

Antitrypanosomal Activity and Acute Toxicity of *Euphorbia hirta* Leaf Extract and a Green-Synthesized Vanadium-Based Nanoparticles against *Trypanosoma brucei brucei* in Mice

**Funmilayo Ibilola Paul^{1,2*}, James Ishaku Maikenti¹, Akwashiki Ombugadu¹,
Mohammed Ahmed Ashigar¹, Hussein Oshomah Ahmed¹ and Victoria Adamu Pam¹**

¹Department of Zoology, Federal University of Lafia, PMB 146, Nasarawa State, Nigeria

²Federal College of Veterinary and Medical Laboratory Technology, Vom, Plateau State, Nigeria

Abstract

African trypanosomiasis remains an important parasitic disease for which limitations associated with existing trypanocidal drugs necessitate alternative therapeutic approaches. This study evaluated the phytochemical composition, in vitro and in vivo antitrypanosomal activity, and acute toxicity of aqueous *Euphorbia hirta* leaf extract and green-synthesized vanadium nanoparticles against *Trypanosoma brucei brucei* in mice. Aqueous *E. hirta* leaf extract was subjected to phytochemical and GC–MS analyses and used for green synthesis of vanadium nanoparticles, which were characterized by FTIR and SEM. Acute toxicity was assessed using Lorke's method. In vitro and in vivo antitrypanosomal activities were evaluated against *T. brucei brucei*, with parasitaemia, body weight, PCV, and histopathological changes assessed in infected mice. Phytochemical screening detected alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, coumarins, carbohydrates, and proteins, while GC–MS identified 25 constituents. In vitro, both treatments inhibited parasite motility in a concentration- and time-dependent manner, with complete inhibition at 10 mg/mL. Acute toxicity testing indicated LD₅₀ values ≥ 3000 mg/kg for the synthesized nanoparticles and ≥ 5000 mg/kg for the leaf extract. In vivo, significant differences were observed among treatment groups for parasitaemia ($H = 18.30$, $p = 0.006$), body weight ($H = 19.19$, $p = 0.004$), and PCV ($H = 16.09$, $p = 0.012$). Histopathological examination showed reduced tissue alterations in treated mice compared with infected untreated controls. *E. hirta* extract and green-synthesized vanadium nanoparticles demonstrated preliminary parasite-suppressive activity but were less effective than diminazene aceturate. Further studies incorporating dose optimization, mechanistic investigations, pharmacokinetics, and long-term safety assessment are suggested.

Keywords: *Euphorbia hirta*; Vanadium nanoparticles; Anti-trypanosomal activity; *Trypanosoma brucei brucei*; Phytochemicals; African trypanosomiasis

Article History

Submitted

June 04, 2026

Revised

September 23, 2026

First Published Online

October 18, 2026

*Correspondences

F. I. Paul ✉

paulfunmilayo9@gmail.com

doi.org/10.62050/ljsir2026.v4n2.963

Introduction

Despite its continued threat to human life and economic development in sub-Saharan Africa, African trypanosomiasis, also known as sleeping sickness, continues to be underestimated. Besides its health risks, the condition also entails significant economic losses, especially to the rural populations engaged in agriculture. The causative agent of the disease is a *Trypanosoma* parasite (specifically the subspecies of *T. brucei*) that is transferred from one individual to another by the bite of the tsetse fly (*Glossina* spp.) [1]. The two sub-species responsible for human African trypanosomiasis are *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*. The major species of veterinary importance include *T. congolense*, *T. vivax*, and *T. b. brucei*, causing anemia, weight loss, reduced milk production, infertility, abortion, and death in livestock [2]. Treatment and control of the disease have become challenging because the existing trypanocidal drugs are limited by toxicity, cost, difficult administration, and emerging resistance. There are many medicinal plants, such as *Euphorbia hirta*, which is commonly used in

traditional African medicine to treat infections and inflammation [3].

Euphorbia hirta contains numerous bioactive phytochemicals including flavonoids, alkaloids, tannins, and terpenoids, which are capable of efficiently protecting the body against bacteria and viruses [4, 5]. Apart from phytotherapy, advances in nanotechnology make it possible to develop more efficient therapeutics due to enhanced bioavailability and better absorption of drug particles. Green-synthesized vanadium nanoparticles have attracted considerable research interest as promising antimicrobial and antiparasitic compounds with promising biological activity attributed to specific physical and chemical properties [6]. The effectiveness of both treatment approaches, however, remains unclear. There is a shortage of studies exploring whether they can be used as potential alternatives in coping with African trypanosomiasis in real-life settings. Therefore, this study investigated the efficacy and safety of *Euphorbia hirta* extract and green-synthesized vanadium nanoparticles in mice experimentally infected with *Trypanosoma brucei brucei*.

The objectives of the study include determining the phytochemical composition, in vitro and in vivo antitrypanosomal activity, and acute toxicity of aqueous *Euphorbia hirta* leaf extract and green-synthesized vanadium nanoparticles against *Trypanosoma brucei* in mice. The novelty of the work lies in the sense that it provides an alternative candidate treatment agent for trypanosomiasis although this requires further studies to understand the mechanism of action of the compounds.

Materials and Methods

Study location

The study was conducted at the Laboratory of Biochemistry and Chemotherapy, Nigerian Institute for Trypanosomiasis Research (NITR), Vom, Jos South Local Government Area, Plateau State, Nigeria (Fig. 1).

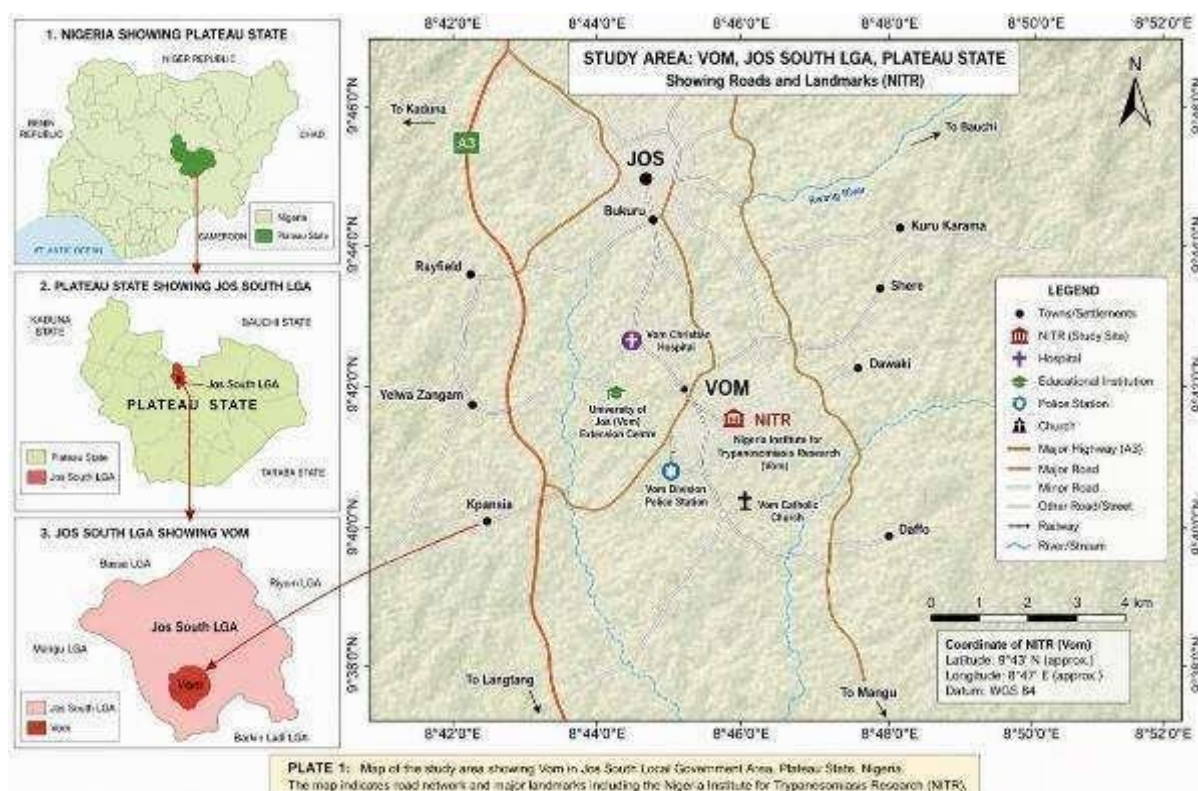


Figure 1: Nigeria Institute for Trypanosomiasis Research (NITR), Vom, Jos South LGA, Plateau State, Nigeria

Ethical approval

This study was conducted in accordance with the guidelines for the care and use of laboratory animals. Ethical approval was obtained from the Animal Care and Use Research Ethics Committee of the Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna, Nigeria, under approval number HREC/2026/002. All experimental procedures complied with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and institutional ethical requirements.

Sample size for the study

Sample size was determined using the resource equation method described by Mead [7], which is recommended for animal experiments where prior estimates of effect size are unavailable. According to this method, the error degrees of freedom ($E = N - G$) should lie between 10 and 20. In the present study, 21 mice were allocated to seven experimental groups, giving an E-value of 14 ($21 - 7 = 14$), which falls within the recommended range and indicates an adequate sample size for the study.

Collection and preparation of plant materials

Fresh leaves of *Euphorbia hirta* were collected from fresh plant materials in Jos North Local Government Area (Lat. 9.9348, Lon. 8.9065), Plateau State, Nigeria, at the beginning of the early rainfall season in May, 2025 in the morning. Samples from the plant were taken to the College of Forestry, Jos and identified by a plant taxonomist and herbarium samples were preserved with the voucher number: FHJ913. Afterwards, the remaining plant materials were transported immediately to the Biochemistry and Chemotherapy Division laboratory of the National Institute for Trypanosomiasis Research, Vom, Jos South LGA, Plateau State, Nigeria. The leaves were washed thoroughly to remove adhering soil and debris and then shade-dried at room temperature for 21 days in order to avoid fungal contamination. The dried plant materials were finely ground to a powder using a pestle and mortar and kept in airtight containers prior to extraction.

