ANTI-PLASMODIAL AND ANTI-TRYPANOSOMAL ACTIVITIES OF WHOLE PLANT N-BUTANOL EXTRACT OF *THESIUM VIRIDE* IN BALB/c MICE MODEL

Supported tetfund

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Received: February 11, 2023 **Accepted:** April 15, 2023

Abstract

Drug resistance is a serious issue that hampers efforts to control or eradicate malaria and the African sleeping sickness worldwide. In light of this growing problem, immediate efforts are required to find brand-new antiprotozoal drugs that are very effective and don't have any harmful side effects. This study was designed to determine the pharmacological activities of a whole-plant n-butanol extract of *Thesium viride* against *Plasmodium berghei* or *Trypanosoma brucei* in BALB/c mice. Forty (40) adult BALB/c mice (*Mus musculus*) were randomly allocated to two major groups, A and B. In group A, each mouse in subgroups I to V was inoculated with 0.1 mL containing 10^6 *Plasmodium berghei* merozoites. In group B, each mouse in subgroups I to V was inoculated with 0.1 mL containing 10^6 *Trypanosoma brucei* trypomastigotes. Following a patency period of 3 days, mice in all groups, A and B, were either non-treated, treated with the standard drug, or treated with varying dosages of n-butanol extracts for four days. Standard methods and techniques measured the parasitemia level, packed cell volume, rectal temperature, weight, and other clinical indices. After the four-day oral treatment, the whole plant n-butanol extract showed significant ($p < 0.01$) anti-plasmodial and anti-trypanosomal activities. However, no haematinic activity was observed against *Plasmodium berghei* or *Trypanosoma brucei* in all infected mice administered the plant extract. In conclusion, *Thesium viride* showed better suppressive ability against *Plasmodium berghei* than *Trypanosoma brucei*, suggesting its potential as an anti-plasmodial agent.

Keywords: *Thesium viride*, *Plasmodium berghei Trypanosoma brucei*, Pharmacological activity, N-butanol extract, Mouse model

Introduction

The protozoan parasites, *Plasmodium* spp. and *Trypanosoma* spp. are of major public health concern, responsible for malaria and trypanosomiasis, respectively (Ungogo *et al*., 2020; Kotepui *et al*., 2021). They cause high morbidity and mortality in humans, animals, and wildlife (Ungogo *et al*., 2020; Ayawa *et al*., 2021).

Malaria is most common in tropical and subtropical areas and is especially prevalent in sub-Saharan Africa (WHO, 2022). *Plasmodium vivax*, *P. malariae*, *P. ovale*, and *P. falciparum* are the only four species of the *Plasmodium* parasite that cause human malaria. However, *P. falciparum* is mainly found in Nigeria and is the most lethal and virulent malaria parasite. The burden of malaria in the world is still disproportionately heavy in the African Region. About 95% of all malaria cases and 96% of all malaria deaths occurred in the region in 2021 (WHO, 2022). More than 80% of all malaria deaths in the African region occurred in children under five. Just over half of all malaria deaths happened in four African countries: Nigeria (31.3%), the Democratic Republic of the Congo (12.6%), the United Republic of Tanzania (4.1%), and Niger (3.9%) (WHO, 2022). Malaria is a major burden on Nigerian families, communities, the health system, and the workforce, accounting for roughly 60% of outpatient visits, 30% of hospitalisations, 25% of infant deaths, and 11% of maternal fatalities. *Plasmodium* parasites, which individual contracts through the bites of infected female Anopheles mosquitoes, are the source of the acute fever sickness known as malaria (WHO, 2022). The initial signs of malaria, including fever, headache, and chills, can be mild and challenging to diagnose. They typically show up 10 to 15 days after the infected insect bite. If *P. falciparum* malaria is not treated, it can lead to severe sickness and death in less than 24 hours (WHO, 2022).

African trypanosomiasis is one of the most debilitating protozoan infections with a high impact on human health, cattle health, agricultural production, and rural development (FAO, 2008). African trypanosomiasis, or sleeping sickness, is caused by two subspecies of *T. brucei: T. brucei gambiense* and *T. brucei rhodesiense.* The third subspecies, *T. brucei brucei*, is only infectious to animals (WHO, 2005). The chronic form of sleeping sickness is caused by *T. brucei gambiense* in West and Central Africa, while the acute condition is caused by *T. brucei rhodesiense* in East and Southern Africa (WHO, 2005). African trypanosomiasis induces haematological changes that produce severe anaemia via decreased packed cell volume, haemoglobin concentration and leucocyte counts (Wada *et al*., 2016a, 2016b).

