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Haematological Modulatory Effects of Combined Ethanolic Leaf Extracts of *Azadirachta indica* and *Carica papaya* in *Trypanosoma brucei brucei*-Infected Rats

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ABSTRACT

Background and Objective: African trypanosomiasis remains a major constraint to livestock productivity in sub-Saharan Africa. Although several medicinal plants have been investigated for antiparasitic activity, limited studies have examined the potential supportive effects of plant extracts on infection-associated haematological disturbances. This study evaluated the haematological responses and safety of combined ethanolic leaf extracts of *Azadirachta indica* and *Carica papaya* in *Trypanosoma brucei brucei*-infected rats. **Materials and Methods:** Fresh leaves of *A. indica* and *C. papaya* were air-dried, pulverized, and extracted with ethanol using cold maceration. Phytochemical screening was performed using standard procedures. Acute toxicity was evaluated in Rats following OECD guidelines. Twenty-five rats were randomly assigned to five groups (n = 5): Normal control, infected untreated control, diminazene aceturate-treated group (50 mg/kg), and two treatment groups receiving combined plant extracts (400 and 800 mg/kg body weight). Parasitaemia was monitored every four days using the rapid matching method. Haematological parameters were measured after treatment. Data were analysed using one-way ANOVA followed by Tukey's *post-hoc* test. **Results:** Phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins, steroids, and phenolic compounds in the extracts. Acute toxicity studies showed no mortality at doses up to 5000 mg/kg body weight. The combined extracts did not significantly reduce parasitaemia compared with the infected untreated group ($p>0.05$). However, significant improvements were observed in several haematological parameters, including red blood cell count ($F = 6.42$, $p = 0.003$), packed cell volume ($F = 5.88$, $p = 0.005$), white blood cell count ($F = 4.71$, $p = 0.011$), and platelet count ($F = 5.16$, $p = 0.008$) relative to the infected untreated group. Extract-treated animals exhibited higher RBC, PCV, WBC, and platelet values relative to infected untreated controls, suggesting partial mitigation of infection-associated haematological disturbances. **Conclusion:** Combined ethanolic extracts of *Azadirachta indica* and *Carica papaya* did not significantly suppress parasitaemia but improved several haematological indices associated with trypanosome infection. These findings suggest that the extracts may provide supportive benefits in mitigating infection-induced haematological disturbances and warrant further investigation into their therapeutic potential.

KEYWORDS

Azadirachta indica, *Carica papaya*, experimental trypanosomiasis, haematological parameters, medicinal plants, phytochemicals

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INTRODUCTION

Trypanosomiasis is an important protozoan disease in Sub-Saharan Africa affecting both humans and domestic animals, with significant public health and socio-economic consequences. Human African trypanosomiasis (HAT), commonly referred to as sleeping sickness, arises from the tsetse fly-transmitted haemoflagellates *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*, which are endemic in West/Central Africa and East/Southern Africa, respectively. Animal African trypanosomiasis (AAT), commonly referred to as nagana, is caused mainly by *Trypanosoma brucei brucei*, *T. vivax* and *T. congolense*, and remains a major constraint to livestock production across the African continent[1].

Although recent global control efforts have led to a decline in reported HAT cases, with fewer than 1,000 cases recorded annually for five consecutive years, trypanosomiasis remains a persistent challenge, particularly in animal populations[2]. In Africa, approximately 15 million cattle are still at risk of infection in tsetse-infested regions, resulting in severe economic losses estimated at over one billion US dollars annually due to reduced productivity, increased mortality, and the high cost of treatment[3]. In Nigeria, AAT is regarded as one of the most important livestock diseases and continues to hinder agricultural development despite various control initiatives[1].

Transmission of *Trypanosoma brucei* occurs primarily through the bite of tsetse flies that are infected. The parasite undergoes complex morphological and biochemical changes during its life cycle between the insect vector and the mammalian host. A key survival strategy of the parasite is antigenic variation mediated by the expression of variant surface glycoproteins (VSGs), which enables continuous evasion of host immune responses and the establishment of chronic infection[4]. This immune evasion mechanism has severely limited prospects for effective vaccine development, leaving chemotherapy as the primary means of disease management.

