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Journal of Animal Science and Veterinary Medicine
Volume 10(2), pages 185-196, April 2025
Article Number: 7759A79710
ISSN: 2536-7099
https://doi.org/10.31248/JASVM2025.550
https://integrityresjournals.org/journal/JASVM

Full Length Research

Antitrypanosomal effect of ethanolic stem extract of *Cassytha filiformis* on *Trypanosoma congolense* infection in albino mice

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Received 19th March 2025; Accepted 29th April 2025

ABSTRACT: African trypanosomiasis is a parasitic infection caused by single-celled protozoan parasites of the genus *Trypanosoma* that is primarily transmitted by the bite of infected tsetse flies. This study aimed to investigate the antitrypanosomal effect of ethanolic stem extract of *Cassytha filiformis* on *Trypanosoma congolense* infection in albino mice. Crude extract of the plant was obtained by maceration in absolute ethanol, its phytochemical and acute toxicity studies were carried out following standard procedures. Thereafter, the antitrypanosomal effect of the extract was investigated in albino mice. Haematological, biochemical and histopathological changes in the experimental animals were assessed. Phytochemical screening revealed the presence of glycosides, tannins, terpenoids, alkaloids, flavonoids, and steroids. The extract of *C. filiformis* showed a mean lethal dose (LD_{50}) >5000 mg/kg body weight. *T. congolense* savannah parasites used in this study showed a prepatent period of 96 hours. Lower doses of the extract (30 and 60 mg/kg body weight) produced better antitrypanosomal effects. Resistance of *T. congolense* parasite to standard trypanocide (*Diminazine aceturate*) was noted. The animal survival study showed that the higher the parasite load, the lower the survivability of the infected animals. Packed cell volume (PCV) of the treated groups showed a dose-dependent increase. The serum aspartate amino transferase (AST) activity was significantly ($p<0.05$) higher in groups 2, 3, 4, 5 and 6 compared to groups 1, 7 and 8 while alanine amino transferase (ALT) activities were significantly higher ($P<0.05$) in groups 3 and 4 compared to groups 1, 2, 7 and 8. The histopathological changes in the kidney and liver were moderate in the worst scenario. *C. filiformis* extract has significant potential as an antitrypanosomal agent due to its efficacy in reducing parasite load, improving survival rates, and showing manageable side effects.

Keywords: *C. filiformis*, ethanolic stem extract, Trypanosomiasis, *T. congolense*.

INTRODUCTION

African trypanosomiasis is a parasitic infection caused by single-celled protozoan parasites of the genus *Trypanosoma* that is primarily transmitted by the bite of infected tsetse flies (Feyera *et al*., 2014). Both human and animal trypanosomiases negatively affect the whole economy of Africa by weakening both human and animal health (John *et al*., 2012). Human African Trypanosomiasis (HAT), also known as sleeping sickness is classified as one of the world's classical neglected tropical diseases representing a major public health threat in Sub-Saharan African countries (WHO, 2020). It is caused by the bite of blood sucking tsetse fly of the genus *Glossina* (Büscher *et al*., 2017). Among the protozoan species affecting the Sub-Saharan African countries, *Trypanosoma brucei gambiense (T.b gambiense)* is the most infectious in West and Central Africa, causing chronic form of the disease (Franco *et al*., 2020). *Trypanosoma brucei rhodesiense* (*T. brhodesiense)* is endemic in Eastern

Africa and is the pathogenic agent for more acute form of the disease (Barret, 2003). African Animal Trypanosomiasis (AAT) is caused by the following species of trypanosome: *Trypanosoma vivax, T. congolense, T.b brucei, T. simiae, T. theileri,* and *T. evansi* which affect domestic animals including cattle, goats, dogs etc (Hamill *et al*., 2013; Salim *et al*., 2011).

Clinical signs of animal trypanosomiasis are not sufficiently specific to support a clinical diagnosis and, therefore, laboratory tests are required to confirm clinical suspicions (Desquesnes *et al*., 2022). Consequently, case confirmations and epidemiological studies can only be done using laboratory facilities (Desquesnes *et al*., 2022). The unavailability of vaccines against trypanosomiasis and escalating costs of initiating and maintaining tsetse control campaigns have led to the vast tsetse -infected areas of Africa being almost completely reliant on the use of trypanocidal drugs (Geerts and Holmes, 1998). However, only a small group of chemoprophylactic and chemotherapeutic trypanocidal compounds are currently in use to which resistance has developed (Delespaux *et al*., 2008). Contrast to the situation of HAT, where the nifurtimox-eflornithine combination therapy (NECT) is now the preferred first line treatment for second stage disease (Priotto *et al*., 2009; Alirol *et al*., 2013), no drug combinations are currently used for animal trypanosomiasis. Instead, alternative compounds, particularly diminizine and isometamidium (called a sanative pair), with low risk of cross-resistance, is recommended where possible (Giordani *et al*., 2016). Some of the trypanocides that are used for the management of African Animal Trypanosomiases are as follows; Homidium Salt, Suramin and Pentamidine (WHO, 2013).

Furthermore, the registered trypanocides are beset with several drawbacks such as toxicity, rigorous administration procedure, lack of efficacy and high cost (Legros *et al*., 2002). Therefore, the need for alternative new molecules that are safe, effective, affordable is urgent. Consequently, it has been observed that natural products derived from plants offer novel possibilities to obtain new drugs that are active against trypanosomes and investigation of antitrypanosomal activity of traditionally used plants has been a major area of contemporary research focus (Hoet *et al*., 2004).