Chemotherapeutic or chemoprophylactic drugs are frequently used to treat trypanosomiasis or malaria. Treatment for malaria and African trypanosomiasis is challenging due to drug shortages, drug resistance, high prices, unpleasant side effects, and toxicity (Matovu *et al*., 2001; Toya, 2010; Barrett *et al*., 2011). Drug resistance is a serious issue that hampers efforts to control or eradicate malaria and the African sleeping sickness worldwide. An unsettling trend that impedes an efficient control plan is the development of antibiotic resistance. In light of this growing problem, immediate efforts are required to find brand-new antiprotozoal drugs that are very effective and don't have any harmful side effects. In Africa's traditional healthcare system, medicinal plants play a significant role (Mahomoodally, 2013). These include substances that can be used medicinally, or that can be used as building blocks to synthesise useful medicines in part or their entirety (Sofowora *et al*., 2013). The Hausa ethnic group in Nigeria refers to *Thesium viride* as "Huntu" (Moore *et al*., 2010). It has long been known that plants can treat several illnesses. The leaves are cooked in water and

used as a decoction to cure jaundice, fever, and intestinal worms (Belakhdar *et al*., 2014; Kamaruding *et al*., 2020). *Thesium viride* extract and fractions have been shown to have antibacterial and antiulcer properties and improve and safeguard the liver's antioxidant enzymes against CCl4-induced liver damage (Shehu *et al*., 2016, 2022). Research has yet to take advantage of the intriguing properties of *Thesium viride* fully. Thus, it is necessary to employ mouse models to conduct scientific research on the possibility of using a whole plant extract of *Thesium viride* to treat malaria or trypanosomiasis. Hence, this study was designed to determine the pharmacological activities of whole plant n-butanol extract of *Thesium viride* on a *Plasmodium berghei* and *Trypanosoma brucei* in BALB/c mouse models.

Materials and Methods

Ethical Considerations

The study protocol and permission to do the study were obtained with the approval number ABUCAUC/2021/078 from the Ethical Committee on Animal Use and Care at Ahmadu Bello University in Zaria, Nigeria.

Study Locations

The research was conducted in the Parasitology Laboratory, Department of Veterinary Parasitology and Entomology, Ahmadu Bello University in Zaria, Nigeria.

Collection and Identification of the Plant Material

The whole plant-dried sample of *T. viride* was collected from Zaria local government, Kaduna State, Nigeria, in February 2020. The Taxonomist identified the plant at the herbarium section of the Department of Botany, Ahmadu Bello University, Zaria (voucher number ABU06986).

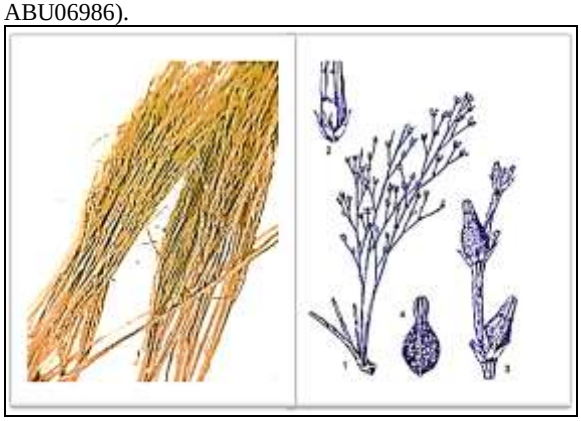

Plate I: The dried whole plant of *Thesium viride* – 1. Flowering branch; 2. Flower; 3. Young fruit; 4. Mature fruit (Bosch, 2008)

Preparation of Extracts

Two hundred (200g) pulverised dried sample of *Thesium viride* was filled in a thimble and extracted using 500 ml of distilled n-butanol, isopropanol, and water in a soxhlet apparatus for 8–10 hours. The extract was filtered through Whatman filter paper to remove all unextractable matter, including cellular materials and other insoluble constituents in the extraction solvent. The extract was concentrated to dryness using a rotary flash evaporator under reduced pressure. The dried extract was redissolved in n-Butanol, isopropanol, and water to yield solutions containing 200, 400, and 800mg of leaf extract per ml of solvent.

Acute Oral Toxicity Study

An acute toxicity study was carried out following recommendations from the Organization for Economic Cooperation and Development (OECD) (423; OECD guideline, 2002). The LD_{50} value was calculated using the equation:

$$LD_{50} = \sqrt{D0 \text{ X } D100}$$

Where D0 = the highest dose that gave no mortality
D100 = lowest dose that produced no mortality

Experimental Animals

Forty (40) healthy adult male and female mice weighing 17 to 23g were obtained from the animal house, Department of Physiology, Faculty of Medicine, Ahmadu Bello University (ABU), Zaria, Nigeria. They were allowed to acclimatise for two weeks in the Laboratory at the Department of Zoology, A.B.U., Zaria. They were housed in clean plastic cages with wood shavings as bedding, which were changed twice a week. The mice were fed standard feed and access to clean water *ad libitum.*

Experimental Parasites

The *Trypanosoma brucei* used for this experiment was obtained from the Department of Veterinary Parasitology and Entomology, A.B.U., Zaria. The parasites were maintained in mice by continuous passage. Each cycle of passage was done when parasitaemia was in the range of 35 to 40 parasites per field of a prepared wet mount.