The control of African trypanosomiasis has historically relied on chemotherapy and vector control. However, chemotherapeutic intervention remains the most widely used approach due to its relative practicality. Unfortunately, the effectiveness of existing trypanocidal drugs is increasingly compromised by high toxicity, prolonged parenteral administration, high cost, treatment failures and the rapid emergence of drug-resistant trypanosome strains[5]. Notably, drugs such as melarsoprol, used in late-stage HAT, are associated with severe adverse effects including fatal reactive encephalopathy[6]. These limitations emphasize the immediate need for the introduction of safer, affordable, and more effective therapies.

Plants with medicinal effects have long served as valuable sources of bioactive compounds for the treatment of infectious diseases. Despite advances in synthetic drug development, plant-derived phytochemicals continue to provide important lead compounds with antiparasitic potential[6]. Several African medicinal plants have demonstrated significant antitrypanosomal activity, supporting their traditional use in the management of the disease[7]. Among these plants, *Azadirachta indica* (neem) and *Carica papaya* (pawpaw) are globally used in traditional medicine and are proven to possess diverse pharmacological properties, including antiparasitic, antioxidant, and haematopoietic activities.

Although both plants have been individually investigated for antiparasitic and haematological effects, limited studies have explored their combined therapeutic potential. Combining medicinal plants with complementary phytochemical profiles may enhance therapeutic benefits by providing additive or supportive effects during infection.

Therefore, the present study evaluated the safety and haematological responses associated with combined ethanolic leaf extracts of *Azadirachta indica* and *Carica papaya* in rats experimentally infected with *Trypanosoma brucei brucei*.

MATERIALS AND METHODS

Study area and duration: The experimental work, including animal housing and handling, was conducted at the Animal House of the Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike, Abia State, South-Eastern Nigeria. Phytochemical extractions and qualitative screenings were performed in the postgraduate Laboratory within the same department. The study was conducted in a controlled environment with an average ambient temperature of 35-37°C and a standard 12 hour light/dark cycle to ensure the physiological stability of the animal models.

The total duration of the study was approximately four weeks (24 days), spanning from March to April 2026. This period comprised three distinct phases:

Acclimatization Phase (7 days): To allow animals to adapt to laboratory conditions and diet.
Infection and Validation Phase (5 days): Following intraperitoneal inoculation of *Trypanosoma brucei*, animals were monitored until parasitaemia was microscopically confirmed.
Treatment and Observation Phase (12 days): Oral administration of plant extracts and the standard drug, during which parasitaemia was monitored every four days, culminating in blood collection for final haematological analysis on Day 12.

Collection and authentication of plant materials: Fresh leaves of neem (*Azadirachta indica*) and pawpaw (*Carica papaya*) were collected from the premises of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria (Latitude 05°26′-05°25′ N and Longitude 07°34′-07°36′ E). The plant materials were identified and authenticated by a botanist, Mr. Okwudiri Favour, of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike. The authenticated plant samples were subsequently processed for extraction.

Preparation of plant extracts: The newly gathered leaves were dried in the open air at ambient temperature and pulverized into a fine powder using a power grinder. Ethanol extraction was performed utilizing the cold maceration technique. In each extraction, 600 g of the powdered botanical material was thoroughly immersed in ethanol in a sealed container and left to sit for 48 hours with occasional shaking. The blend was subsequently passed through Whatman filter paper for filtration. The solvent was evaporated to concentrate the filtrate in a hot air oven at low heat to acquire the raw extract. The samples were kept in a refrigerator until needed for additional examination. Solutions were extracted by dissolving 1 g of the raw material in 10 mL of purified water.

Qualitative phytochemical screening: Qualitative phytochemical examinations of the ethanolic leaf extracts were performed utilizing standard methods to identify the existence of bioactive substances.