One of the plants that has been reported to have medicinal values is *C. filiformis* (Eluu *et al*., 2019). *C. filiformis* belongs to the family Lauraceae (Jaiswal *et al*., 2021). It is also known to parasitize a wide range of host plants, causing significant damage to crops, forests, and natural habitats. It has been reported to infest various plant species, including trees, shrubs, and herbaceous plants (Rosli *et al*., 2024). The damage it causes to host plants is well documented, with effects ranging from reduced sexual reproduction to decreased survival (Zhang *et al*., 2022). *C. filiformis* seedlings are autotrophic and can survive up to a month before parasitizing a viable host (Furuhashi *et al*., 2016). Like other parasitic vines, they grow in a creeping form, and upon encountering a potential host, they twine around the stem. If the host is viable, haustoria develops to penetrate the host tissue and once established, *C. filiformis* can cover the host completely as it grows (Nelson, 2008; Debabrata 2018). Even though it can establish on a host, *C. filiformis* may still move toward better-quality hosts, where it eventually reproduces sexually (Zhang *et al*., 2022). This hemiparasitic species does not produce leaves, and its stems slightly vary in color, displaying tonalities that range from yellowish or brown to light or dark greenish depending on the stem type (mature holding stems or young growing stems) (Anchevida *et al*., 2024).

The plant is used in the traditional treatment of many diseases, such as vermifuge, and also in the suppression of lactation after stillbirth by several tribes in Nigeria (Burkill, 1995). The plant (stem and false leaves) is boiled in water and administered for varying lengths of time to treat jaundice (Adamu *et al*., 2017). Aqueous and alcoholic extracts of *C. filiformis* were found to exhibit significant diuretic activity by causing a marked increase in the sodium ion and potassium excretion (Sharma *et al*., 2009). Furthermore, ocoteine, a phytochemical from *C. filiformis* is an anticancer agent (Nelson, 2008). Extracts of *Cassytha filiformis* were also shown to have antibacterial action against *staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, antifungal activity against *Candida albicans* (Adonu *et al.*, 2013; Mythili, 2011).

This study, therefore, investigated the antitrypanosomal effect of ethanolic stem extracts of *C. filiformis* on *T. congolense* infection in Albino mice (*Mus musculus).*

MATERIALS AND METHODS

Plant material and experimental animal

Fresh stem samples of *C. filiformis* were collected from Zabi area of Sabon Gari Local Government, Kaduna State, Nigeria in December 2023 and identified by a plant taxonomist in the Department of Botany, Ahmadu Bello University, Zaria with identification number: ABUH016761. Laboratory albino mice (*M. musculus)* of both sexes, having similar weight (±2-4 g) were used. They were obtained from a breeding colony of the Department of Pharmacognosy and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna, Nigeria. Standard protocol was followed in handling the animals.

Preparation of plant extract

The collected plant material was shade dried at 25°C and crushed into powder using a clean laboratory mortar and

pestle. The crude extract was obtained by the maceration technique in absolute ethanol. 1 kg of the powdered plant material was soaked in 5000 ml of the solvent for 7 days with occasional shaking. The mixture was first filtered using gauze, and the filtrate was passed through a sterile filter (Whatman, England). The filtrate was dried in an oven at a temperature of 40°C. The resulting extract was weighed, recorded, and kept in a refrigerator until required for use (Feyera *et al*., 2014).

Phytochemical analysis

Qualitative phytochemical analysis was carried out using the standard methods described by Harborne (1998).

Test for the presence of alkaloids

Exactly, 1 ml of the filtrate was added to a test tube, followed by the addition of 1 ml of Wagner's reagent. The solution was mixed properly, and a colour change was observed. A reddish brown precipitate indicated the presence of an alkaloid (Harborne, 1998).

Test for the presence of steroids

Liebermann-Burchard's test was used to test for steroids. Briefly, 1 ml of the extract was treated with 0.5 ml of acetic anhydride and cooled. This was later mixed with 0.5 ml of chloroform, and 1 ml of concentrated sulphuric acid was carefully added using a pipette. There was the formation of a reddish brown ring, which indicated the presence of steroids (Harborne, 1998).

Test for the presence of flavonoids

The presence of flavonoids in each sample was investigated using the method described by Harborne (1998). Briefly, 4 ml of filtrate was mixed with 1 ml of dilute ammonia solution. The layers were allowed to separate, and the yellow colour in the ammonical layer indicated the presence of flavonoids.

Test for the presence of terpenoids

Salkowski's test was used to test for the presence of terpenoids. This was done by mixing 5 ml of plant extract with 2 ml of chloroform, followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate was produced immediately, indicating the presence of terpenoids (Harborne, 1998).

Test for the presence of saponin

The presence of saponins in the samples was determined using Harborne (1998) method, in which 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable, persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for emulsion.

Test for the presence of tannin

The presence of tannins in the samples was determined using the ferric chloride test. To 3 ml of the filtrate in the test tube, few drops of ferric chloride were added. A greenish black precipitate indicated the presence of tannins.

Test for cardiac glycosides (Keller Killani test)

Exactly 5 ml of the filtrate was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates presence of a deoxysugar characteristic of cardenolides (Harborne, 1998).

Acute toxicity study of ethanolic stem extract of *C. filiformis*

Acute toxicity study was done using the method of Lorke (1983). The study was conducted in two phases. In the first phase, nine rats were divided into 3 groups of 3 rats each. Groups 1, 2 and 3 animals were given a single dose of 10, 100 and 1000 mg/kg body weight (b.w.) of the extract, respectively and monitored for 24 hours for possible signs of toxicity. In the second phase, specific doses (1600, 2900 and 5000 mg/kg b.w.) of the extract were administered to three rats (one rat per dose) to further determine the correct LD_{50} value. All animals were observed frequently on the day of treatment and surviving animals were monitored for 24 hours for signs of acute toxicity. The LD_{50} value was then calculated as the geometric mean of the highest dose that gave no mortality and the lowest dose that produced mortality as shown below:

$$LD_{50} = \sqrt{(D0 \text{ x } D100)}$$

Where: D_0 = Highest dose that gave no mortality, and D_{100} = Lowest dose that produced mortality.