Preparation of extract

The leaves were washed, air-dried at room temperature, and ground into a fine powder. The aqueous extract was prepared using a maceration process where 500 g of the powdered leaves were macerated in distilled water for 48 h with occasional stirring. The mixture was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator at 40 °C. The percentage yield of the extract after concentrating in the rotary evaporator was 13 %.

Phytochemical screening

Qualitative phytochemical screening of the aqueous extract was done using standard methods [8-10] in order to determine the presence of alkaloids, carbohydrates, saponins, flavonoids, tannins, steroids, terpenoids, and coumarins.

GC-MS analysis

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the sample was done using a Shimadzu GC-MS QP 2010 Plus system interfaced with a mass spectrometer. The column used in the study was Elite-1 fused silica capillary (30 m × 0.25 mm; 1.0 µm df; 100 % dimethyl polysiloxane). The electron ionization (EI) energy used was 70 eV. Helium (99.99 %) was used as the carrier gas with a flow rate of 1 mL/min. The injection volume used was 2 µL, and the split ratio was 10:1. The injector temperature was set at 250 °C, while the ion source temperature was set at 280 °C. The initial oven temperature was 110 °C (isothermal for 2 min), increasing at 10 °C/min to 200 °C, further increasing at 5 °C/min to 280 °C, and finally isothermal at 280 °C for 9 min. Mass spectrum was obtained using 70 eV and a scan rate of 0.5 sec. Fragments were detected in the range of 40–550 Da. Total run time was 60 min. Relative amounts of compounds were determined using the peak area. Peak analysis was done using TurboMass-OCPTVS-Demo SPL software.

Green synthesis of vanadium nanoparticles

Nanoparticles were synthesized using the aqueous extract that was prepared by boiling 10 g of powdered plant sample in 100 mL of double-distilled water for 20 min. Then cooled and filtered using Whatman No. 1 filter paper. 0.02 M solution of ammonium metavanadate was used as the metal precursor. The extract was introduced dropwise into the precursor solution with constant stirring until the color change in the solution showed nanoparticle formation. For better stability, the reaction was continued for a very short time to allow complete reduction. The nanoparticles were precipitated by centrifuging the reaction mixture at 12,000 rpm for 15 min. The precipitate was washed three to four times using distilled water and absolute ethanol before re-centrifuging. The nanoparticles were finally dried at 50 °C using a hot-air oven and stored in airtight vials for further use [6].

Characterization of the vanadium oxide nanoparticles

Fourier transform infrared (FTIR) spectroscopy

The functional groups and metal-oxygen bonds in the synthesised vanadium oxide nanoparticles were identified by FTIR spectroscopy using a Fourier Transform Infrared spectrometer. The dried nanoparticle powder was ground to a fine, uniform powder. Before the measurement, the sample compartment and crystal were cleaned with ethanol, and a background spectrum was recorded and automatically subtracted to remove contributions from atmospheric CO₂ and water vapour. A small amount of the powder was placed on the ATR crystal and pressed down firmly to make good contact. The spectrum was recorded in transmittance mode over the range 4000 to 650 cm⁻¹ at a resolution of 4 cm⁻¹, averaging 16 or 32 scans. After the measurement, baseline correction was applied, the peak positions were labelled, and the bands were assigned to functional groups and V-O bonds by comparison with values in the literature.

Scanning electron microscopy (SEM)

The surface morphology of the vanadium oxide nanoparticles was examined using a Zeiss scanning electron microscope. A small amount of the dried powder was fixed on an aluminium stub with double-sided carbon adhesive tape, and loose particles were removed with a gentle stream of air so that they would not contaminate the chamber. The stub was loaded into the chamber, which was evacuated before imaging. Micrographs were acquired at an accelerating voltage (EHT) of 15 kV and a working distance of 7.7 mm, using a high-definition backscattered electron (HDBSD) detector, at several magnifications. The images were used to describe the shape, surface texture, agglomeration and porosity of the particles.

Procurement of laboratory mice

Three-week-old (juvenile) albino mice (*Mus musculus*), both sexes, of approximate weight between 14 and 24 g, were supplied from the animal house, NITR. The animals were housed in clean cages with good ventilation and sawdust as bedding material at the Animal House, Vector and Parasitology Division, NITR, Vom, Plateau State. They were offered commercial feed and tap water ad libitum. The animals were allowed for a week to acclimatize at a room temperature of 22 – 26 °C, relative humidity between 30 – 60 % and a photoperiod of 12:12 light/dark cycle.

Inclusion and exclusion criteria

Apparently healthy albino mice of both sexes, approximately three weeks old and weighing between 14 and 24 g, were included in the study. Only animals that were free from visible signs of injury or disease at the commencement of the experiment and that successfully established *Trypanosoma brucei brucei* parasitaemia following inoculation were enrolled. Animals showing signs of illness before inoculation, those that failed to establish parasitaemia after infection, those that



sustained accidental injuries unrelated to the experimental procedures, and any animals that died before the commencement of treatment were excluded from the study.

Test organism and parasite inoculation

Trypanosoma brucei brucei (Federe strain) was obtained from the Parasitology and Molecular Diagnostics Division, Nigeria Institute for Trypanosomiasis Research (NITR), Vom, Plateau State. The organism was serially passage into a donor mice that about 35–40 parasites could be seen per microscopic field after six days of infection [11]. According to the rapid matching method for estimating host parasitemia by Herbert and Lumsden [12], parasite inoculation was done when parasite density was 2 – 3 parasites in 5 fields when viewed under the microscope at x40 objective lens, which is equivalent to 0.5 parasites per field or 1.995×10^6 organisms per mL of infected blood. 0.1 mL of the infected blood was then inoculated into each mice intraperitoneally. Parasitaemia was then monitored daily for 4 days pre-patent period. After confirming the onset of parasitaemia, treatment was initiated on the fifth day post-infection.

Determination of parasitaemia

Parasitaemia was determined daily using blood samples obtained from tail snips. Blood was smeared on a clean slide and viewed using a x40 objective lens. Counts were done using the rapid matching technique of Herbert and Lumsden [12] using either undiluted blood or blood diluted with normal saline (NS). The logarithmic value of the parasite count was compared to a reference table, and its antilogarithm was taken as the parasite count per milliliter of blood.

Acute toxicity study (LD₅₀)

The acute toxicity of the aqueous extract of *Euphorbia hirta* and the green-synthesized vanadium nanoparticles was evaluated using the method described by Lorke [13]. In the first phase, nine mice were randomly assigned to three groups of three animals each and administered intraperitoneally with 10, 100, and 1000 mg/kg of the test materials, respectively. The animals were observed continuously during the first 24 h and thereafter monitored daily for 14 days for signs of toxicity and mortality. In the second phase, three additional mice were used for each test material (*Euphorbia hirta* extract and vanadium nanoparticles). Each mouse received a single intraperitoneal dose of 1600, 2900, or 5000 mg/kg, respectively, and was monitored for clinical signs of toxicity and mortality and were used to determine the median lethal dose (LD₅₀). Gross necropsy was performed on any animal that died during the study, while surviving animals were examined at the end of the observation period.

In vitro antitrypanosomal activity

The in vitro antitrypanosomal activity assay was done using blood from a highly infected mouse (about 100 parasites per field). Approximately 0.1 of blood was

drawn from the tail vein of an infected mouse and collected in EDTA-containing tubes to prevent clotting. The infected blood was diluted with dextrose-normal saline to produce a suspension of trypanosomes. This was further diluted to produce workable concentrations of approximately 2–3 parasites per field. The reaction mixture was produced by mixing the diluted blood with Ringer's solution, which was put into five test tubes each having different concentrations of the plant extract of 10, 1, 0.5, 0.05, and 0.005 mg/mL. All these mixtures were kept at around 37 °C. Microscopic observations were made immediately (time zero) and at 15 min intervals up to 120 min. Small amounts were drawn from the reaction mixture and put onto a slide and examined using a x40 objective lens. A decrease in motility was considered to be an indication of parasite death due to the plant extract.

In vivo antitrypanosomal experiment

Twenty-one healthy mice were acclimatized for one week prior to experimental infection with *Trypanosoma* spp. through intraperitoneal inoculation. Following confirmation of parasitaemia, the animals were randomly assigned into seven experimental groups (n = 3 per group) using a simple randomization procedure. Each mouse was assigned a unique identification number, and treatment allocation was performed by random selection to minimize selection bias. The experimental groups comprised a non-infected untreated group (negative control), an infected untreated group (positive control), an infected group treated with diminazene aceturate (standard drug control), two infected groups treated orally with the plant extract at doses of 250 and 500 mg/kg body weight, and two infected groups treated intraperitoneally with vanadium nanoparticles at doses of 100 and 200 mg/kg body weight.

The plant extract and vanadium nanoparticles were administered at their respective doses, while diminazene aceturate was administered intramuscularly at 3.5 mg/kg body weight as the reference drug. Parasitaemia was monitored daily using the rapid matching method of Herbert and Lumsden [12], whereas packed cell volume (PCV) and body weight were measured weekly throughout the experimental period.

The use of three animals per group was based on the exploratory nature of the study and the principle of Reduction within the 3Rs (Replacement, Reduction and Refinement) framework for the ethical use of laboratory animals, which recommends the minimum number of animals necessary to achieve the study objectives while minimizing unnecessary animal use.

Histopathological studies

Liver and kidney tissues of the mice were harvested after being killed at the end of the experiments. The tissues were then prepared and stained using histopathological techniques with H and E staining according to Carleton [14].

Animal welfare monitoring

Animals were observed at least twice daily throughout the study for clinical signs of distress, including reduced

activity, abnormal posture, ruffled fur, weight loss, dehydration, and impaired feeding. Body weight was monitored weekly, while food and water intake were observed daily. Animals exhibiting severe distress or reaching predefined humane endpoints were humanely euthanized to minimize suffering.