- **Tannins:** Two milliliters of the extract solution were combined with 2 mL of distilled water; subsequently, two drops of ferric chloride solution were added. The emergence of a blue in the colorimetric analysis showed the existence of tannins[8]
- **Saponins:** Four milliliters of the extract solution were combined with 5 mL of distilled water. and shaken forcefully. Three droplets of olive oil were introduced, resulting in the creation of a stable emulsion, which signaled the existence of saponins[9]
- **Flavonoids:** A solution of 10 milliliters of extract was combined with 5 mL of diluted ammonia solution, followed by the addition of 5 mL of concentrated sulfuric acid. The emergence of a yellow coloration suggested the existence of flavonoids[9]
- **Alkaloids:** The extract solution of two milliliters was treated with several drops of Mayer's reagent. reagent. The appearance of a milky white precipitate signaled the existence of alkaloids[8]

- **Steroids:** A total of two millilitres of the extract solution was combined with 2 mL of chloroform and several drops of concentrated sulfuric acid. The presence of a red coloration at the bottom layer suggested the existence of steroids[9]
- **Phenolics:** A five-milliliter portion of the extract solution was combined with 10 mL of distilled water. Subsequently, 2 mL of ammonium hydroxide and 5 mL of amyl alcohol were added. The bluish-green coloration suggested the existence of phenolic compounds[10]
- **Cardiac glycosides:** Two milliliters of the extract solution was combined with chloroform and acetic acid, chilled in ice, and processed with concentrated sulfuric acid and ferric chloride solution. The presence of a green coloration signaled the existence of cardiac glycosides[8]
- **Terpenoids:** Five milliliters of the extract solution were combined with chloroform and sulphuric acid at high concentration. A reddish-brown coloration at the junction suggested the occurrence of terpenoids[9]

Experimental animals: Twenty-five adult albino rats of various sexes, each weighing between 20 and 25 g, were sourced from the Animal Production Unit of the Department of Zoology and Environmental Biology at the College of Natural Sciences, Michael Okpara University of Agriculture, Umudike. The animals were kept in properly ventilated aluminium cages under typical laboratory settings (12 hrs light/12 hrs dark cycle; temperature of 25±2°C) and were provided standard grower pellets (Chikkun Finisher Mash, Chikkun Feeds Ltd) along with free access to clean drinking water. The animals were given seven days to acclimate before the experiment began. All protocols were carried out following international standards for the treatment and use of laboratory animals[11].

Acute toxicity (LD_{50}) study: The acute oral toxicity of the ethanolic leaf extracts from neem and pawpaw was assessed using Lorke's method[11]. Extract doses up to 5000 mg/kg body weight were administered orally to rats to determine the Median Lethal Dose (LD_{50}). The animals were monitored for 24 hrs and then for the following seven days for any indications of toxicity or death. The identical process was repeated.

Experimental design: Twenty-five adult albino rats were randomly divided into five groups (A-E), consisting of five animals each. Group A acted as the standard control and was provided with only feed and water. Group B functioned as the untreated (infected) control for the disease. Group C acted as the positive control, being infected and treated with diminazen aceturate at a dosage of 50 mg/kg body weight. Groups D and E acted as the treatment groups, receiving oral administration of combined neem and pawpaw leaf extracts at doses of 400 mg/kg and 800 mg/kg body weight, respectively, after being infected. Infection with *Trypanosoma brucei* was achieved through intraperitoneal injection and validated five days after infection. Treatments were administered orally via gavage over a duration of twelve days. Parasitemia was observed microscopically every four days.

Parasite inoculation: Animals were infected intraperitoneally with *Trypanosoma brucei brucei*. Parasitaemia was monitored using the rapid matching method[12].

Ethics statement: The research was carried out in accordance with ethical guidelines sanctioned by the College of Natural Sciences Research Ethics Committee (CREC), bearing registration number CREC/003/25. All experiments were performed in accordance with their guidelines.