The *Plasmodium berghei* (NK45) employed in this study was sourced from the Nigerian Institute of Medical Research, Lagos state, Nigeria. The parasites were maintained in rats by continuous passage and transported by road to Zaria, Kaduna state.

Experimental Design, Parasite Inoculation, and Treatment

The experimental design was set up in a simple complete randomised design (CRD) following toxicity studies and establishing safe lethal doses for n-butanol extract. Forty (40) adult BALB/c mice (*Mus musculus*) of both sexes weighing 17-23g were randomly allocated to two major groups, A and B, of twenty (20) mice each. Group A was further subdivided into five groups, I (negative control), II (positive control), III (N200 mg/kg), IV (N400 mg/kg), and V (N800 mg/kg) in a simple complete randomised design, to determine the anti-plasmodial activity of the n-butanol extract of *Thesium viride* in mice (Figure 2A). Each mouse in groups I to V was inoculated with 0.1 mL containing 10^6 *P. berghei* merozoites. Following three days of patency, mice in groups I, II, III, IV, and V were given either normal saline (NC-negative control), a standard drug (PC-positive control, chloroquine 25 mg/kg), or n-butanol extracts at N200, N400, or N800 mg/kg (Figure 1A).

Mice in group B were also split into five groups: I (negative control), II (positive control), III (N200 mg/kg), IV (N400 mg/kg), and V (N800 mg/kg). This was done in a simple randomised design to see how well the n-butanol extract of Thesium viride worked against *Trypanosoma brucei* (Figure 2B). Each mouse in groups I to V was given 0.1 mL of T. brucei trypomastigotes containing 106 cells. After three days of patency, mice in groups I, II, III, IV, and V were given either normal saline (NC-negative control), standard drug (PC-positive control, diminazene aceturate 3.5 mg/kg), or n-butanol extracts at N200, N400, or N800 mg/kg (Figure 1B).

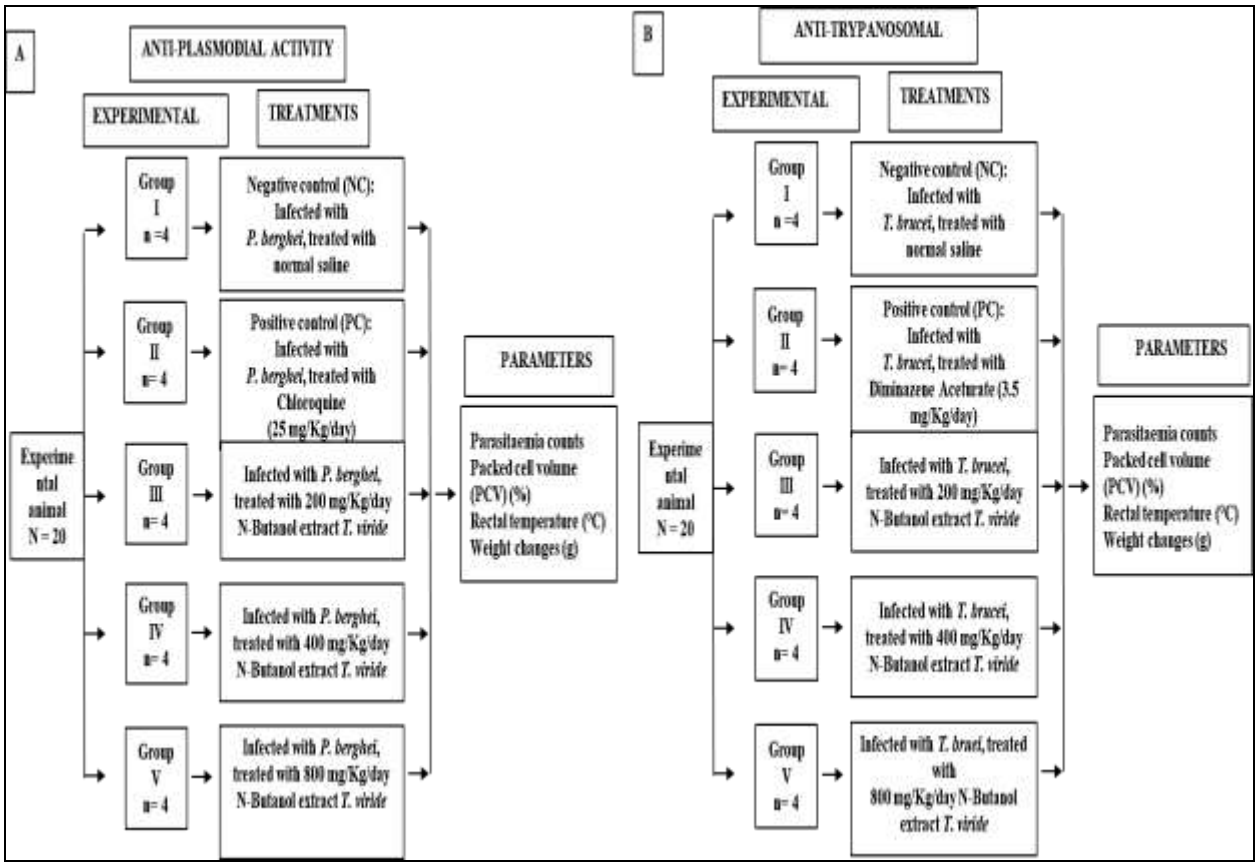

Figure 1: Simple complete randomized experimental design to assess the effect n-Butanol whole plant extract against (A)- *Plasmodium berghei* (B)- *Trypanosoma brucei* in BALB/c mice model

Observation of Clinical Signs

Rectal temperature, feed intake, physical condition, weakness and dullness, rough hair coat, and weight changes were among the clinical symptoms examined during the trial, according to Ayawa *et al*. (2021).