Statistics

The obtained results were managed in Microsoft Excel and thereafter imported into R statistical software version 4.6.0 for statistical analysis. The results were presented using mean $\pm$ standard deviation (SD). Since the data did not satisfy the conditions for a parametric test, Kruskal-Wallis test was performed to ascertain if there was a significant difference in the mean parasitaemia levels as well as weight and PCV level of the treated mice across the different treatment groups. Where a significant difference was obtained, Dunn's multiple-comparison post-hoc test with Holm adjustment was performed to identify the specific treatment groups that differed significantly. Statistical significance was set at $p < 0.05$.

Results and Discussion

Phytochemical composition of *Euphorbia hirta* extract

Qualitative phytochemical composition of the crude aqueous leaf extract of *Euphorbia hirta* indicated the presence of nine (9) bioactive compounds, which include, alkaloids, flavonoids, tannins, saponins, and terpenoids, as shown in Table 1.

Table 1: Qualitative phytochemical constituents of *Euphorbia hirta* leaf extract

Phytocompound	Aqueous Extract
Alkaloids	+
Carbohydrates	+
Saponins	+
Steroids	+
Flavonoids	+
Coumarins	+
Tannins	+
Proteins	+
Terpenoids	+

+ = Present

GC–MS analysis of *Euphorbia hirta* extract

Gas chromatography mass spectrometry (GC/MS) analysis (Fig. 2) identified several bioactive constituents in the extract (Table 2).

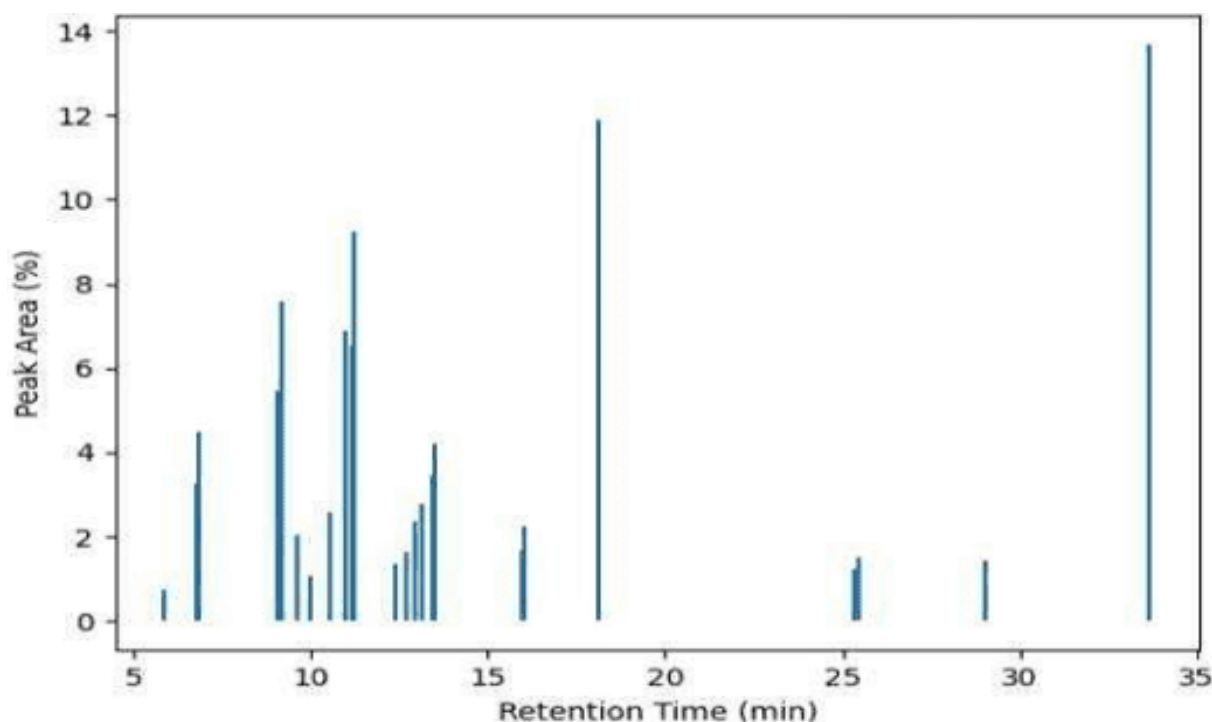


Figure 2: GC/MS chromatogram of whole *Euphorbia hirta* crude extract

**Table 2: Major compounds identified in *Euphorbia hirta* extract by GC–MS**

Peak	RT (min)	Area (%)	Compound	Ref	CAS	Similarity Index
1	6.772	3.27	2-Tetradecene, (E)-	61855	035953-54-9	95
2	6.854	4.46	Hexadecane	89843	000544-76-3	94
3	9.083	5.46	3-Eicosene, (E)-	140277	074685-33-9	94
4	9.155	7.56	Octadecane	115547	000593-45-3	97
5	10.540	2.58	Hexadecanoic acid, methyl ester	130822	000112-39-0	93
6	11.182	6.52	1-Docosene	167463	001599-67-3	93
7	11.241	9.25	Nonadecane	128834	000629-92-5	91
8	12.677	1.64	Methyl stearate	157883	000112-61-8	90
9	13.412	3.47	Dichloroacetic acid, heptadecyl ester	217449	1000282-98-2	93
10	13.476	4.19	Hexacosane	217890	000630-01-3	91
11	15.965	1.67	17-Pentatriacontene	265112	006971-40-0	90
12	18.116	11.91	Bis(2-ethylhexyl) phthalate	233372	000117-81-7	91

Characterization of synthesized vanadium nanoparticle

The functional groups on the synthesised vanadium oxide nanoparticles were identified using FTIR spectroscopy. The spectrum (Fig. 3) shows several absorption bands that correspond to particular functional groups and chemical bonds. The peaks were labelled at 3153.3, 3026.6, 2340.8, 1560.4, 1401.5, 1066.0, 961.7 and 805.1 cm^{-1} . The weak, broad bands at 3153.3 cm^{-1} and 3026.6 cm^{-1} fall in the region assigned to O-H stretching vibrations. They indicate surface-bound hydroxyl groups and adsorbed water on the nanoparticles, possibly together with phenolic O-H from the organic extract used in the synthesis. In vanadium oxides, these bands are also commonly linked to V-OH groups and water molecules held in the structure. This feature is important because surface hydroxyl groups affect the reactivity of the nanoparticles and their interaction with the surrounding environment. The small band at 2340.8 cm^{-1} is attributed to atmospheric CO_2 ($\text{O}=\text{C}=\text{O}$ asymmetric stretching). It is a common background artefact in FTIR measurements and is not part of the nanoparticle structure.

The band at 1560.4 cm^{-1} corresponds to the asymmetric stretching of carboxylate (COO^-) groups. It may also arise from $\text{C}=\text{C}$ stretching or from H-O-H bending of adsorbed water. The band at 1401.5 cm^{-1} is assigned to the symmetric stretching of COO^- groups or to C-H bending. Together, these bands suggest that biomolecules such as phenolics, proteins or organic acids are bound to the nanoparticle surface as capping and stabilising agents. The band at 1066.0 cm^{-1} is attributed to C-O stretching vibrations of alcohols, ethers or carboxylic groups, which again points to organic residues from the synthesis medium. It may also overlap with V-O-H bending modes.

The bands in the lower wavenumber region are the most important for identifying the oxide phase. The band at 961.7 cm^{-1} is characteristic of the V=O stretching

vibration of vanadyl groups. This is the signature band of vanadium oxides, and it is usually reported between about 950 and 1020 cm^{-1} for V_2O_5 . The strong band at 805.1 cm^{-1} , together with the rising absorption towards lower wavenumbers, corresponds to the asymmetric stretching of V-O-V bridges. These are generally observed between about 500 and 850 cm^{-1} and confirm the formation of the vanadium-oxygen framework.

The surface morphology of the synthesised vanadium oxide nanoparticles is shown in Fig. 4. The image was acquired at an accelerating voltage of 15 kV and a working distance of 7.7 mm, using a high-definition backscattered electron detector (HDBSD). The micrograph shows that the material is made up of irregular, densely packed aggregates with no uniform shape. The aggregates have rough, porous surfaces and a granular, flaky texture, and many of them appear as lumpy clusters joined together into larger agglomerates. This kind of morphology is typical of metal oxide nanoparticles prepared by chemical or green synthesis routes. It arises because the high surface energy of the small primary particles drives them to stick together, and organic capping molecules on the surface can further bind them into clusters. This agrees with the hydroxyl, carboxylate and C-O bands seen in the FTIR spectrum, which show that organic residues remain on the particle surface. The rough, open structure with visible voids and crevices between the aggregates indicates a porous surface. A structure like this offers a large surface area, which is useful for applications such as catalysis, sensing, adsorption and energy storage. The brightness variation across the image is due to the backscattered electron signal. Brighter regions are edges, raised features and areas of higher average atomic number, while darker regions are recessed areas and gaps between particles. This contrast confirms that the sample is heterogeneous and has a topographically complex surface.