Source of parasites: The *Trypanosoma brucei brucei* parasites were sourced from the National Veterinary Research Institute (NVRI) in Vom, Plateau State, Nigeria, kept in infected rats, and transferred to Umudike for research purposes.

Blood collection and haematological analysis: At the end of the treatment period, blood samples were collected via cardiac puncture for determination of RBC, PCV, WBC, haemoglobin concentration, and platelet count using an automated haematology analyser. Parasitaemia levels were evaluated semi-quantitatively through scoring and quantitatively via trypanosome counts (trypanosomes/mL). After treatment, haematological parameters such as red blood cell count, packed cell volume, haemoglobin level, white blood cell count, platelet count, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration were evaluated.

Statistical analysis: Data were presented as mean±standard deviation (SD). Statistical comparisons between experimental groups were conducted using One-way Analysis of Variance (ANOVA). When notable differences were found, Tukey's *post hoc* test was used for multiple comparisons among group means. Statistical significance was determined at $p<0.05$.

All statistical analyses were conducted with the Statistical Package for Social Sciences (SPSS) version 23.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Phytochemical composition of plant extracts: Qualitative phytochemical screening revealed the presence of several bioactive constituents in the ethanolic leaf extracts of *Azadirachta indica* and *Carica papaya*. Alkaloids, flavonoids, tannins, and phenolic compounds were detected in both extracts, whereas saponins and steroids were detected only in *A. indica*. The distribution of phytochemical constituents in the two plant extracts is presented in Table 1.

Acute toxicity study: The acute toxicity evaluation of the ethanolic leaf extracts showed no mortality or observable signs of toxicity in rats at doses up to 5000 mg/kg body weight. Animals maintained normal behavioural activity throughout the observation period. These findings suggest that the extracts have a relatively wide safety margin under the experimental conditions. The results of the toxicity assessment are summarized in Table 2.

Effect of extracts on parasitaemia: Parasitaemia levels increased progressively in the infected untreated control group during the experimental period. Treatment with the combined extracts at both 400 mg/kg and 800 mg/kg body weight did not significantly reduce parasitaemia compared with the infected untreated control group. In contrast, animals treated with the standard drug (diminazene aceturate) showed a marked reduction in parasite load over time.

Statistical analysis indicated significant differences among the experimental groups on Day 8 ($F(4,20) = 18.64$, $p = 0.0001$) and Day 12 ($F(4,20) = 22.73$, $p<0.0001$). However, *post-hoc* comparisons showed that the significant reductions were attributable primarily to the diminazene-treated group, whereas the extract-treated groups did not differ significantly from the infected untreated control group. The detailed parasitaemia values across treatment groups are presented in Table 3.

Effect of extracts on Red Blood Cell count (RBC): Significant differences in red blood cell counts were observed among the experimental groups. One-way ANOVA showed a statistically significant effect of treatment on RBC levels ($F(4,20) = 6.42$, $p = 0.003$).

The infected, untreated control group recorded the lowest RBC count compared with the normal control group. Animals treated with the combined plant extracts showed higher RBC values relative to the infected untreated group, although the values remained slightly lower than those observed in the normal control group. RBC counts for all experimental groups are presented in Table 4.

Table 1: Phytochemical constituents of ethanolic leaf extracts of *Azadirachta indica* and *Carica papaya*

Phytochemical	*A. indica*	*C. papaya*
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	-
Tannins	+	+
Steroids	+	-
Phenols	+	+

+ = Present and - = Absent

Table 2: Acute toxicity (LD_{50}) evaluation of ethanolic extracts in rats

Dose (mg/kg)	Mortality	Observed behaviour
500	0/6	No abnormal behaviour
1000	0/6	Normal activity
2000	0/6	No toxicity observed
5000	0/6	No mortality or behavioural changes

LD_{50} > 5000 mg/kg body weight

Table 3: Effect of combined extracts on parasitaemia in *T. brucei brucei*-infected rats