Determination of Parasitemia: Plasmodium berghei

Thin smears of blood from Plasmodium berghei-infected mice were made from the tail of each mouse. The smears were fixed with absolute methanol and stained with 10% Giemsa's stain at pH 7.2 for 15 min. The stained slides were washed gently using distilled water and were air-dried at room temperature. Two stained slides for each mouse were examined under a light microscope using a ×100 objective lens with oil immersion. Ten different fields on each slide were examined at random, and the average percentage parasitaemia was determined by counting the number of parasitised RBCs out of 500 erythrocytes and thus calculated using the formula (Mekonnen, 2014):

% Parasitaemia =

$$\frac{\text{Number of infected RBCs}}{\text{Total number of RBCs}} \times 100$$

Determination of Parasitaemia: Trypanosoma brucei

Blood drawn from the tail of Trypanosoma brucei-infected mice was tested for parasitaemia. The number of parasites was determined by preparing a wet film to mount each day, covering it with a coverslip, and looking at slides with a 400-objective lens. The number of fields counted is based on how many parasites are present in each field (Herbert and Lumsden, 1976).

Evaluation of Packed Cell Volume (PCV)

Blood was partially drawn into heparinised capillary tubes due to capillary action. A cotton wool ball was used to wipe up extra blood. After that, a Bunsen flame was used to seal the dry end of the tubes. It was centrifuged for four minutes at 12,000 revolutions per minute using a microhaematocrit centrifuge (rpm). According to Ochei and Kolhatkhar, the spinning tubes were then read and expressed as a percentage using a microhaematocrit reader (2000).

Determination of Rectal Temperature

Every morning between 7:00 am and 8:00 am, a clinical digital thermometer was used to measure the rectal temperature of each experimental animal (KRIS-ALOY CE 0197). The rectal mucosa was touched with the thermometer as it was tilted into the rectum. As the thermometer beeped, it was withdrawn, and the variations in body temperature were read and recorded in degrees Celsius (°C).

Determination of Live Body Weight

The live body weight of the mouse was measured with a precise digital scale in grams (g) to track weight changes.

Data Analyses

Pre- and post-treatment descriptive assessments of clinical parameters—parasitaemia, packed cell volume, rectal temperature, and weight changes—were shown as means with standard errors (SE) in bar charts for each experimental setting. Clinical parameters' pre- and post-treatment means were compared using the student-paired t-test for each therapy, and the clinical indices were compared using a one-way analysis of variance. A significance criterion of $p = 0.05$ was used to assess whether any group's means or treatment conditions substantially varied from those of one or more other groups using the Tukey post-hoc test. The IBM SPSS statistical software version 20 and Microsoft Excel 2019 were used for the statistical analysis.

Results and Discussion

All animals that were given lower and higher doses of the different fractions of the extracts showed no signs of toxicity, and no mortality was recorded. Hence, the median lethal dose was calculated to be greater than 5000 mg/kg. According to Loomis and Hayes' (1996) toxicity categorisation, when given orally to mice, the extract is essentially non-toxic and has a high level of safety. The results of Shehu *et al*. (2017) for oral administration of *Thesium viride* extract in rats showed a similar outcome and concordance with the current findings in mice.

Figure 2 depicts the effect of a *Thesium viride* n-butanol extract on the parasitaemia level of *Plasmodium berghei* or *Trypanosoma brucei*-infected mice. All infected mice in this study developed acute malaria and trypanosomiasis three days after infection, exhibiting the classic signs and symptoms of *Plasmodium* and *Trypanosoma* spp. Animal infections include intermittent pyrexia, weakness, lethargy, dull and rough coats, slight weight losses, and reduced packed cell volumes (Oluyemi *et al*., 2020; Ungogo *et al*., 2020; Ayawa *et al*., 2021). Following the four days of treatment for the anti-plasmodial activity, the percent parasitaemia suppression of treatment was highly significant ($p < 0.01$) for the positive control (-63.94%), N400 (-29.89%), N800 (-29.35%), and N200 mg/kg (-23.49%) in comparison to the negative control, which showed an increase in parasitaemia level of 156.95 percent (Figure 1A). In terms of anti-trypanosomal activity, the positive control demonstrated a significant ($p= 0.001$) percent reduction in parasitaemia level (-77.45%), followed by doses of N400 (-22.66%), N200 (14.44%), and N800 mg/kg (-14.03%). No parasite suppressions were observed for the negative control (27.21%) (Figure 2B).