Test organism collection, parasite inoculation, animal grouping, and treatment

The stock of *T. congolense* used in this study was obtained from stabilates maintained in liquid nitrogen at the National Veterinary Research Institute (NVRI), Vom, Plateau State,

Nigeria. A total of 80 healthy mice of both sexes were randomly selected and assigned into 8 groups of 10 mice each. The isolate was inoculated heavily by interperitoneal injection of 0.1 ml of blood containing 10^4 cells/ml trypanosomes (*T. congolense*) to mice of all the 8 groups except Groups 1 and 8 which were the control groups and Group 7 which was administered high dose of the extract 3 days before being infected with the parasite and treatment continued daily for 5 days. The study was terminated on the 8th day of the experiment for all groups. GRP 1: Uninfected-untreated but given 2 ml/kg body weight of phosphate buffered saline (PBS). GRP 2: Infected-untreated but given 2 ml/kg body weight of PBS. GRP 3: Infected-treated with 3.5 mg/kg body weight of standard trypanocide (Diminazine aceturate). GRP 4: Infected-treated with 30 mg/kg body weight of extract daily for 4 days and terminated on day 8. GRP 5: Infected-treated with 60 mg/kg body weight of extract daily for 4 days and terminated on day 8. GRP 6: Infected-treated with 100 mg/kg body weight of extract daily for 4 days and terminated on day 8. GRP 7: Prophylactic was administered 100 mg/kg of body weight of extract 3 days before infection and treatment continued daily for 4 days after infection and terminated on day 8. GRP 8: Uninfected-treated with 100 mg/kg of body weight of extract for 5 days and terminated on day 8.

Determination of parasitaemia

Parasitaemia was determined by the procedure described by Herbert and Lumsden (1976). Briefly, the method involves microscopic counting of parasites per field in blood smears from the peripheral blood obtained from tail vein of each mouse. Wet smears were prepared from each animal and examined microscopically. Logarithm values of these counts were obtained by matching with the table given by Herbert and Lumsden (1976).

Determination of mean survival time

Mortality was monitored daily, and the number of days from the time of inoculation of the parasite up to death was recorded for each mouse in the treatment and control groups throughout the follow-up period of 8 days (Feyera *et al*., 2014).

Sample collection

On the final day of the experiment (Day 8), the mice of all the groups were euthanized by anesthetizing them using gaseous chloroform in a 70 liter airtight plastic jar. A mass of cotton wool was placed in the jar, and 50 ml of chloroform was added, in which the cotton wool was soaked just before introducing the experimental mice. Blood samples were collected into EDTA and plain containers for the assessment of haematological and biochemical parameters, respectively. Kidney and liver samples were collected and placed in 10% buffered formaldehyde for histopathological analysis.

Determination of packed cell volume (PCV)

The peripheral blood obtained from the tail vein of each mouse was used for the determination of packed cell volume. The PCV was measured on day 8 using the Wintrobe method to predict the effectiveness of the test extracts in preventing hemolysis resulting from increasing parasitaemia associated with trypanosomiasis (Feyera *et al*., 2014).

Assessment of biochemical parameters

After centrifugation, serum obtained from the blood of the mice was used for the determination of biochemical parameters. Serum urea and creatinine levels were determined using Agappe Kits (Agappe Diagnostics Switzerland GmbH) while aspartate amino transferase (AST) and Serum alanine amino transferase (ALT) were determined using Randox Kits (Randox Laboratories, UK) following the manufacturer's instructions.

Histopathological studies

Livers and kidneys from different experimental groups were harvested and kept in 10% buffered formaldehyde. Tissue samples of the kidney and liver harvested were retrieved from the 10% buffered formaldehyde and analyzed for histopathological changes according to the method described by Avwioro (2002).

Statistical analysis

Statistical analysis was done with the aid of the Statistical Package for Social Science (SPSS), version 20.0. The data obtained from the study were summarized and expressed as mean ± standard error of mean (SEM). Comparison of results obtained from different groups was done by one-way ANOVA. Duncan's post hoc test was used to identify the pairs of means that differ. A p-value less than 0.05 was considered significantly different.

RESULTS

Phytochemicals

The data from phytochemical screening of the ethanolic extract of *C. filiformis* revealed the presence of glycosides, tannins, terpenoids, alkaloids, flavonoids, and steroids.

Table 1. Phytochemical composition of the ethanolic stem extract of *C. filiformis.*

Phytoconstituents	Abundance
Glycoside	++++
Steroids	+
Terpenoids	+++
Alkaloids	++
Resins	-
Tannins	++++
Saponin	-
Flavonoid	++
Protein	-
Fat	-

Key: ++++ = Abundantly present**,** +++ = copiously present**,** ++ = moderately present**,** + = slightly present**,** **-** = absent.

Glycosides and tannins were the most abundant (++++) phytochemicals in the plant sample. Terpenoids were copiously present (+++), alkaloids and flavonoids were observed in moderate (++) amounts, while steroids were slightly (+) present. Other phytochemicals tested but not found in the sample include: Resins, saponins, proteins, and fats (Table 1).

Acute toxicity of *C. filiformis*

The acute toxicity study of the ethanolic stem extract of *C. filiformis* revealed no sign of toxicity at 10, 100, and 1000 mg/kg doses. All the experimental mice administered the above doses were apparently normal after 24 hours post-extract administration. Similarly, the administration of 1600, 2900 and 5000 mg/kg body weight of the plant extract resulted in neither mortality nor any observable toxicity in the animals after 24 hours of extract administration. In the present study, 5000 mg/kg body weight of the extract was the highest dose that gave no mortality, this therefore implies that the lowest dose that would produce mortality must be greater than 5000 mg/kg body weight of the extract. Hence, the LD_{50} of the ethanolic extract of *C. filiformis* must be greater than 5000 mg/kg body weight.