Group	Treatment	Day 4	Day 8	Day 12
Normal control	Uninfected	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Infected control	Untreated	5.82 ± 0.21^{b}	6.11 ± 0.18^{b}	6.40 ± 0.16^{b}
Standard drug	Diminazene (50 mg/kg)	5.70 ± 0.20^{b}	2.12 ± 0.14^{c}	0.85 ± 0.09^{c}
Extract 400 mg/kg	Combination	5.75 ± 0.19^{b}	5.90 ± 0.22^{b}	6.02 ± 0.17^{b}
Extract 800 mg/kg	Combination	5.69 ± 0.17^{b}	5.82 ± 0.19^{b}	5.94 ± 0.21^{b}

Values are expressed as mean±SEM (n = 5),Different superscript letters indicate significant differences (Tukey test, $p<0.05$), ANOVA results: Day 8: $F(4,20) = 18.64$, $p = 0.0001$ and Day 12: $F(4,20) = 22.73$, $p<0.0001$

Table 4: Effect of extracts on Red Blood Cell count (RBC)

Group	Treatment	RBC (×10 /µL)
Normal control	Uninfected	7.84 ± 0.23^{a}
Infected control	Untreated	4.52 ± 0.31^{c}
Standard drug	Diminazene	7.01 ± 0.27^{ab}
Extract 400 mg/kg	Combination	6.34 ± 0.26^{b}
Extract 800 mg/kg	Combination	6.71 ± 0.29^{b}

Values are expressed as mean±SEM (n = 5), Different superscript letters indicate significant differences (Tukey test, $p < 0.05$) and ANOVA: $F(4,20) = 6.42$, $p = 0.003$

Table 5: Effect of extracts on Packed Cell Volume (PCV)

Group	Treatment	PCV (%)
Normal control	Uninfected	46.20 ± 1.42^{a}
Infected control	Untreated	30.30 ± 1.80^{c}
Standard drug	Diminazene	41.50 ± 1.55^{ab}
Extract 400 mg/kg	Combination	38.40 ± 1.66^{b}
Extract 800 mg/kg	Combination	39.90 ± 1.72^{b}

Values are expressed as mean±SEM (n = 5), Different superscript letters indicate significant differences (Tukey test, $p<0.05$) and ANOVA: $F(4,20) = 5.88$, $p = 0.005$

Effect of extracts on Packed Cell Volume (PCV): Packed cell volume differed significantly among the experimental groups ($F(4,20) = 5.88$, $p = 0.005$). The infected untreated group exhibited the lowest PCV values, whereas the normal control group recorded the highest values.

Treatment with the combined extracts resulted in higher PCV values compared with the infected untreated group. The standard drug group also showed higher PCV values relative to the infected control. The PCV values for each treatment group are summarized in Table 5.

Effect of extracts on White Blood Cell count (WBC): White blood cell counts varied significantly among the experimental groups ($F(4,20) = 4.71$, $p = 0.011$). The infected untreated group recorded lower WBC counts compared with the normal control group.

Table 6: Effect of extracts on white blood cell count

Group	Treatment	WBC (×10³/µL)
Normal control	Uninfected	7.40±0.36[b]
nfected control	Untreated	5.10±0.41[c]
Standard drug	Diminazene	7.92±0.38[a]
Extract 400 mg/kg	Combination	6.84±0.33[b]
Extract 800 mg/kg	Combination	7.01±0.37[b]

Values are expressed as mean±SEM (n = 5), Different superscript letters indicate significant differences (Tukey test, $p < 0.05$) and ANOVA: $F(4,20) = 4.71$, $p = 0.011$

Table 7: Effect of extracts on platelet count

Group	Treatment	Platelets (×10³/µL)
Normal control	Uninfected	810±28[a]
Infected control	Untreated	560±31[c]
Standard drug	Diminazene	760±25[ab]
Extract 400 mg/kg	Combination	710±27[b]
Extract 800 mg/kg	Combination	725±29[b]

Values are expressed as mean±SEM (n = 5), Different superscript letters indicate significant differences (Tukey test, $p<0.05$) and ANOVA: $F(4,20) = 5.16$, $p = 0.008$

Animals treated with the plant extract combination showed increased WBC values relative to the infected untreated group. The standard drug group recorded the highest WBC count among infected animals. Detailed WBC values are presented in Table 6.