Figure 3 depicts the mean percent parasitaemia suppression of *Plasmodium berghei* or *Trypanosoma brucei*-infected mice treated with n-butanol extract of *Thesium viride* daily for four days. The antiprotozoal activity of the n-butanol extract of *Thesium viride* was significantly higher ($p<0.01$) for *Plasmodium berghei* in all mice administered 200 mg/kg/day (-23.49%), 400 mg/kg/day (-29.89%) and 800 mg/kg/day (-29.35%), compared to its anti-trypanosomal activity for *Trypanosoma brucei*-infected mice administered 200 mg/kg/day (-14.44%), 400 mg/kg/day (-22.66%) and 800 mg/kg/day (-14.03%), respectively (Figure 3).

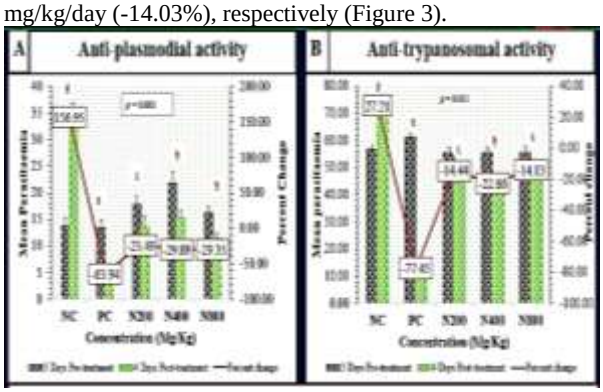

Figure 2: Effect of n-Butanol extract of *Thesium viride* on the parasitaemia level of *Plasmodium berghei* and *Trypanosoma brucei*-infected mice.NC = Negative Control (Normal Saline), PC = Positive Control (*Plasmodium berghei*, Chloroquine 25 mg/Kg), PC = Positive Control (*Trypanosoma brucei*, Diminazene Aceturate 3.5 mg/Kg), N = N-Butanol Extract. Superscripts across bars with different alphabets differed significantly ($p< 0.01$) in percent parasitaemia suppression

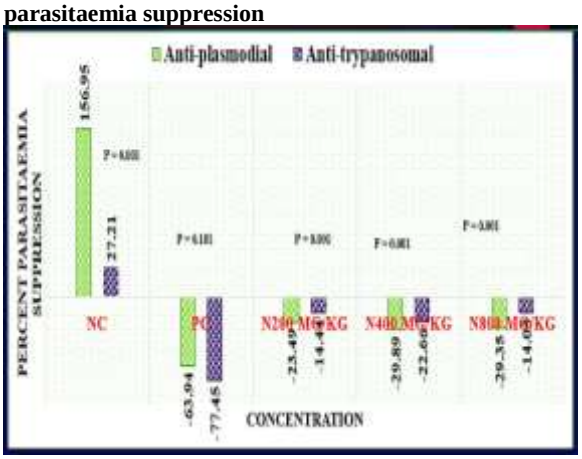

Figure 3: Student-t test for comparing the mean percent parasitaemia suppression of *Plasmodium berghei* and *Trypanosoma brucei*-infected mice treated daily for four days with N-butanol extract *Thesium viride*. PC = Positive Control (*Plasmodium berghei*, Chloroquine 25 mg/Kg), PC = Positive Control (*Trypanosoma brucei*, Diminazene Aceturate 3.5 mg/Kg), N = N-Butanol Extract

There was no apparent stoppage or total eradication of parasites from the bloodstream of any infected mice after the four-day treatment with a whole plant n-butanol extract of *Thesium viride* against either *Plasmodium berghei* and *Trypanosoma brucei*. However, compared to mice infected with *Trypanosoma brucei*, there was a considerable decrease in parasitemia, particularly in all groups that received the n-butanol extract. This suggests that *Trypanosoma brucei* in the mouse model was more resistant to the suppressive effects of *Thesium viride* whole-plant n-butanol extract than *Plasmodium berghei*. The short treatment period, insufficient for the treatment regimen to be finished, may be responsible for the partial clearance of parasitaemia, as seen for all the positive controls in all treatment groups. Also, the oral route of administration of the plant extract may have prevented the active chemicals in the extracts from reaching the site of action or prevented them from undergoing rapid metabolisation within the host, which could very well explain why the plant extract in this study was unable to eliminate the parasite from the blood (Wurochekke *et al*., 2004, 2005; Ayawa *et al*., 2021). This is perhaps the first pharmacological investigation of antiprotozoal prospects of n-butanol extract of *Thesium viride* against *Plasmodium berghei* and *Trypanosoma brucei* parasites in different mice model.