Effect of ethanolic stem extract of *C. filiformis* on the parasitaemia of *T. Congolense* infected mice

The *T. congolense* parasite showed a prepatent period of 96 hours. Treatment commenced on Day 4 to Day 7. The negative control group (Group two), which was infected but not treated with plant extract, recorded a very high parasite load on days 5, 6, and 8 compared to other groups. A sharp decline in parasitaemia was also noted in the same group on day 7. Group three, which was treated with Diaminazine acteturate, a standard trypanocide, showed a parasite load as high as 9.4×10^6 cells per ml of blood on day 8. This value was only second to group six, which recorded 1.26×10^6 cells per ml of blood on day 8 among all the extract-treated groups. Groups 4, 5, and 7 had comparable parasitaemia reduction effects in the infected and treated groups. The effects of these three treatment groups were significantly lower than all other treatment groups, including the diminazine aceturate-treated group on day 8 (Figure 1).

Effects of the extract on the percentage survival of *T. congolense* infected mice

The survival rate of the experimental animals following challenge with *T. congolense* infection and subsequent treatment is shown in Figure 2. The uninfected-untreated (group 1) and Group 8 (uninfected but given 100 mg/kg of body weight of extract) recorded 100% survival of the experimental animals. Group 2 (infected-untreated group), as well as group 5 (Infected-treated with 60 mg/kg body weight of the extract) recorded 30 % survival at day 8 of the study. Both the group treated with standard drug (group 3) and the prophylactic group (group 7) had 70% animal survival on day 8. While groups 4 and 6 had 50% and 20% survival rates, respectively (Figure 2).

Effect of extract on haematological and biochemical parameters of *T. congolense-infected* mice

The uninfected control groups (1 and 8) showed the highest values for the packed cell volume. The PCV of group 1 was significantly higher ($p<0.05$) than other treatment groups except group 8 ($P>0.05$). Figure 3 also showed that there was no significant change ($P>0.05$) in the PCV of groups 2, 3, 4, 5, 6, and 7. There was a dose-dependent increase in packed cell volume of the extract-treated groups (Figure 3). The serum alanine aminotransferase (ALT) activities were significantly higher ($P<0.05$) in groups 3 and 4 compared to groups 1, 2, 7, and 8. The serum AST activities were significantly higher ($P<0.05$) in groups 2, 3, 4, 5, and 6 compared to groups 1, 7, and 8. However, the changes in AST activity were not different statistically ($p>0.05$) in groups 2, 3, 4, 5, and 6. The serum levels of urea and creatinine showed insignificant change in all the treatment groups (Table 2).

Effect of ethanolic extract of *C. filiformis* on kidney histology of mice infected with *T. congolense* parasite (H & E stain, x 250 magnification)

Normal glomerulus and tubules were noticed in treatment groups 1, 7, and 8. The infected-untreated control (Group 2) showed pigment deposit as well as tubular necrosis. Other histological changes noticed were slight hyperplasia

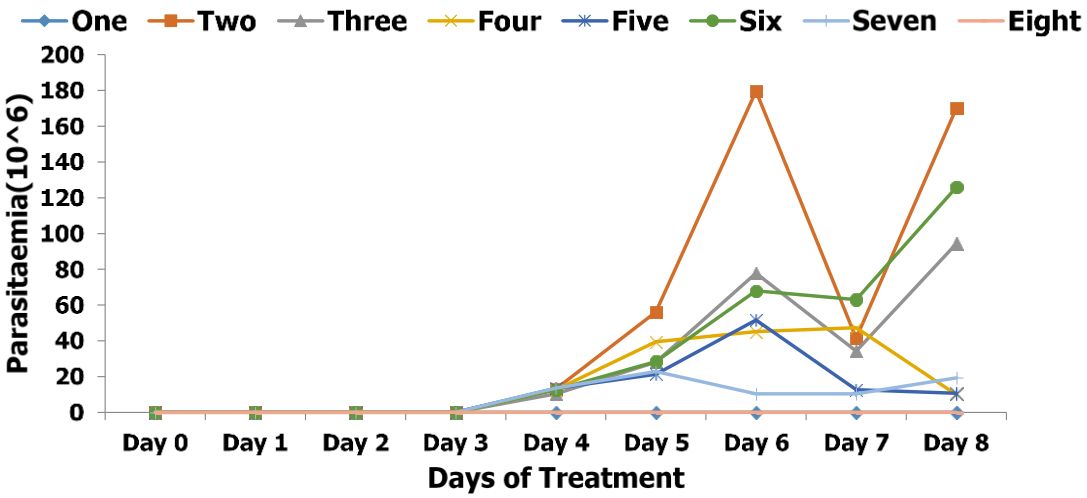

Figure 1. Effect of ethanolic stem extract of *C. filiformis* on parasitaemia of *T. congolense* infected mice day 8 post-infection (After treatment for 3 days). **Key:** GRP 1: Uninfected-untreated but given 2 ml/kg body weight of PBS, GRP 2: Infected-untreated but given 2 ml/kg body weight of PBS, GRP 3: Infected-treated with 3.5 mg/kg body weight of standard trypanocide (Diminazine aceturate), GRP 4: Infected-treated with 30 mg/kg body weight of extract daily for 4 days, GRP 5: Infected-treated with 60 mg/kg body weight of extract daily for 4 days, GRP 6: Infected-treated with 100 mg/kg body weight of extract daily for 4 days, GRP 7: Prophylactic was administered 100 mg/kg of body weight of extract 3 days before infection and treatment continued daily for 5 days after infection, GRP 8: Uninfected-treated with 100 mg/kg of body weight of extract for 4 days. The experiment was terminated on day 8 for all the groups.