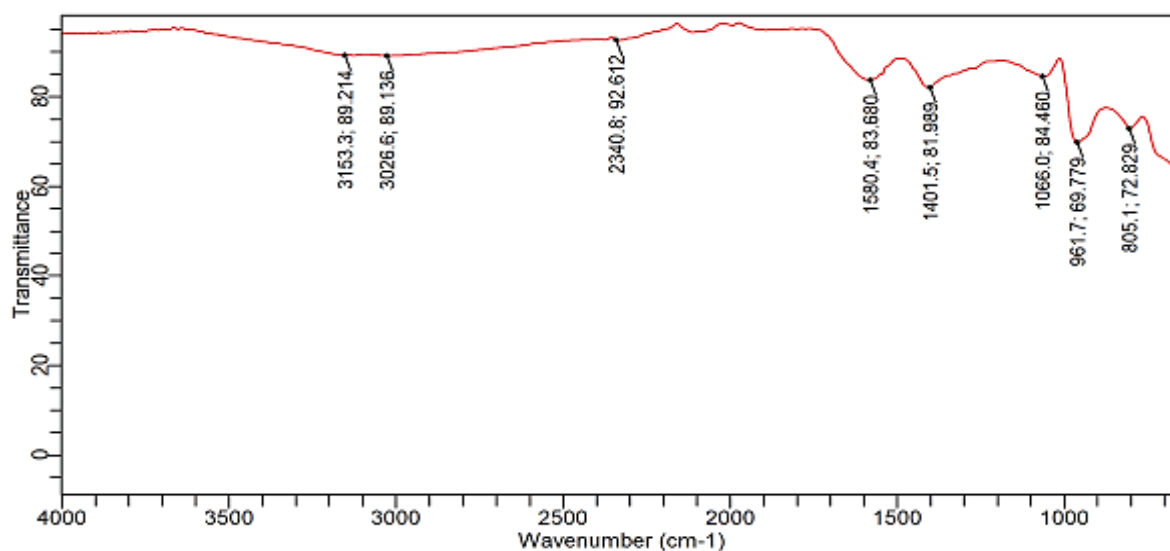


Figure 3: FTIR analysis of vanadium nanoparticles

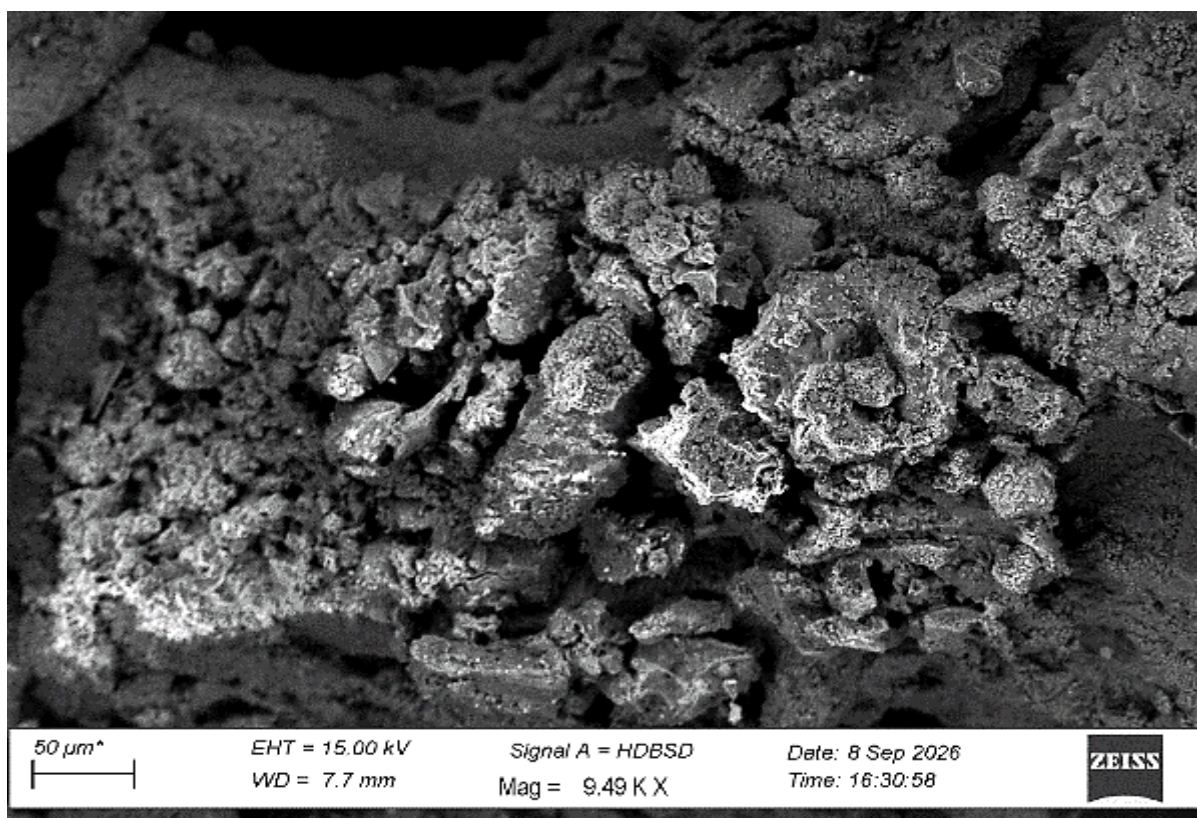


Figure 4: SEM Image of the synthesized vanadium nanoparticles

Table 3: Acute oral toxicity of aqueous extract of *Euphorbia hirta* and vanadium nanoparticles in mice (Lorke's Method)

Phase	No. of mice	Dose (mg/kg)	Mortality for <i>E. hirta</i>	Mortality for vanadium nanoparticles
1	3	10	Zero	Zero
1	3	100	Zero	Zero
1	3	1000	Zero	Zero
2	1	1600	Zero	Zero
2	1	2900	Zero	Zero
2	1	5000	Zero	one
Control	1	Distilled water	Zero	Zero



Acute toxicity (LD₅₀) of extract and nanoparticles

Acute toxicity studies revealed that the crude aqueous extract of *Euphorbia hirta* and green vanadium nanoparticle had an LD₅₀ $\geq$ 5000 mg/kg and $\geq$ 3000 mg/kg respectively indicating relatively low toxicity (Table 3).

In vitro antitrypanosomal activity

Both *Euphorbia hirta* extract and vanadium nanoparticles demonstrated dose-dependent inhibition of parasite motility. Complete cessation of motility was observed at 10 mg/mL for both treatments, with nanoparticles exhibiting faster activity at lower concentrations, as shown in Table 4.

Table 4: Motility assessment of *Trypanosoma brucei* against Aqueous *Euphorbia hirta* Leaf Extract and Vanadium Nanoparticles

Vanadium Nanoparticles											
Treatment	Concentrations (mg/ml)	Motility Rate (minutes)									
		0	15	30	45	60	75	90	105	120	
<i>Euphorbia hirta</i>	10	+	-	-	-	-	-	-	-	-	
	1	+	+	+	-	-	-	-	-	-	
	0.5	+	+	+	+	+	+	+	+	+	
	0.05	+	+	+	+	+	+	+	+	+	
	0.005	+	+	+	+	+	+	+	+	+	
Nanoparticles	10	+	-	-	-	-	-	-	-	-	
	1	+	+	-	-	-	-	-	-	-	
	0.5	+	+	+	+	+	+	+	-	-	
	0.05	+	+	+	+	+	+	+	+	+	
	0.005	+	+	+	+	+	+	+	+	+	
Negative Control		+	+	+	+	+	+	+	+	+	

+ = Active Motility; - = No Motility

In vivo antitrypanosomal activity

Overall, the result indicated that there was a significant difference ($p < 0.05$) in parasitaemia level across the different treatment groups (Table 5). The standard drug (Diminazene aceturate) completely eliminated the parasites in the course of the study (0.00 ± 0.00). Although, both *Euphorbia hirta* extract and vanadium nanoparticles caused a decline in parasitaemia level (3.20 ± 0.30 and 2.80 ± 0.25 respectively) compared to the positive control (8.50 ± 0.45), the observed effect was not statistically significant ($p > 0.05$) based on the results of the post hoc analysis performed.

Table 5: Effect of treatments on parasitemia levels in infected mice

Group	Treatment	Parasitemia Level (Mean $\pm$ SD)
Group I	Positive control	8.50 ± 0.45^b
Group II	<i>E. hirta</i> extract	3.20 ± 0.30^{ab}
Group III	Vanadium nanoparticles	2.80 ± 0.25^{ab}
Group IV	Diminazene aceturate	0.00 ± 0.00^a

Values are mean $\pm$ standard deviations (SD) of the mean; Values in the same column followed by different superscript letter are significantly different at $p < 0.05$ based on Dunn's post hoc test with Holm adjustment

Kruskal-Wallis: $H = 10.1158$, $df = 3$, p -value = 0.02

In vivo antitrypanosomal effect of treatment on body weight and packed cell volume (PCV)

Table 6 showed significant differences among treatment groups for parasitaemia ($H = 18.30$, $p = 0.006$), body weight ($H = 19.19$, $p = 0.004$) and PCV ($H = 16.09$, $p = 0.012$). The least parasitaemia was recorded in the standard drug group (0.31 ± 0.16), which differed only from that recorded in the nanoparticle group at 200 mg/kg (3.25 ± 0.15). Body weight was highest in the standard drug group (18.41 ± 0.27 g) and differed from the NP group at both concentrations administered: 200 mg/kg group (14.22 ± 0.26 g) and 100 mg/kg (14.88 ± 0.32). PCV was highest in the standard drug (58.33 ± 0.72 %) and Ext 250 mg/kg (58.50 ± 0.63 %) groups, although the post-hoc test indicates no significant pairwise differences in PCV among the groups.

Table 6: Changes in body weight and PCV of experimental mice

Group	Parasitemia (LEV)	Weight (g)	PCV (%)
NP 100 mg/kg	2.71 ± 0.17^{ab}	14.88 ± 0.32^b	56.17 ± 0.65^b
NP 200 mg/kg	3.25 ± 0.15^b	14.22 ± 0.26^b	55.78 ± 0.71^b
Ext 250 mg/kg	2.45 ± 0.20^{ab}	15.47 ± 0.24^{bc}	58.5 ± 0.63^b
Ext 500 mg/kg	2.94 ± 0.16^{ab}	16.2 ± 0.30^{abc}	55.83 ± 0.62^b
Standard Drug	0.31 ± 0.16^a	18.41 ± 0.27^c	58.33 ± 0.72^b
Positive Control	2.85 ± 0.21^{ab}	16.12 ± 0.31^{bc}	54.67 ± 0.71^b
Negative Control	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
K-Wallis (p-value)		18.30(0.006)	19.19(0.004)
		16.09(0.012)	

Values are mean $\pm$ standard deviations (SD) of the mean; Values in the same column followed by different superscript letters are significantly different at $p < 0.05$ based on Dunn's post hoc test with Holm adjustment

Table 7: Survival rate of mice following treatment

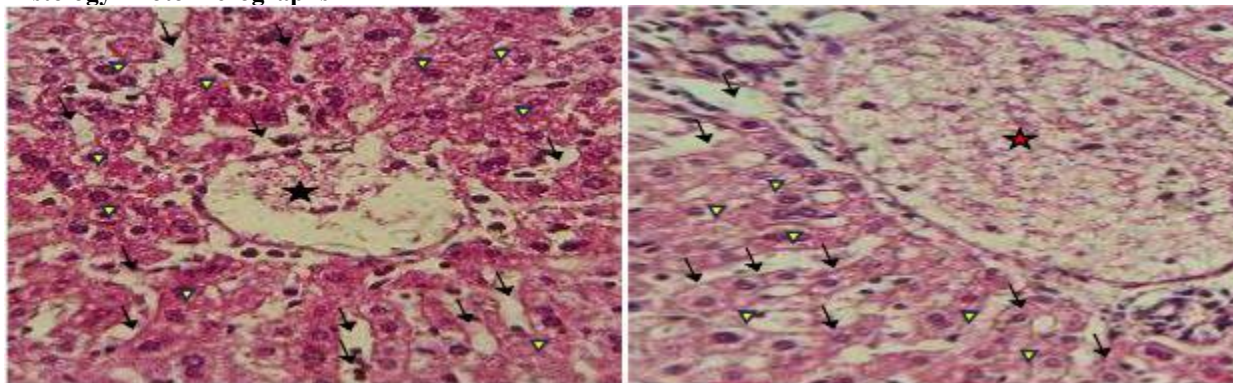
Group	Treatment	Survival Rate (%)
Control	Untreated	20
Extract	<i>E. hirta</i>	70
Nanoparticles	Vanadium NP	80
Standard drug	Diminazene	100

Survival rate of experimental animals

The survival rate of treated mice was significantly higher compared to untreated controls (Table 7). The standard drug group recorded 100 % survival.