Figure 4 shows the effect of the N-butanol extract of *T. viride* on the packed cell volume (PCV) of *P. berghei* and *T. brucei*-infected mice. In the anti-plasmodial activity of the n-butanol extract, there was a decrease in the packed cell volume across all the doses administered. However, a student t-test paired analysis for each of the paired doses revealed a non-significant difference ($p> 0.05$) between the pre-treatment and post-treatment PCV values. In comparison, the anti-trypanosomal activity of the N-Butanol fractions administered at doses of 200, 400, and 800 mg/kg/day all revealed a significant decrease ($p < 0.05$) in the PCV values across all the treated groups, except for the negative and positive

control groups, which were statistically significant ($p < 0.05$) (Figure 4B).

The mean percent change in packed cell volume (PCV) of *P. berghei* and *T. brucei*-infected mice treated daily for four days with n-butanol extract of *Thesium viride* is shown in Table 1. Following a four-day treatment of *P. berghei*-infected mice with N-butanol extract of the whole plant of *T. viride* at 200, 400, and 800 mg/kg/day, packed cell volumes decreased by 29.24%, 7.50%, and 5.31%, respectively. In comparison with *T. brucei*-infected mice, the percent decrease in packed cell volume was higher at doses of 400 mg/kg/day (37.77%) and 800 mg/kg/day (18.22%) in comparison to the values obtained for *P. berghei* administered similar doses of the extract (Table 1).

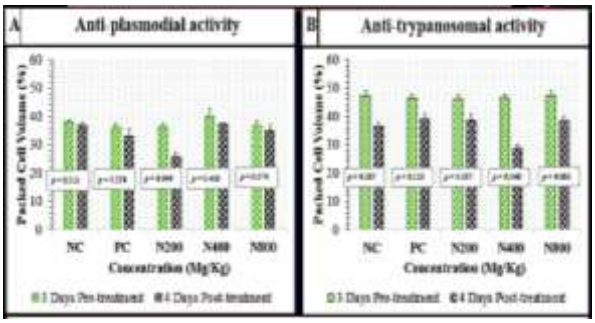

Figure 4: Effect of n-butanol extract of *Thesium viride* on the packed cell volume (PCV) of *Plasmodium berghei* and *Trypanosoma brucei*-infected mice.NC = **Negative Control (Normal Saline), PC = Positive Control (*Plasmodium berghei*, Chloroquine 25 mg/Kg), PC = Positive Control (*Trypanosoma brucei*, Diminazene Aceturate 3.5 mg/Kg), N = N-Butanol Extract**

Table 1: Mean percent change in packed cell volume (PCV) of *Plasmodium berghei* and *Trypanosoma brucei*-infected mice treated daily for four days with n-butanol extract of *Thesium viride*

Pathogen	Concentration	3 Days Pre-treatment	4 Days Post-treatment	Percent change
Plasmodium berghei				
	NC	38.00±0.71	36.93±0.81	-2.82
	PC	36.25±1.32	33.15±2.41	-8.55
	N200 (mg/Kg)	36.25±1.11	25.65±1.67	-29.24
	N400 (mg/Kg)	40.00±2.58	37.00±0.79	-7.50
	N800 (mg/Kg)	36.75±1.75	34.80±1.93	-5.31
Trypanosoma brucei				
	NC	47.20±2.08	36.40±1.36	-22.88
	PC	46.40±1.72	39.00±1.98	-15.95
	N200 (mg/Kg)	46.00±1.58	38.75±2.21	-15.76
	N400 (mg/Kg)	46.60±1.50	29.00±1.08	-37.77
	N800 (mg/Kg)	47.20±1.59	38.60±1.44	-18.22

NC = Negative Control (Normal Saline), PC = Positive Control (*Plasmodium berghei*, Chloroquine 25 mg/Kg), PC = Positive Control (*Trypanosoma brucei*, Diminazene Aceturate 3.5 mg/Kg), N = N-Butanol Extract

The packed cell volume decreased in all doses due to the n-butanol extract's anti-plasmodial and anti-trypanosomal actions. None of the extract dosages produced increased packed cell volume. Due to a high parasite load and immune-mediated hemolysis caused by the parasites' destruction of erythrocytes, the packed cell volumes for mice infected with *Plasmodium berghei* and *Trypanosoma brucei* decreased (Chamond *et al*., 2010). Reduction in packed cell volume causes severe anaemia, a symptom of African trypanosomiasis (Wada *et al*., 2016a, 2016b), and malaria, indicating the severity of both infections (WHO, 2022). *Thesium viride* whole plant extracts exhibit little to no haematinic action against P*lasmodium* and *Trypanosoma* parasites in mice, as evidenced by their inability to stop the reduction in packed cell volume.

Figure 5 shows the effect of the n-butanol extract of *Thesium viride* on the rectal temperature of *Plasmodium berghei* and *Trypanosoma brucei*-infected mice. In the anti-plasmodial activity of the n-butanol extract, there was a general decrease in the rectal temperatures between the pre-treatment and post-treatment values across all the doses administered (Figure 5A). However, with *Trypanosoma brucei*-infected mice, there was a general increase in the mean rectal temperature across all doses except for the positive control (Figure 5B).