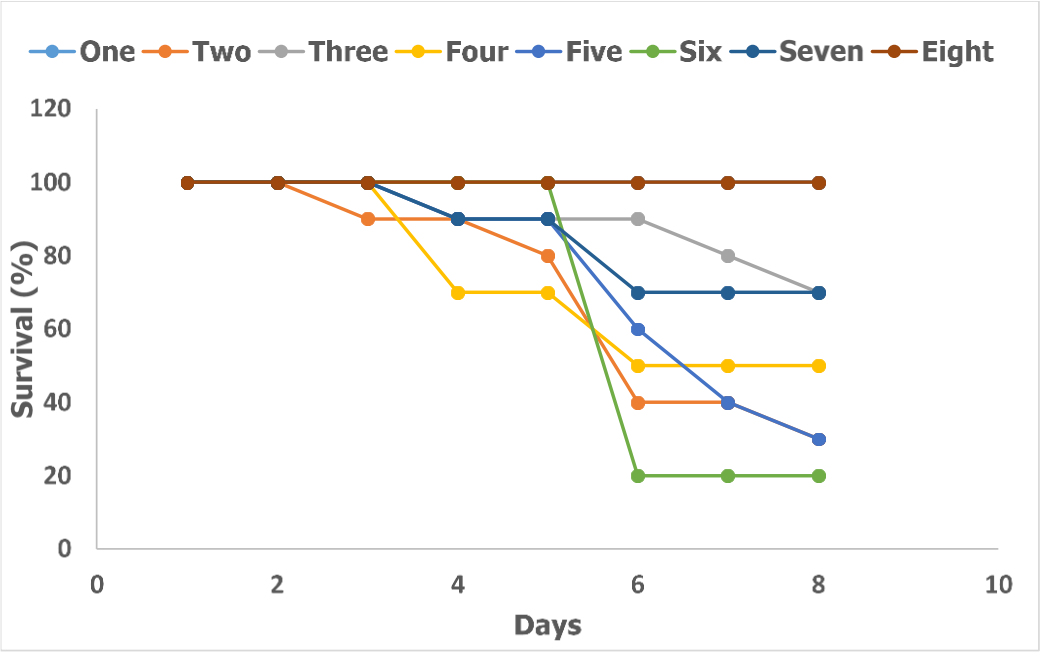

Figure 2. Effects of the extract on the percentage survival of *T. congolense* infected mice. **Key:** GRP 1: Uninfected-untreated but given 10 ml/kg body weight of PBS, GRP 2: Infected-untreated but given 2 ml/kg body weight of PBS, GRP 3: Infected-treated with 3.5 mg/kg body weight of standard trypanocide (Diminazine aceturate), GRP 4: Infected-treated with 30 mg/kg body weight of extract daily for 4 days, GRP 5: Infected-treated with 60 mg/kg body weight of extract daily for 4 days, GRP 6: Infected-treated with 100 mg/kg body weight of extract daily for 4 days, GRP 7: Prophylactic was administered 100 mg/kg of body weight of extract 3 days before infection and treatment continued daily for 5 days after infection, GRP 8: Uninfected-treated with 100 mg/kg of body weight of extract for 4 days. The experiment was terminated on day 8 for all the groups.

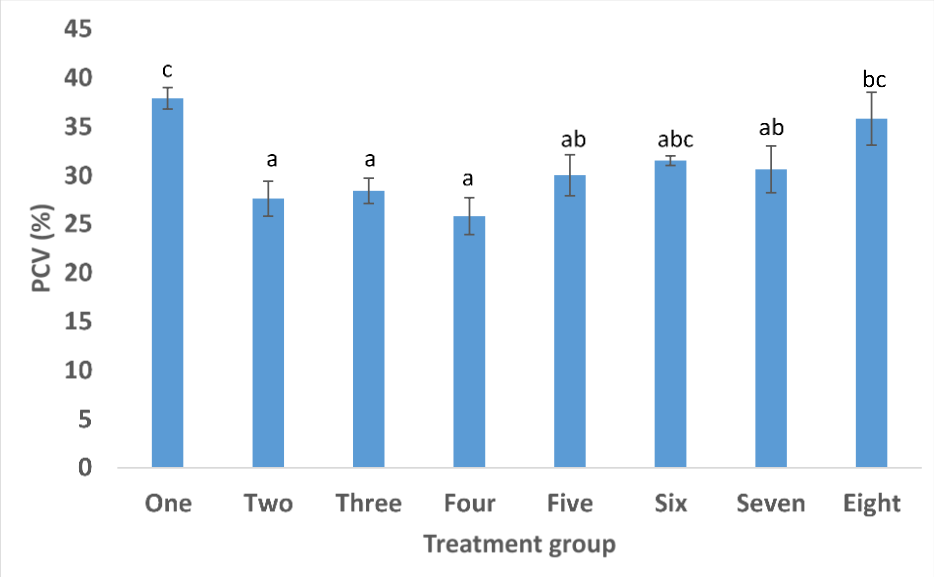

Figure 3. Effect of extract on Packed Cell Volume (PCV) of *T.congolense* infected mice Day 8 Post-infection (3 days after treatment). Values are expressed as Mean ± SEM (n=3). **Key:** GRP 1: Uninfected-untreated but given 10 ml/kg body weight of PBS, GRP 2: Infected-untreated but given 2 ml/kg body weight of PBS, GRP 3: Infected-treated with 3.5 mg/kg body weight of standard trypanocide (Diminazine aceturate), GRP 4: Infected-treated with 30 mg/kg body weight of extract daily for 4 days, GRP 5: Infected-treated with 60 mg/kg body weight of extract daily for 4 days, GRP 6: Infected-treated with 100 mg/kg body weight of extract daily for 4 days, GRP 7: Prophylactic was administered 100 mg/kg of body weight of extract 3 days before infection and treatment continued daily for 5 days after infection, GRP 8: Uninfected-treated with 100 mg/kg of body weight of extract for 4 days. The experiment was terminated on day 8 for all the groups.