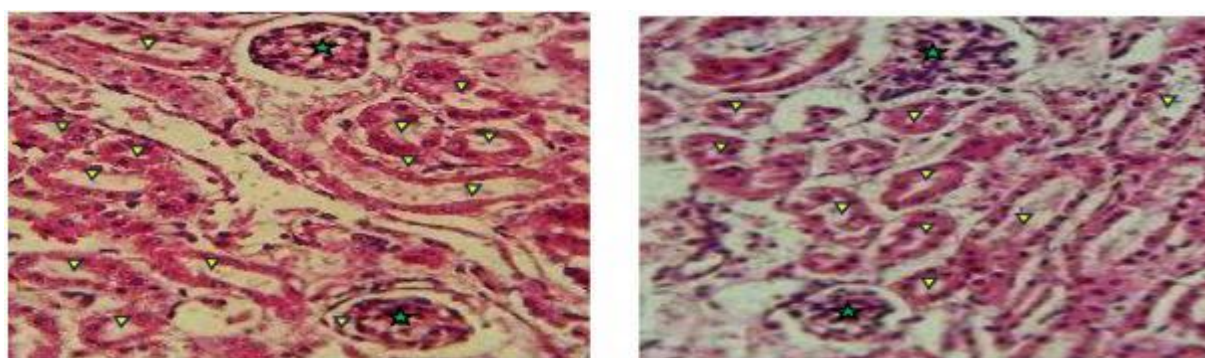
Histopathological Output

Histopathological examination revealed severe tissue damage (necrosis and congestion) in untreated infected mice, while treated groups showed reduced pathological alterations and signs of tissue recovery, as shown in Plates (1 – 14).

Histology Photomicrographs


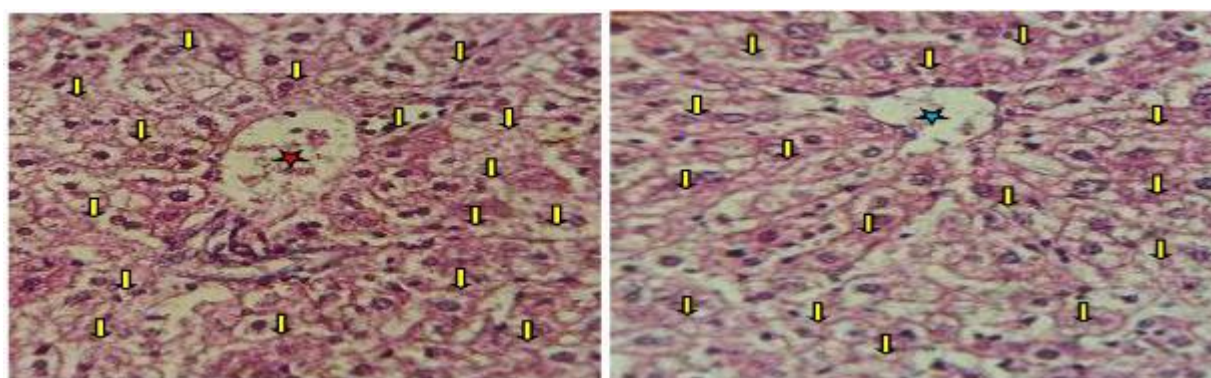
Positive Control 1 (Uninfected Mice)

Plate 1: Liver: The Central vein (Black Star) and the Portal Vein (Red Star) are clear of congestion; sinusoids (Black Arrow); hepatocytes (Yellow arrowheads). No pathology observed. Hematoxylin and eosin stain. Magnification x400



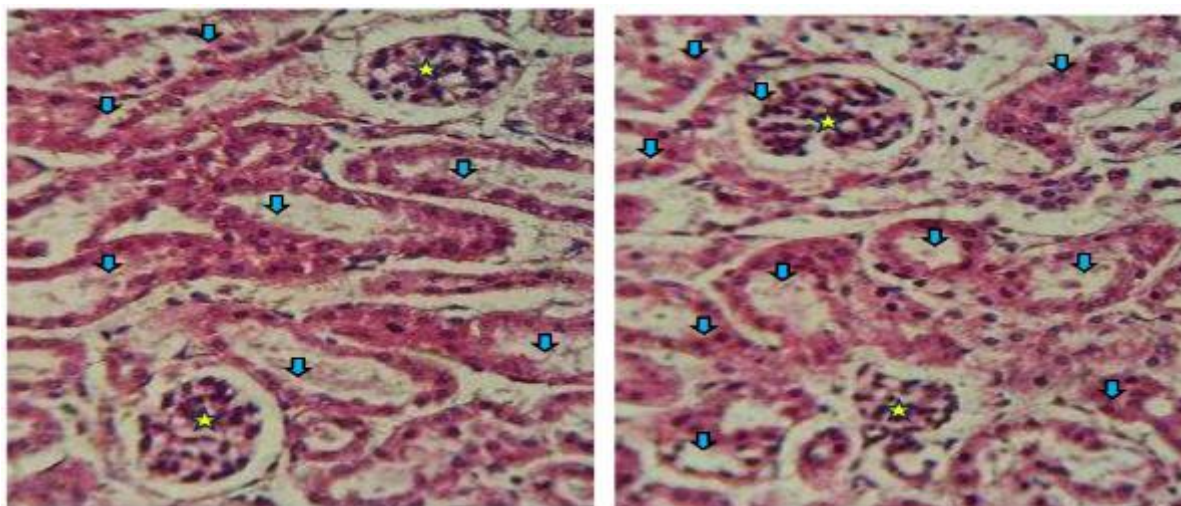
Positive Control 1 (Uninfected Mice)

Plate 2: Kidney: renal corpuscles (green stars) and renal tubules (yellow arrowheads). No pathology observed. Hematoxylin and eosin stain. Magnification x400



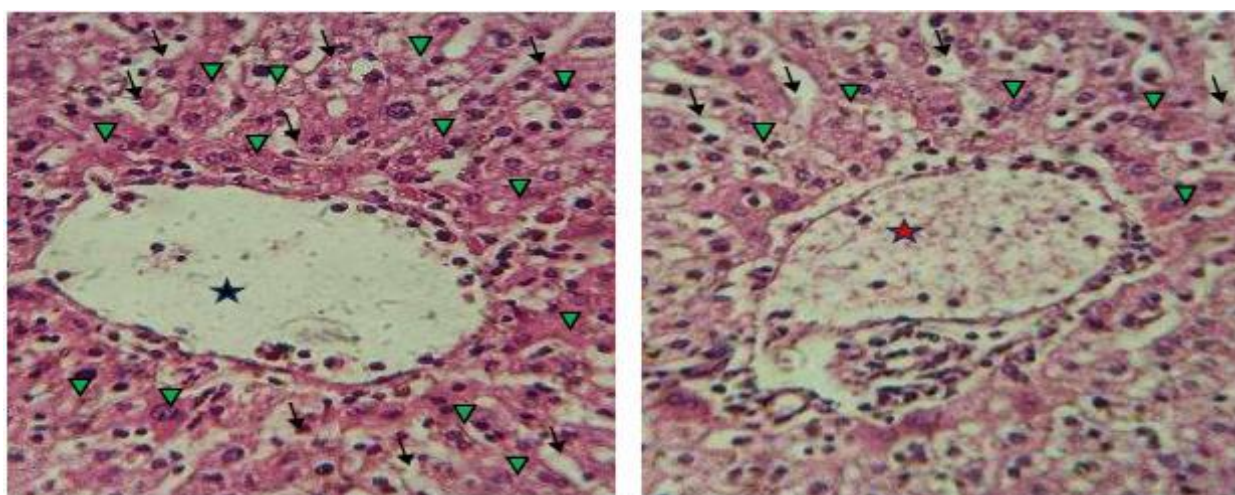
Negative Control (infected and not treated Mice)

Plate 3: Liver: necrosis of the hepatocytes (yellow arrows) is observed. The portal vein (red star) and central vein (blue star) are free of congestion. Hematoxylin and eosin stain. Magnification x400