Table 2 shows the mean percent change in rectal temperature of *Plasmodium berghei* and *Trypanosoma brucei*-infected mice treated daily for four days with n-butanol extract of *Thesium viride*. Following the four days of treatment at doses of 200, 400, and 800 mg/kg/day, a percent decrease in rectal temperature was observed from the pre-treatment values of 1.17%, 1.63%, and 2.31%, respectively, for *Plasmodium berghei* infection. In contrast, for *Trypanosoma brucei*, a percent increase of 5.41%, 4.40%, and 0.14% in rectal temperature was observed for doses administered at 200, 400, and 800 mg/kg/kg/day, respectively (Table 2).

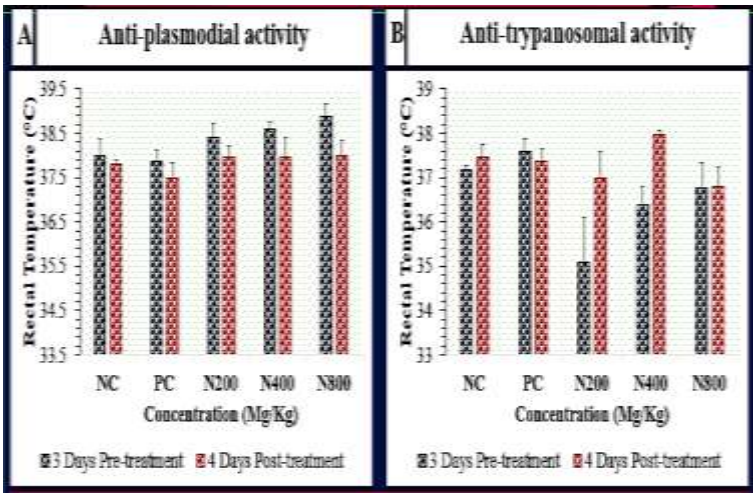

Figure 5: Effect of n-butanol extract of *Thesium viride* on the rectal temperature of *Plasmodium berghei* and *Trypanosoma brucei*-infected mice.NC = Negative Control (Normal Saline), PC = Positive Control (*Plasmodium berghei*, Chloroquine 25 mg/Kg), PC = Positive Control (*Trypanosoma brucei*, Diminazene Aceturate 3.5 mg/Kg), N = N-Butanol Extract

Table 2: Mean percent change in rectal temperature of *Plasmodium berghei* and *Trypanosoma brucei*-infected mice treated daily for four days with n-butanol extract of *Thesium viride*

Pathogen	Concentration	3 Days Pre-treatment	4 Days Post-treatment	Percent change
Plasmodium berghei				
	NC	38.00±0.37	37.80±0.10	-0.53
	PC	37.85±0.26	37.50±0.33	-0.92
	N200 (mg/Kg)	38.40±0.33	37.95±0.27	-1.17
	N400 (mg/Kg)	38.58±0.17	37.95±0.45	-1.63
	N800 (mg/Kg)	38.88±0.29	37.98±0.38	-2.31
Trypanosoma brucei				
	NC	37.17±0.09	37.47±0.26	0.81
	PC	37.60±0.27	37.38±0.28	-0.59
	N200 (mg/Kg)	35.10±0.97	37.00±0.58	5.41
	N400 (mg/Kg)	36.37±0.42	37.97±0.09	4.40
	N800 (mg/Kg)	36.75±0.59	36.80±0.44	0.14

NC = Negative Control (Normal Saline), PC = Positive Control **(***Plasmodium berghei***,** Chloroquine 25 mg/Kg), PC = Positive Control **(***Trypanosoma brucei***,** Diminazene Aceturate 3.5 mg/Kg), N = N-Butanol Extract

The mean daily rectal temperatures of all treated animals did not significantly change. In vulnerable animals, elevated body temperature is a defining sign and symptom of African trypanosomiasis and malaria fever. After being given an n-butanol extract of *T. viride*, mice maintained rectal temperatures within the usual range despite undulating parasitaemia. Studies have reported decreased rectal temperature in infection with either *Plasmodium berghei* and *Trypanosoma brucei* in mice, and this could be attributed to the host's immune response in response to parasitaemia in suppressing the high parasitaemia leading to a sudden change in body temperature or hypothermia (Mekonnen, 2014; Ayawa *et al*., 2021).

Figure 6: Effect of n-butanol extract of *T. viride* on the live weight of *Plasmodium berghei* and *Trypanosoma brucei*-infected mice. In the anti-plasmodial activity of the n-butanol extract, the mean weights of all mice administered 200 and 800 mg/kg doses decreased after post-treatment compared to the controls and the 400 mg/kg group (Figure 6A). In contrast, all the groups administered 200, 400 and 800 mg/kg/day for *Trypanosoma brucei* showed a reduction in the mean weight following the four days post-treatment, except for the negative control group (Figure 6B).