Table 2. Biochemical parameters of *T. congolense* infected mice treated with ethanolic extract of *C. filiformis* Day 8 post-infection (after treatment for 5 days).

Groups	ALT (U/L)	AST (U/L)	Urea(mg/dL)	Creatinine (mg/dL)
1	97.67±11.3^{ab}	95.67±13.9^{a}	34.80±4.8^{a}	2.50±0.3^{a}
2	95.33±4.4^{a}	204.00±13.7^{bcd}	31.33±16.8^{a}	2.20±1.2^{a}
3	154.00±9.2^{c}	157.33±25.1^{abc}	38.50±3.1^{a}	1.40±0.7^{a}
4	160.00±10.6^{c}	225.67±37.3^{cd}	23.47±6.4^{a}	1.97±0.3^{a}
5	119.00±5.0^{abc}	247.50±19.5^{d}	31.30±12.4^{a}	2.05±0.4^{a}
6	140.00±8.3^{bc}	207.00±18.2^{bcd}	38.33±9.8^{a}	5.13±2.4^{a}
7	111.33±12.0^{ab}	133.00±32.4^{ab}	30.30±4.6^{a}	2.47±0.8^{a}
8	107.00±24.6^{ab}	90.00±3.0^{a}	34.03±6.6^{a}	2.87±1.2^{a}

Results are expressed as Mean ± SD triplicates values. Values with different superscripts alphabets are significantly different from each other down the column for any given parameter P<0.05 (One- way ANOVA, Duncan- HSD multiple range *post hoc* test). **Key**: GRP 1: Uninfected-untreated but given 10 ml/kg body weight of PBS, GRP 2: Infected-untreated but given 2 ml/kg body weight of PBS, GRP 3: Infected-treated with 3.5 mg/kg body weight of standard trypanocide (Diminazine aceturate), GRP 4: Infected-treated with 30 mg/kg body weight of extract daily for 4 days, GRP 5: Infected-treated with 60 mg/kg body weight of extract daily for 4 days, GRP 6: Infected-treated with 100 mg/kg body weight of extract daily for 4 days, GRP 7: Prophylactic was administered 100 mg/kg of body weight of extract 3 days before infection and treatment continued daily for 5 days after infection, GRP 8: Uninfected-treated with 100 mg/kg of body weight of extract for 4 days. The experiment was terminated on day 8 for all the groups.

of inflammatory cells, which occurred in the group that received diminazine aceturate (Group 3), and moderate hyperplasia of inflammatory cells in the group that received the least dose of *C. filiformis* extract (Group 4). The histology of Groups 5 and 6 indicated slight tubular necrosis and slight glomerular necrosis, respectively (Figure 4).

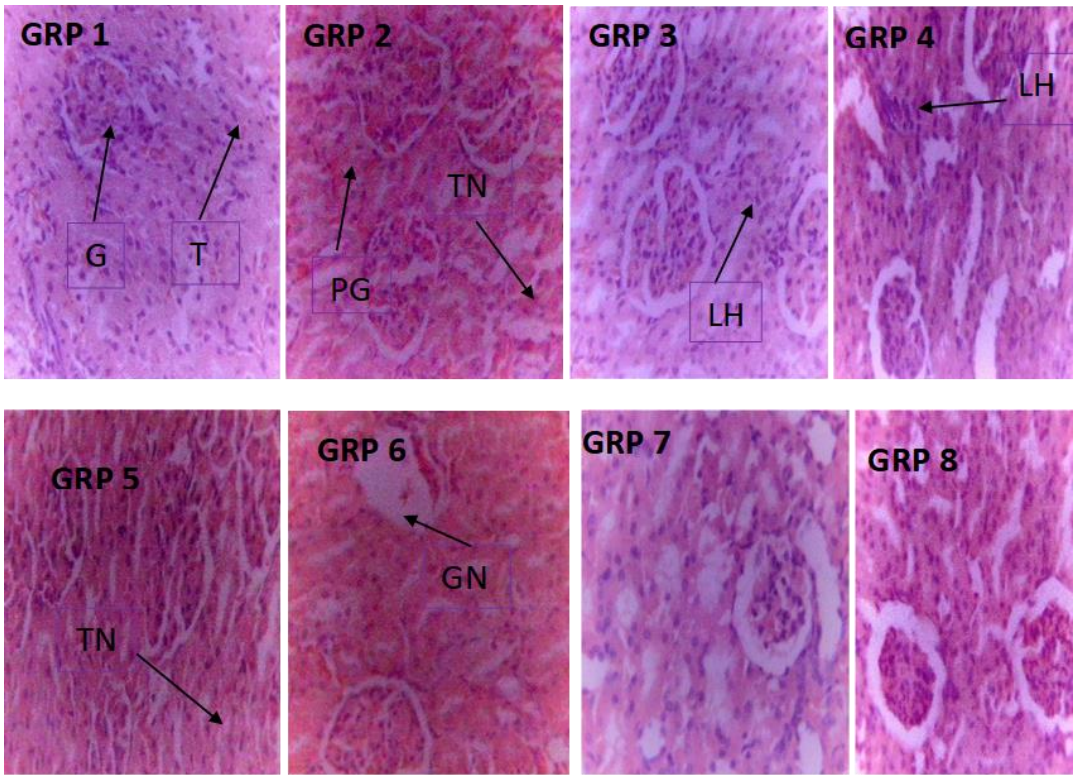

Figure 4. Histological changes in kidney tissues of *C. filiformis* treated mice infected with *T. congolense* parasite (H & E stain, x 250 magnification), day 8 post-infection (after treatment for 3 days). **Key: G:** Normal glomerulus**,** T: Normal Tubules, PG: Pigment Deposit, TN: Tubular Necrosis, LH: Hyperplasia of Inflammatory cells, GN: Glomerular Necrosis, GRP 1: Uninfected-untreated but given 10 ml/kg body weight of PBS, GRP 2: Infected-untreated but given 2 ml/kg body weight of PBS, GRP 3: Infected-treated with 3.5 mg/kg body weight of standard trypanocide (Diminazine aceturate), GRP 4: Infected-treated with 30 mg/kg body weight of extract daily for 4 days, GRP 5: Infected-treated with 60 mg/kg body weight of extract daily for 4 days, GRP 6: Infected-treated with 100 mg/kg body weight of extract daily for 4 days, GRP 7: Prophylactic was administered 100 mg/kg of body weight of extract 3 days before infection and treatment continued daily for 5 days after infection, GRP 8: Uninfected-treated with 100 mg/kg of body weight of extract for 4 days. The experiment was terminated on day 8 for all the groups.