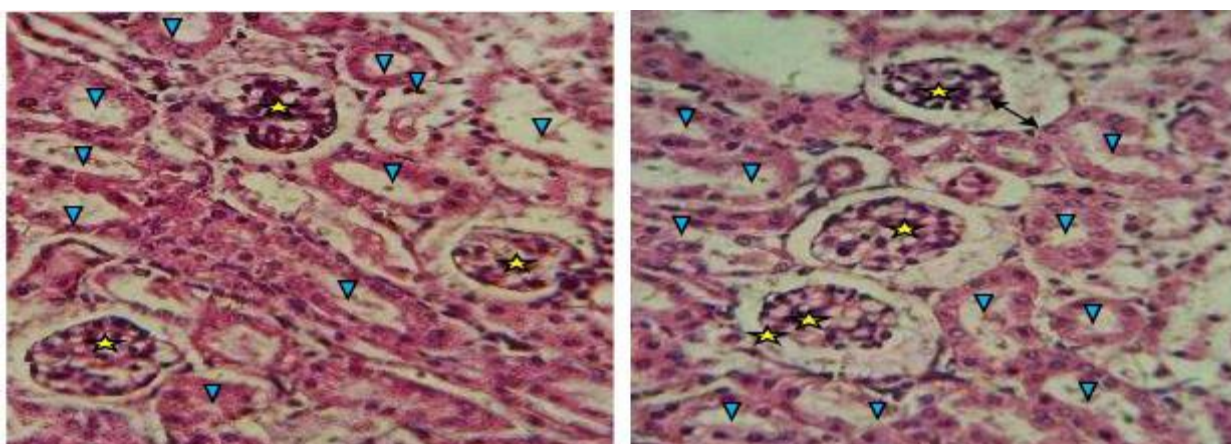
Negative Control (infected and not treated Mice)

Plate 4: Kidney: necrosis of the renal corpuscles (yellow stars) and renal tubules (blue arrows). Hematoxylin and eosin stain. Magnification x400



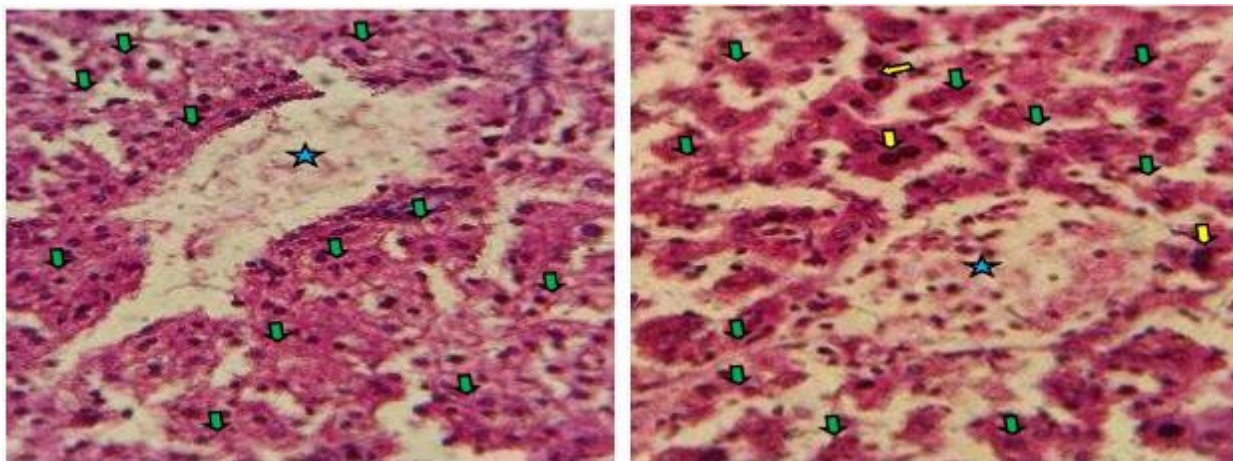
Positive Control 2 (infected mice and treated with standard drug). Diminazene aceturate

Plate 5: Liver: The central vein (black star) and the portal vein (red star) are clear of congestion; sinusoids (black arrows); necrosis of the hepatocyte (green arrowheads) is observed. Hematoxylin and eosin stain. Magnification x400



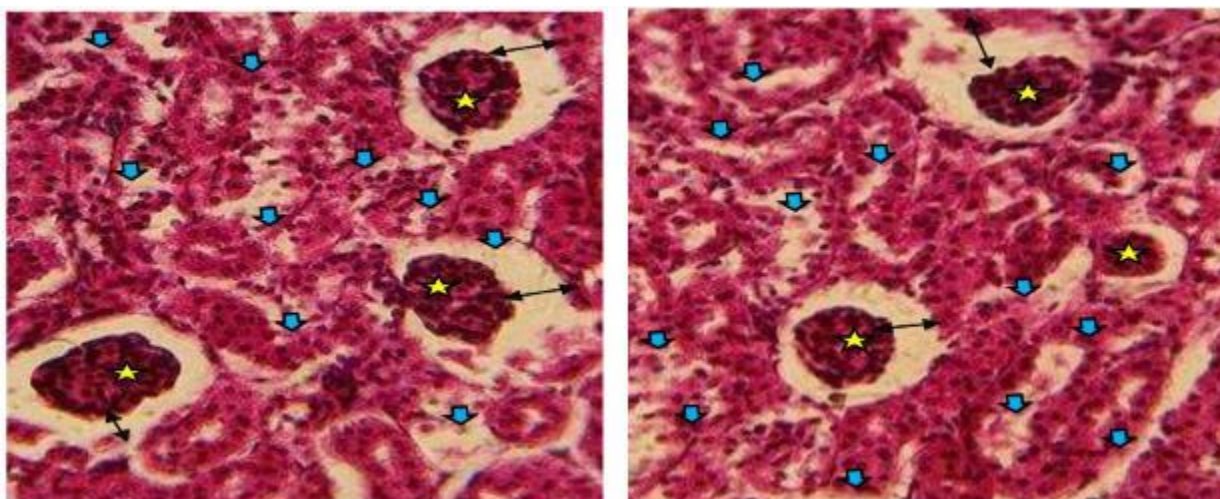
Positive Control 2 (infected mice and treated with standard drug). Diminazene aceturate

Plate 6: Kidney: renal corpuscles (yellow stars) and renal tubules (blue arrows). No pathology observed. Hematoxylin and eosin stain. Magnification x400



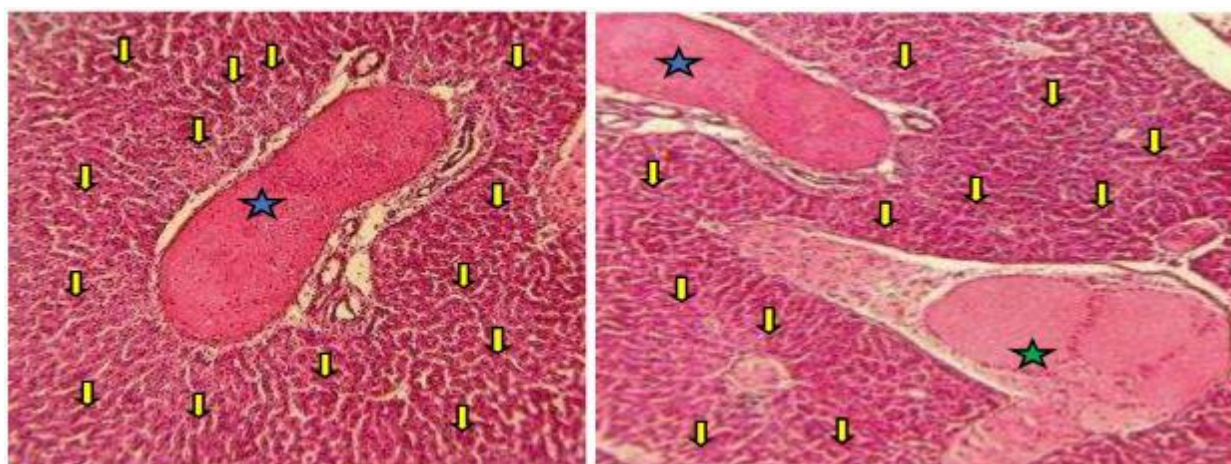
Mice Treated with 100 mg/kg Nanoparticles

Plate 7: Liver: necrosis of the hepatocytes (green arrows); the central veins (blue stars) are clear of congestion. Replicating hepatocytes (yellow arrows) signifying the healing process. Hematoxylin and eosin stain. Magnification x400



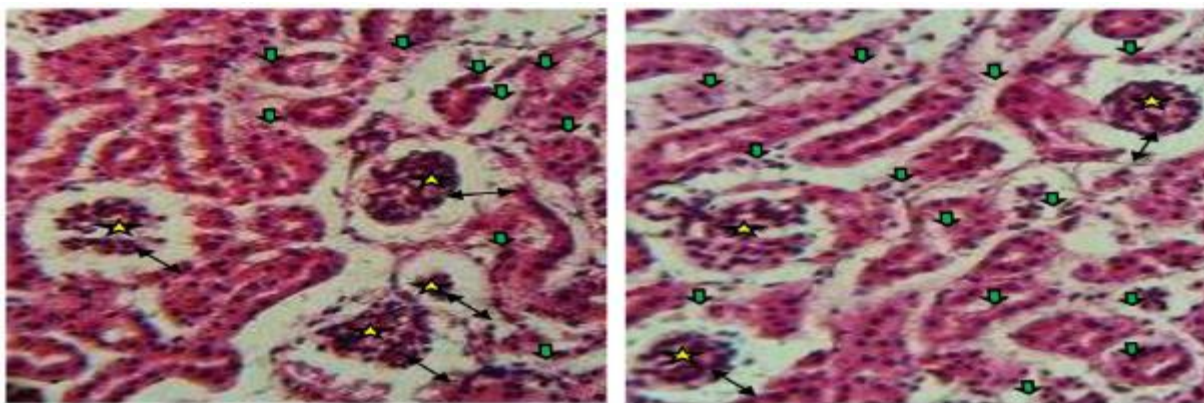
Mice Treated with 100 mg/kg Nanoparticles

Plate 8: Kidney: necrosis and atrophy of the renal corpuscles (yellow stars) and tubules (blue arrows); widened Bowman's space (line arrow: double) due to the atrophied renal corpuscles. Magnification x400