The mean percent change in the live weight of *P. berghei* and *T. brucei*-infected mice treated daily for four days with the n-butanol extract of *T. viride* is shown in Table 3. For the anti-plasmodial activity of the N-Butanol extract, the percent mean weights of all mice administered doses of 200 and 800 mg/kg following four days post-treatment decreased by 8.66% and 9.66%, respectively, in comparison to the controls and the group administered 400 mg/kg dosage, which showed an increase in percent weight (13.84%) (Table 4.8). In comparison, the anti-trypanosomal activity of the extract showed a general percent decrease in the mean weights of mice administered 200 mg/kg/day (-18.38%), 400 mg/kg/day (-25.26%), and 800 mg/kg/day (-10.48%) for *T. brucei* (Table 3).

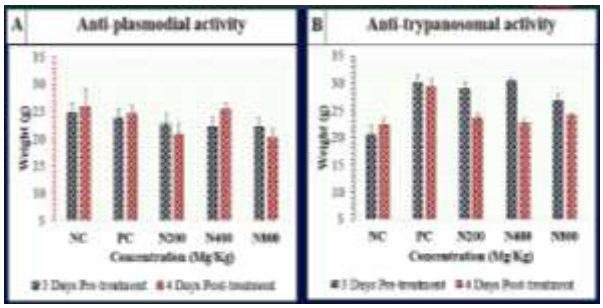

Figure 6: Effect of n-butanol extract of *Thesium viride* on the live weight of *Plasmodium berghei* and *Trypanosoma brucei*-infected mice. **NC = Negative Control (Normal Saline), PC = Positive Control (*Plasmodium berghei*, Chloroquine 25 mg/Kg), PC = PositiveControl (*Trypanosoma brucei*, Diminazene Aceturate 3.5 mg/Kg), N = N-Butanol Extract**

Table 3: Mean percent change in live weight of *Plasmodium berghei* and *Trypanosoma brucei*-infected mice treated daily for four days with N-butanol extract of *Thesium viride*

Pathogen	Concentration	3 Days Pre-treatment	4 Days Post-treatment	Percent change
Plasmodium berghei				
	NC	25.87±3.20	24.75±1.75	-4.33
	PC	23.75±1.80	24.55±1.62	3.37
	N200 (mg/Kg)	22.75±1.89	20.78±2.02	-8.66
	N400 (mg/Kg)	22.25±1.65	25.33±1.11	13.84
	N800 (mg/Kg)	22.25±1.70	20.10±1.83	-9.66
Trypanosoma brucei				
	NC	22.33±1.20	20.67±1.67	-7.43
	PC	30.00±1.58	29.25±1.55	-2.50
	N200 (mg/Kg)	29.00±1.15	23.67±0.88	-18.38
	N400 (mg/Kg)	30.33±0.67	22.67±0.88	-25.26
	N800 (mg/Kg)	26.80±1.20	24.00±0.58	-10.45

NC = Negative Control (Normal Saline), PC = Positive Control **(***Plasmodium berghei*, Chloroquine 25 mg/Kg), PC = Positive Control (*Trypanosoma brucei*, Diminazene Aceturate 3.5 mg/Kg), N = N-Butanol Extract

In general, the extract was more effective at preventing the weight loss that comes with *P. berghei* infection than *T. brucei* infection in mice. Compared to the animals in the negative control groups, the trypanosome-infected groups displayed a progressive loss in body weight. There was a decrease in feed intake and a loss in body weight in the experimental animals despite administering the *T. viride* extract and the usual diet. Increased body temperatures and the extravascular nature of *T. brucei* in the host may be to blame for reducing body weight, especially in *T. brucei*-infected mice. Also, the progressive rise in parasitemia and ensuing muscle degeneration in the *T. brucei*-treated mice may have contributed to their weight loss, indicating the n-butanol extract's incapacity to eradicate and reverse the parasite-induced weight loss. According to several studies, infected mice with *T. brucei* and *P. berghei* have both experienced weight loss (Toma *et al*., 2015; Trindade *et al*., 2016; Ungogo *et al*., 2020; Oluyemi *et al*., 2020; Yun *et al*., 2021).

Conclusion

The n-butanol extract showed better activity in inhibiting the weight loss in *Plasmodium berghei*-infected mice than their activities in *T. brucei*-infected mice; no haematinic activity against *P. berghei* or *T. brucei* was observed by administering the n-butanol extract orally in the mouse model. Interestingly, this is the first time the study has demonstrated the therapeutic prospects of n-butanol extracts of *T. viride*. The n-butanol extract showed better antiplasmodial activity in *P. berghei*-infected mice than in *T. brucei*-infected mice. However, the anti-plasmodial and anti-trypanosomal evaluations were limited to only four days of treatment, which is insufficient to complete the treatment regime; thus, future research should consider a complete treatment regime to determine T. viride's full pharmacological potential. Future research should also focus on isolating the individual compounds to assess the individual compound responsible for the antiprotozoal activities. Further studies are recommended to explore the antioxidant and anti-inflammatory capabilities of *T. viride* against *Plasmodium* and *Trypanosoma* parasites.

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