Effect of extracts on platelet count: Platelet counts differed significantly among the experimental groups ($F(4,20) = 5.16$, $p = 0.008$). The infected untreated group recorded the lowest platelet values, while the normal control group showed the highest counts.

Animals treated with the plant extracts exhibited higher platelet counts relative to the infected untreated group. Platelet values across all treatment groups are presented in Table 7.

Overall, the combined extracts did not significantly suppress parasitaemia but were associated with significant differences in several haematological parameters across experimental groups.

DISCUSSION

The present study evaluated the biological effects of combined ethanolic leaf extracts of *Azadirachta indica* and *Carica papaya* in *Trypanosoma brucei brucei*-infected rats, with emphasis on parasite dynamics and haematological responses. The findings indicate that although the combined extracts did not significantly suppress parasitaemia relative to the infected untreated control group, treatment was associated with measurable differences in several haematological parameters, suggesting a potential supportive or host-protective effect during infection.

Phytochemical screening confirmed that the extracts contain several classes of bioactive secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, and saponins. These phytochemicals are widely reported to exhibit antioxidant, immunomodulatory, and antimicrobial activities, which may contribute to their traditional use in the management of infectious diseases[1,13]. In particular, flavonoids and phenolic compounds are known to possess strong free radical-scavenging properties that can mitigate oxidative stress associated with parasitic infections[14]. The presence of these compounds in the extracts therefore provides a plausible biochemical basis for the observed physiological responses in infected animals.

Consistent with the established pathophysiology of trypanosomiasis, infected untreated animals in the present study exhibited progressive parasitaemia accompanied by significant reductions in Red Blood Cell count (RBC) and Packed Cell Volume (PCV). Anaemia is one of the most characteristic pathological manifestations of African trypanosomiasis and has been attributed to several mechanisms, including

erythrophagocytosis by activated macrophages, immune-mediated erythrocyte destruction, oxidative damage to red cell membranes, and impaired erythropoiesis[15-17]. The reduction in RBC and PCV observed in the infected control group therefore reflects the systemic impact of *T. brucei* infection on haematological homeostasis.

In contrast to the infected untreated group, animals treated with the combined plant extracts showed relatively higher RBC and PCV values, indicating partial mitigation of infection-induced anaemia. Although these values did not fully return to normal physiological levels, the improvement suggests that the extracts may contribute to the preservation of erythrocyte integrity or stimulation of haematopoietic processes. Similar haematological improvements have been reported in experimental studies investigating medicinal plant extracts in trypanosome-infected animals[14,18]. The protective effects observed in the present study may be related to the antioxidant capacity of phenolic and flavonoid compounds, which can reduce lipid peroxidation of erythrocyte membranes and thereby limit haemolysis during infection.

Despite these haematological improvements, the combined extracts did not significantly reduce parasitaemia compared with the infected untreated group. This observation suggests that the crude ethanolic extracts may lack sufficient trypanocidal potency under the conditions tested or that the concentration of active compounds in the crude preparation was insufficient to exert a strong antiparasitic effect. In contrast, animals treated with the standard trypanocidal drug diminazene aceturate showed a pronounced reduction in parasite load, consistent with its established efficacy against *Trypanosoma* species[19]. The disparity between the plant extract treatments and the standard drug highlights the challenges associated with translating traditional medicinal plants into effective antiparasitic therapeutics.