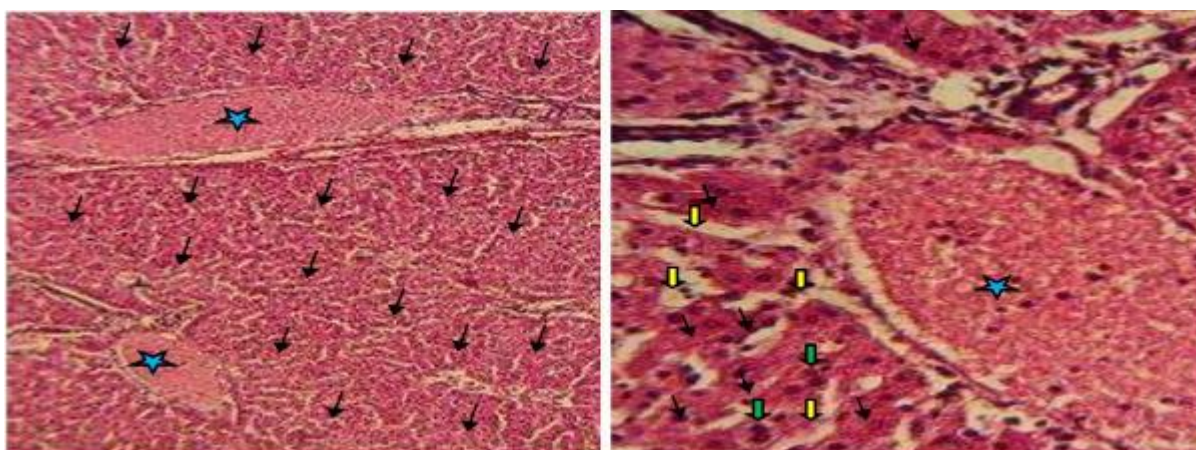
Mice Treated with 200 mg/kg Nanoparticles

Plate 9: Liver: necrosis of the hepatocytes (yellow arrows); congestion of portal veins (blue stars) and central vein (green star). Hematoxylin and eosin stain. Magnification x100



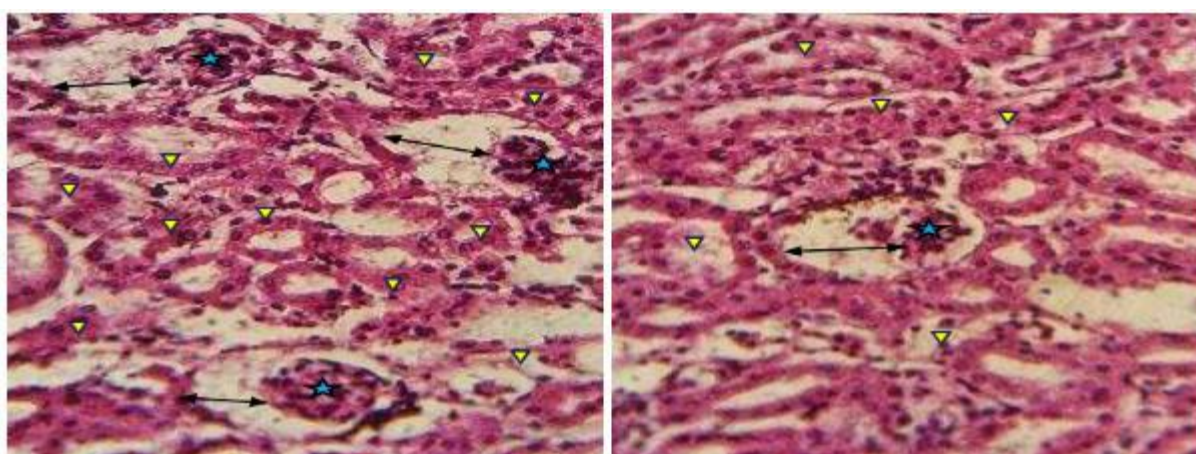
Mice Treated with 200 mg/kg Nanoparticles

Plate 10: Kidney: necrosis and atrophy of the renal corpuscles (yellow stars), mild necrosis of the renal tubules (green arrows). The Bowman's space (line arrow: double) is widened due to the atrophy of the renal corpuscles. Hematoxylin and eosin stain. Magnification x400



Mice Treated with 250 mg/kg *E. hirta*

Plate 11: Liver: congestion of the portal veins (blue stars); hepatocytes (black arrows); sinusoids (yellow arrows). Replicating hepatocytes (green arrows) signifying the healing process. Hematoxylin and eosin stain. Magnification x100 and x400 respectively



Mice Treated with 250 mg/kg *E. hirta*

Plate 12: Kidney: necrosis and atrophy of the renal corpuscles (blue stars) and renal tubules (yellow arrowheads); widened Bowman's space (line arrow: double) due to the atrophied renal corpuscles. Hematoxylin and eosin stain. Magnification x400

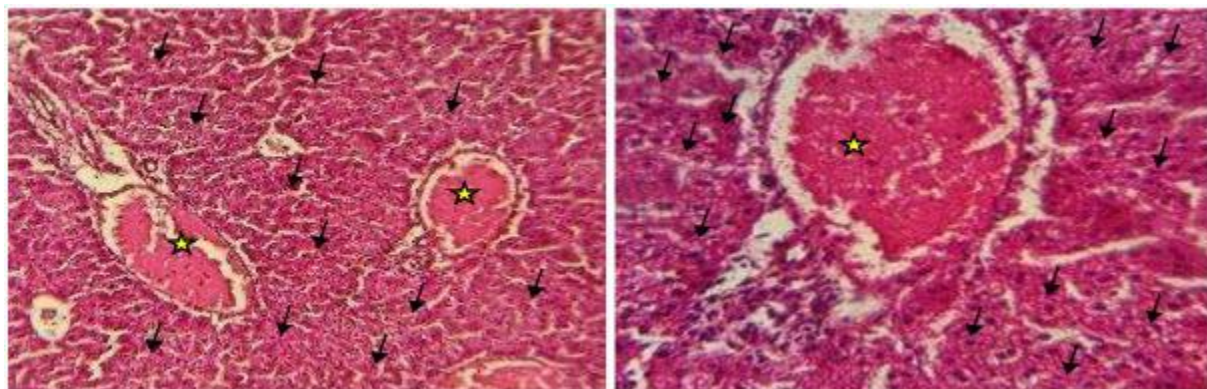
Mice Treated with 500 mg/kg *E. hirta*

Plate 13: Liver: necrosis of the hepatocytes (black arrows) and congestion of the portal veins (yellow stars). Hematoxylin and eosin stain. Magnification x100 and x400 respectively

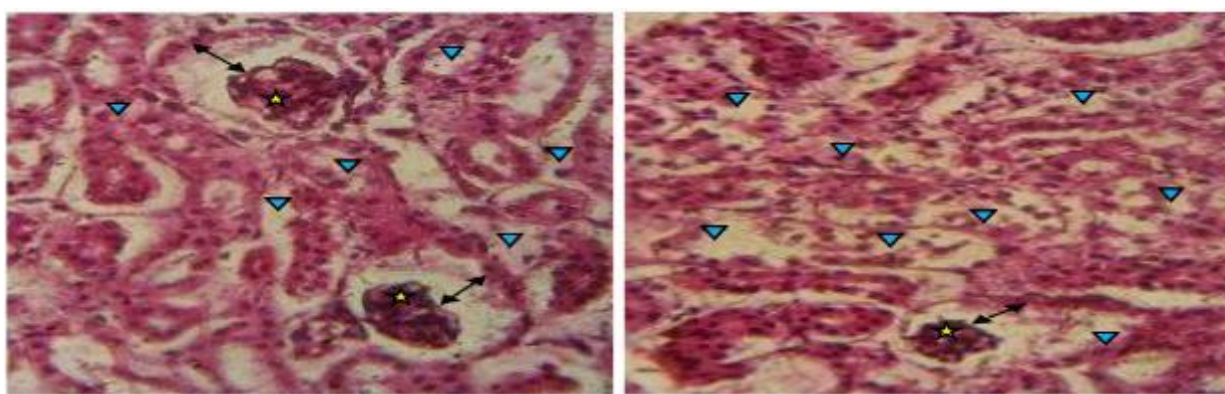
Mice Treated with 500 mg/kg *E. hirta*

Plate 14: Kidney: necrosis and atrophy of the renal corpuscles (yellow stars) and renal tubules (blue arrowheads); widened Bowman's space (line arrow: double) due to the atrophied renal corpuscles. Hematoxylin and eosin stain. Magnification x400

Phytochemical composition of *Euphorbia hirta*

The phytochemical composition of *Euphorbia hirta* identified in the present study is consistent with previous reports describing the species as a chemically diverse medicinal plant [15]. Alkaloids, flavonoids, tannins, saponins, steroids, coumarins and terpenoids have previously been reported in *E. hirta*, although the composition varies according to plant part, geographical origin and extraction procedure [3, 4]. Similar phytochemical classes have also been reported in *E. hirta* preparations investigated for antiparasitic and other biological activities, supporting the reproducibility of the broad phytochemical profile of the plant [4, 16]. Compared to earlier studies, the present study employed an aqueous leaf extract, whereas several earlier investigations used ethanol or methanol extracts. For example, an ethanol extract of whole *E. hirta* demonstrated antiparasitic activity in *Plasmodium berghei*-infected mice, although the activity was considerably lower than that of the reference treatment [16]. Differences in solvent polarity may explain some variation between studies because aqueous extraction may preferentially recover polar constituents, whereas organic solvents can extract a wider range of moderately polar compounds. Thus, differences in biological activity

between the present study and earlier work might not necessarily be contradictory.

The biological relevance of the phytochemical classes detected in this study is supported by previous pharmacological investigations. Alkaloids have been associated with antimicrobial and antiparasitic effects, while flavonoids and tannins possess antioxidant and antimicrobial properties [17, 18]. Saponins have likewise been associated with a range of biological activities, including effects on cellular membranes [19]. The antioxidant capacity previously demonstrated for *E. hirta* leaves, including substantial radical-scavenging activity, further supports the possibility that the plant may provide both direct antiparasitic and host-protective effects [20]. However, the presence of these compounds might not necessarily be a proof that any particular constituent was responsible for the antitrypanosomal activity observed in the present study. Since the extract was crude, interactions among multiple constituents are possible, but such synergy remains to be experimentally established.

The findings also agree with other studies which reported that medicinal plants possess antitrypanosomal activity against *T. b. brucei* [21]. However, the magnitude of activity among medicinal plants varies considerably.