Effect of ethanolic extract of *C. filiformis* on liver histology of mice infected with *T. congolense* parasite (H & E stain, x 250 magnification)

The liver histology of the uninfected-untreated control (Group 1), Group 7, and 8 indicated normal liver features. There was sinusoidal congestion, hyperplasia of inflammatory cells, and vascular congestion in the infected-untreated group (Group 2), while in Group 3 (diminazine aceturate-treated group) there was hepatic necrosis and hyperplasia of inflammatory cells. Vascular congestion was observed in Groups 4 and 5, whereas Group 6 presented slight hepatic necrosis and vascular congestion (Figure 5).

DISCUSSION

The objective of this study was to evaluate the *in vivo* antitrypanosomal effect of ethanolic extract of the stem part of *C. filiformis* against the field isolate of *T. congolense* in mice models of infection. Different studies and several literatures have shown that plants are used in traditional medicine (Evbuomwan *et al.,* 2018; Mbaya and Ibrahim, 2011). Due to the fact that trypanosomiasis is a neglected tropical disease, coupled with the available drugs for its treatment being very old and the existence of drug resistant strains of trypanosome parasites, it became imperative to search for natural, reliable, cheap, effective and readily available therapies from plant sources to combat the menace of trypanosomiasis in Africa.

The phytochemical screening of the ethanolic stem extract of *C. filiformis* revealed the presence of glycosides, steroids, terpenoids, alkaloids, tannins, and flavonoids. This finding is in line with the report of Mbaya *et al*. (2010), which showed that the ethanolic extract of the stem bark of *Azadirachta indica* contained glycosides, tannins, alkaloids, and saponins, which produced a remarkable

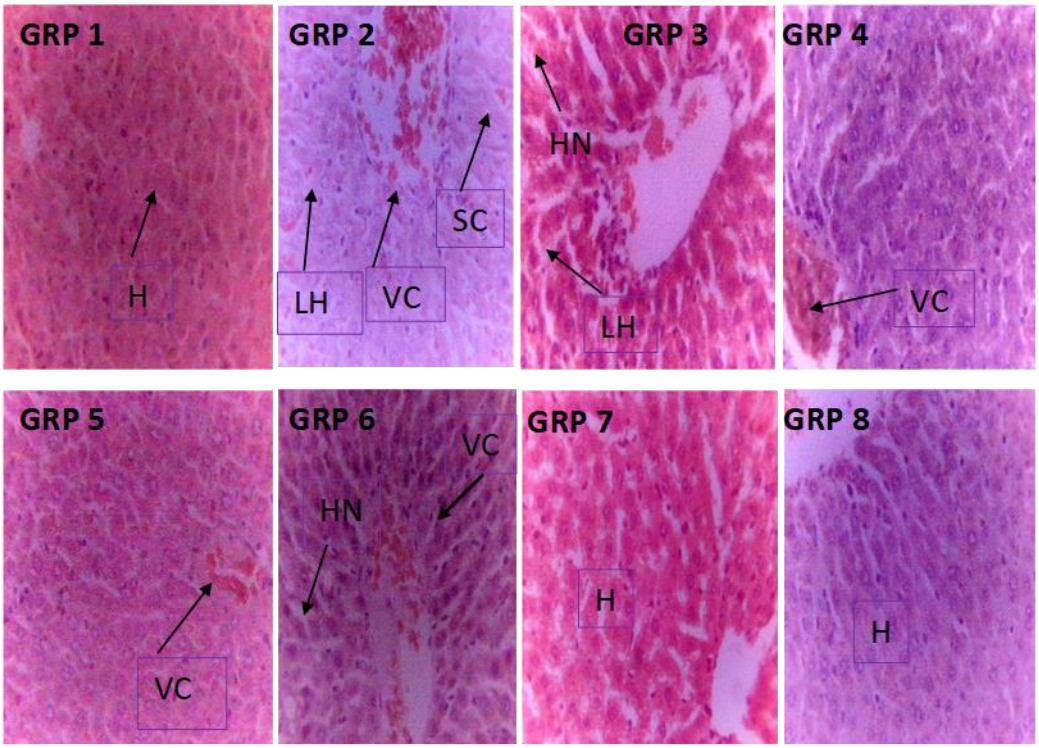

Figure 5. Histological changes in liver tissues of *C. filiformis* treated mice infected with *T. congolense* parasite (H & E stain, x 250 magnification), day 8 post-infection (after treatment for 5 days). **Key:** H: Hepatocytes, LH: Hyperplasia of inflammatory cells, VC: Vascular congestion, SC: Sinusoidal congestion, HN: Hepatic necrosis, NH: Normal hepatocytes. GRP 1: Uninfected-untreated but given 10 ml/kg body weight of PBS, GRP 2: Infected-untreated but given 2 ml/kg body weight of PBS, GRP 3: Infected-treated with 3.5 mg/kg body weight of standard trypanocide (Diminazine aceturate), GRP 4: Infected-treated with 30 mg/kg body weight of extract daily for 4 days, GRP 5: Infected-treated with 60 mg/kg body weight of extract daily for 4 days, GRP 6: Infected-treated with 100 mg/kg body weight of extract daily for 4 days, GRP 7: Prophylactic was administered 100 mg/kg of body weight of extract 3 days before infection and treatment continued daily for 5 days after infection, GRP 8: Uninfected-treated with 100 mg/kg of body weight of extract for 4 days. The experiment was terminated on day 8 for all the groups.