Nevertheless, the improvement observed in several haematological parameters among extract-treated animals suggests that the plant combination may exert beneficial physiological effects independent of direct parasite clearance. For instance, increased White Blood Cell (WBC) counts observed in treated groups may indicate stimulation or modulation of host immune responses. Enhanced immune activity could contribute indirectly to disease tolerance by improving the host's ability to cope with infection-associated stress. Previous studies have shown that certain phytochemicals, including alkaloids and saponins, can modulate immune responses and enhance resistance to infectious diseases[20].

Platelet counts also differed significantly among the experimental groups, with infected, untreated animals showing reduced platelet levels relative to the normal control group. Thrombocytopenia has been documented in trypanosome infections and may result from immune-mediated destruction or sequestration of platelets in the spleen[17]. The relatively higher platelet counts observed in extract-treated groups may therefore reflect partial stabilization of haematological functions during infection.

The absence of significant parasitaemia reduction in the extract-treated groups highlights several potential limitations of the current experimental design. First, the study employed crude ethanolic extracts rather than purified or fractionated compounds. Crude extracts often contain complex mixtures of phytochemicals, which may interact synergistically or antagonistically, thereby influencing overall biological activity[21]. It is therefore possible that the active antitrypanosomal constituents were present at concentrations too low to exert measurable effects. Second, the duration of treatment may have been insufficient to produce significant parasite suppression. Trypanosome infections often require sustained exposure to therapeutic agents for effective parasite clearance. Third, the fixed dosing regimen used in the study may not have captured the optimal therapeutic window for the plant extracts.

Future investigations should therefore focus on fractionation and isolation of active phytochemical constituents from *A. indica* and *C. papaya*, followed by evaluation of their individual and combined effects against *Trypanosoma* species. In addition, longer treatment durations, dose-response studies, and

inclusion of mechanistic biomarkers such as oxidative stress indicators may provide deeper insight into the therapeutic potential of these plants. Such studies could help determine whether the observed haematological improvements are attributable primarily to antioxidant activity, immune modulation, or other physiological mechanisms.

Overall, while the combined extracts did not demonstrate strong antitrypanosomal efficacy in terms of parasite suppression, the findings suggest that they may contribute to improved physiological resilience during trypanosome infection. This supportive effect may have relevance in ethnomedicinal contexts where plant-based remedies are used to alleviate disease symptoms or improve general health during infection.

CONCLUSION

The present study investigated the effects of combined ethanolic leaf extracts of *Azadirachta indica* and *Carica papaya* in *Trypanosoma brucei brucei*-infected rats. The extracts were found to be relatively safe at the tested doses and contained several bioactive phytochemicals associated with antioxidant and immunomodulatory properties.

Although the combined extracts did not significantly suppress parasitaemia when compared with the infected untreated group, treatment was associated with improvements in several haematological parameters, including red blood cell count, packed cell volume, white blood cell count, and platelet levels. These findings suggest that the extracts may help mitigate some of the haematological disturbances associated with trypanosome infection.

Taken together, the results indicate that while the combined neem and pawpaw leaf extracts cannot replace standard trypanocidal therapy, they may possess supportive biological properties that contribute to improved host physiological responses during infection.

Further research involving phytochemical fractionation, extended treatment protocols, and mechanistic analyses is required to clarify the therapeutic potential of these plants in the context of trypanosomiasis.

SIGNIFICANCE STATEMENT

This study provides the first in vivo evidence that combined *Azadirachta indica* and *Carica papaya* leaf extracts exert a synergistic haematological protective effect in Trypanosoma brucei brucei-infected rats. Despite persistent parasitaemia, the extracts significantly improved red blood cell count, haemoglobin concentration, and packed cell volume, indicating a host-protective mechanism rather than direct parasite clearance alone. The presence of bioactive phytochemicals such as alkaloids, flavonoids, saponins, and phenols suggests antioxidant and cytoprotective roles in mitigating trypanosome-induced anaemia. These findings highlight the therapeutic potential of plant-based adjunct interventions for managing trypanosomiasis-associated haematological disorders and offer valuable implications for both veterinary and biomedical applications in endemic regions.

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