Some extracts have produced substantial parasite suppression or clearance, whereas others have produced only weak effects at the doses tested [22]. Such variation may result from differences in phytochemical composition, extraction solvent, plant part, parasite strain, treatment dose and duration.

GC–MS identified compounds and pharmacological significance

The GC–MS analysis in this study demonstrates the chemical complexity of the *E. hirta* extract. The detection of hydrocarbons, fatty acid derivatives and esterified compounds is consistent with previous chemical investigations of the plant, which have identified numerous structurally diverse constituents, including triterpenoids, coumarins, lignans and diterpenes [23, 24]. The difference between the compounds identified in the present GC–MS analysis and compounds reported from other investigations is not unexpected because GC–MS primarily detects compounds amenable to chromatographic volatilization, whereas other analytical approaches can identify less volatile constituents.

The identification of fatty acid derivatives is of potential pharmacological interest because fatty acids and related compounds have been reported to exert antimicrobial effects through interactions with biological membranes [25]. Similarly, other hydrocarbon and ester compounds identified in plant extracts have been associated with antimicrobial activity [26]. Nevertheless, these observations provide only a possible explanation for the biological activity of the extract. The current study identified the presence of bis (2-ethylhexyl) phthalate in the extract. Although phthalate derivatives have been reported in plant extracts, their occurrence can also be associated with environmental or laboratory contamination.

Acute toxicity and safety profile

The acute toxicity profile observed in this study suggests that the preparations were tolerated at the doses investigated, although the safety margin differed between the extract and nanoparticles. The relatively high tolerance of the *E. hirta* extract is consistent with previous studies reporting low acute toxicity of crude *E. hirta* preparations [3, 4]. For example, acute toxicity investigations of *E. hirta* have reported administration of gram-per-kilogram doses without marked acute lethality, although toxicity depends on the preparation and route of administration [27, 28].

The relatively lower toxicity threshold of the nanoparticle preparation compared with the plant extract may reflect differences in particle characteristics and biological distribution. Nanoparticles have a high surface-area-to-volume ratio and may interact more readily with cellular structures than bulk materials [6]. Previous research has demonstrated that nanoparticle-associated toxicity can depend on particle size, surface characteristics, chemical composition and the capacity to generate oxidative stress [29]. Vanadium-containing

nanomaterials have also demonstrated biological activity that may involve oxidative mechanisms.

Pilot in vitro anti-trypanosomal assay

The in vitro findings conducted demonstrate that both preparations possess direct activity against *T. b. brucei*, with inhibition dependent on concentration and exposure time. This finding extends the existing pharmacological evidence for *E. hirta*, for which antimicrobial and antiprotozoal activities have previously been reported [30]. However, direct comparison with earlier antitrypanosomal studies is limited because relatively few studies have specifically investigated *E. hirta* against *T. b. brucei*. Much of the available evidence concerns *Plasmodium* or bacterial and fungal organisms [16, 31]. The observed antitrypanosomal effect in the current finding may be attributable to differences in extract composition and assay conditions. Antitrypanosomal activity can be strongly influenced by extraction solvent, concentration, parasite density, parasite strain and exposure period [32]. For example, plant extracts investigated against *T. b. brucei* have shown considerable variation, ranging from weak suppression to complete parasite clearance, depending on the plant and experimental conditions [21].

The relatively rapid activity of the nanoparticle preparation may be related to its physicochemical characteristics and increased interaction with parasite surfaces. Vanadium-containing nanomaterials have demonstrated antimicrobial activity in other biological systems, with oxidative stress proposed as one possible mechanism [33]. However, the present study did not characterize the nanoparticles sufficiently to establish particle size, morphology, surface charge or oxidation state, and therefore a specific mechanism cannot be assigned.

In vivo antitrypanosomal efficacy

The in vivo findings of this study indicate that the extract and nanoparticles have antitrypanosomal potential but do not possess the same parasitocidal efficacy as the reference drug. This distinction is important because suppression of parasitaemia does not necessarily indicate complete therapeutic clearance. Similar patterns have been reported in experimental studies of other medicinal plants against *T. brucei brucei* [34, 35]. For example, aqueous and methanolic extracts of *Brillantaisia owariensis* suppressed parasitaemia but did not completely eliminate circulating parasites, whereas diminazene aceturate achieved parasite clearance [34]. Similarly, Ogbadoyi et al. [21] reported that some ethnomedicinal extracts produced substantial parasite suppression and, in some cases, clearance, whereas the activity differed according to plant species and dose. Several factors may account for differences in activity among studies. The present investigation used an aqueous leaf extract, whereas previous studies have employed ethanol, methanol, hexane or other solvents. Plant part, geographical origin and extraction conditions can alter the concentration and composition of active constituents. In addition, differences in *T. b. brucei*

strain, infective dose, stage of infection at treatment initiation, treatment duration and animal model can substantially influence therapeutic response. The incomplete parasite clearance observed in this study suggest that the extracts may possess parasite-suppressive activity that warrants further investigation. This is consistent with the continuing need for additional antitrypanosomal agents because existing drugs are affected by limitations including toxicity, administration requirements and resistance [36, 37].

Hematological recovery (PCV and body weight)

The changes in PCV and body weight provide additional information about the physiological consequences of the administered treatments. Trypanosome infection is well recognized to produce anaemia and progressive deterioration in body condition through mechanisms involving erythrocyte destruction, altered erythropoiesis, inflammatory responses and oxidative stress [38]. Consequently, preservation of PCV and body weight may represent an important component of therapeutic benefit even where complete parasite elimination is not achieved.

The present findings are comparable with observations from other experimental plant-based antitrypanosomal studies. In *B. owarensis*-infected mice, treatment with aqueous and methanolic extracts was associated with recovery of PCV following the decline induced by infection, while the untreated infected group experienced continued deterioration [34]. These findings suggests that plant-derived treatments may provide host-protective effects in addition to direct parasite suppression. The antioxidant properties previously demonstrated for *E. hirta* leaves provide a plausible biological basis for such host protection [4]. However, the present study did not directly measure oxidative stress, erythrocyte turnover or inflammatory biomarkers. Therefore, the improvement in physiological parameters cannot be attributed specifically to flavonoids or other individual phytochemicals. Future studies incorporating antioxidant enzymes, lipid peroxidation markers and haematological indices would help determine whether reduction of oxidative injury contributes to the observed response.

Survival rate and disease outcome

The survival pattern observed in the current study further supports the biological relevance of treatment during experimental infection. Previous studies of *T. b. brucei*-infected mice have demonstrated considerable differences in survival according to plant species, extract type and dose, with some treatments delaying disease progression without achieving complete parasite clearance [21]. The relationship between parasite suppression and improved survival is biologically plausible because reduction in parasite burden may delay the systemic effects of infection.

The protective effects of *E. hirta* may also involve its reported antioxidant and anti-inflammatory properties. The leaves have demonstrated substantial antioxidant activity in previous experimental work, suggesting that

the plant may help mitigate oxidative damage associated with infection [4, 5]. Nevertheless, survival is a complex endpoint influenced by parasite burden, host response and treatment toxicity and would require further study to establish the mechanism employed.

Histopathological outcome

The histopathological findings in this study demonstrate that *T. b. brucei* infection was associated with pathological alterations in the liver and kidney, while treatment was associated with varying degrees of tissue preservation and recovery. Similar hepatic and renal involvement have been documented in experimental *T. brucei* infection, including hepatic extramedullary haematopoiesis and renal interstitial nephritis [39], as well as degenerative changes in the liver and kidneys that were ameliorated following trypanocidal treatment [40]. Histopathological assessment of these organs has also been incorporated into experimental studies evaluating medicinal plants for antitrypanosomal activity and toxicity [34].

The residual lesions observed in some treated groups are particularly important because they indicate that parasite suppression did not necessarily result in complete restoration of tissue architecture. This is consistent with the incomplete parasite clearance observed with the natural products. The apparent regenerative changes observed in some tissues may be associated with reduction of parasite-associated stress and the antioxidant properties of plant constituents. *E. hirta* leaves have previously demonstrated antioxidant activity, while flavonoid-rich fractions of the plant have also shown biological activity in experimental systems [4]. However, the present study did not measure biochemical markers of hepatic or renal function, oxidative stress or inflammation. Furthermore, histopathological assessment was descriptive rather than based on a standardized quantitative lesion-scoring system. Consequently, treatment-related differences in tissue protection should be considered preliminary.

Limitation of the study

The study did not investigate the molecular mechanism underlying the observed parasite inhibition. Similarly, pharmacokinetic and biodistribution assessments were not performed, limiting understanding of how the extract and nanoparticles are absorbed, distributed and eliminated.

Conclusion

This study demonstrated that aqueous *Euphorbia hirta* leaf extract and green-synthesized vanadium nanoparticles exhibited preliminary antitrypanosomal activity against *Trypanosoma brucei brucei* in vitro and in vivo. Both treatments inhibited parasite motility and reduced parasitaemia, although neither achieved complete parasite clearance, unlike diminazene aceturate. Treatment was also associated with improved body weight and reduced histopathological alterations, while PCV showed no significant pairwise differences among groups. The acute toxicity findings indicated



relatively low acute lethality of the extract, whereas the nanoparticles showed a comparatively narrower safety margin. In addition, the findings support further investigation of *E. hirta* extract and green-synthesized vanadium nanoparticles as potential parasite-suppressive agents, with emphasis on nanoparticle characterization, dose optimization, mechanistic and pharmacokinetic studies, and long-term safety evaluation.

Conflict of interest: The authors declare no conflict of interest.

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Citing this Article

Paul, F. I., Maikenti, J. I., Ombugadu, A., Ashigar, M. A., Ahmed, H. O., & Pam, V. A. (2026). Antitrypanosomal activity and acute toxicity of *Euphorbia hirta* leaf extract and a green-synthesized vanadium-based nanoparticles against *Trypanosoma brucei brucei* in mice. *Lafia Journal of Scientific and Industrial Research*, 4(2), 173–189. <https://doi.org/10.62050/ljsir2026.v4n2.963>