trypanocidal activity *in vivo* and *in vitro*. Similarly, in a bid to evaluate the phytochemical components of the fresh pulp of some trypanocidal Nigerian plants, *Aloe vera* showed heavy presence of tannins, resins, and alkaloids (Abubakar *et al*., 2005). Whereas other constituents occurred in moderate or light amounts, glycosides and tannins occurred at an abundant level, terpenoids at a copious amount, alkaloids and flavonoids at a moderate level, and steroids occurred at a low level. The presence of these phytochemicals in *C. filiformis* could be responsible for its numerous medicinal values, hence its use in traditional medicine.

The acute toxicity study revealed no mortality in the albino mice even at the highest dose of 5000 mg/kg body weight after 24 hours. A similar observation was made by Bulus *et al*. (2011), who reported that there was no acute lethal effect on rats after 24 hours of intraperitoneal administration of aqueous extract of *Terminalia avicennioides*. This high LD_{50} could be an indication of the safety of the ethanolic extract of *C. filiformis* in albino mice. The finding of the present study agrees with Lork (1983), who suggested that the LD_{50}, being greater than 5000 mg/kg b.w., is an indication of the safety of the test compound.

The prepatent period of 96 hours shown by *T. congolense* parasite in this study slightly varies from the 72 hours prepatency reported by Eze *et al*., 2024. Expectedly, the infected-untreated group showed significantly higher parasite load on days 5, 6, and 8 compared to other groups ($p<0.05$). This is in keeping with the continuous proliferation of the parasite in the group, as it was not challenged with any treatment. However, the sharp decline in parasitaemia of the infected-untreated (Group 2) could be attributed to the effort of the host immune system to combat the evading parasites (Eze *et al*., 2024). The group treated with standard trypanocide recorded a high parasite load on days 6 and 8 compared to other treated groups. This increase in parasitaemia

notwithstanding challenge with a standard drug could be an indication of the parasite's development of resistance to diminazine aceturate. Moreso, the significant reduction in the parasitaemia of Groups 4 and 5 treated with 30 and 60 mg/kg body weight of the extract, respectively, is an indication that the duo could be considered as they gave better antitrypanosomal effect compared to other doses. The prophylactic group also showed no significant change in its parasite load when compared to Groups 4 and 5.

The 100% survival of the experimental animals in the uninfected controls against the varied survival levels in the infected and treated groups is a confirmation that trypanosomiasis is a life-threatening condition with high mortality rates if not properly treated. It can also be inferred that the effectiveness of treatment goes a long way in ensuring the survival of animals infected with trypanosome parasites. The least survival rates were noted in Group 2 (infected-untreated) and Group 6 (infected and treated with 60mg/kg body weight), having 30 and 20 % survival rates, respectively. This development is in agreement with high parasitaemia noted in these groups on day 8 of the experiment. Suggesting that the higher the parasite load, the higher the mortality of the animals. However, the low survivability in group 6 remains an enigma. The 70 % survival noted in the prophylactic and the diminazine aceturate-treated groups disagrees with the findings of Eze *et al*. (2024), who reported 100 % survival in the groups given diminazine aceturate and also the prophylactic group. This, as earlier suggested, could be a result of the development of resistance to the standard drug by the parasites.

The higher PCV levels observed in the uninfected controls are expected. The study showed a dose-dependent increase in the PCV of the extract-treated animals. These changes were also comparable to those of the group treated with standard trypanocide. The hallmark of trypanosomiasis is the decline in packed cell volume of the infected animals. However, this study observed a higher PCV in the infected control group compared to the group treated with the least dose of the extract, though the change was not significant.

The elevated serum ALT activity in the extract-treated groups above that of the infected control could be an indication that the extract does not have hepatoprotective potential at the tested doses, hence the increase in serum ALT relative to the negative control. Similarly, the serum AST activity in all the treated groups except the prophylactic (Group 8) was up than that of the infected untreated group, suggesting possible damage to liver cells. Urea and creatinine levels did not show a statistical difference in all the treated groups, suggestive of impaired renal clearance of the extracts. According to Lawal *et al.* (2016), damage to liver tissue could be indicated by an increase in the serum levels of biomarker enzymes; thus, assessment of serum activity of such enzymes could give insight into the integrity of liver tissues.

The histological changes in kidney and liver tissues were further investigated to ascertain the effect of the extract on the kidney and liver tissues. The uninfected-untreated control, the prophylactic group, as well as the group that received the highest dose of the plant extract without infection, presented normal kidney histology, which suggests the safety of the *C. filiformis* extract. Other treatment groups impacted the kidney histology moderately in the worst cases. The hepatocytes of the uninfected-untreated group, the prophylactic and the uninfected but treated groups were all normal, suggesting that the plant extracts on their own had no adverse effect on the liver architecture. Expectedly, the histological changes that occurred in the infected-untreated group include sinusoidal congestion, hyperplasia of inflammatory cells, and vascular congestion. These pathological features support the high morbidity and mortality usually recorded in trypanosomiasis.

Conclusion

C. filiformis extract has significant potential as an antitrypanosomal treatment due to its efficacy in reducing parasite load, improving survival rates, and showing manageable side effects. The phytochemical profile is also highlighted as a basis for future phytopharmaceutical treatments. However, the hepatoprotective and nephroprotective effects of ethanolic extracts of *C. filiformis* should be studied in albino mice without any infection to rule out or implicate it in the mixed results obtained for the biochemical parameters.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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