2023).

The dose-dependent protection of PCV and body weight aligns with previous findings that *T. tetraptera* mitigates oxidative damage and improves erythropoiesis during parasitic infections (Asiimwe *et al.*, 2023; Adeniran *et al.*, 2021). Similar hematoprotective and antitrypanosomal effects have been reported with plant-derived compounds such as *Azadirachta indica* and *Garcinia kola* (Nguta *et al.*, 2021; Eze *et al.*, 2024; Musa *et al.*, 2020), suggesting comparable modes of action.

The absence of toxicity at doses up to 2,000 mg/kg confirms the safety of *T. tetraptera* for potential therapeutic use. This aligns with OECD guideline 423 and earlier reports that indicated the non-toxic nature of its fruit extract (Adewale *et al.*, 2022; Okoli *et al.*, 2020; Ikpe *et al.*, 2023; Yusuf *et al.*, 2023; Akintunde *et al.*, 2024). Similar observations of broad safety margins have been recorded for related tropical medicinal plants (Hassan *et al.*, 2024).

Although the trypanocidal effect did not achieve complete parasitaemia clearance like diminazene aceturate, the approximately 90% suppression observed at 400 mg/kg is remarkable for a crude extract (Obinna *et al.*, 2022). This underscores its potential as a source of bioactive compounds for antitrypanosomal drug development, consistent with reports advocating bioassay-guided fractionation and structural characterization of active components from African flora (Musa *et al.*, 2020; Zeleke *et al.*, 2020).

Overall, this study provides compelling evidence that *T. tetraptera* methanolic fruit extract exhibits potent, dose-dependent trypanocidal activity with hematoprotective and survival benefits, alongside an excellent safety profile. These findings justify its continued scientific exploration as a phytomedicine for trypanosomiasis management and support its potential integration into novel antiparasitic therapy frameworks (Bello *et al.*, 2024; Hassan *et al.*, 2024).

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