

## Therapeutic Potential of *Parkia biglobosa* Crude Aqueous Seed Extract against Trypanosomiasis: An *In vivo* Investigation

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### ABSTRACT

African trypanosomes are protozoans of the order Kinetoplastida of which species are transmitted by tsetse flies which are responsible for the disease trypanosomiasis. Commonly called *Nagana* (in domestic cattle) and “sleeping sickness” in humans, it has considerable impact on public health and economy in Africa. Crude aqueous seed extract of *Parkia biglobosa* (Jacq.) was evaluated for its antitrypanosomal activity in wistar rats experimentally infected with *Trypanosoma brucei brucei*. Phytochemical screening of the crude extract was carried out using standard procedures. The extract contained carbohydrates, cardiac glycosides, triterpenes, tannins and flavonoids. Acute toxicity testing using Lorke’s method was carried out. The LD<sub>50</sub> was found to be non-lethal at 5000mg/kg/bw. Twenty-five wistar rats were divided into five groups of five rats each. Some groups were inoculated intraperitoneally and parasitemia was established after a prepatent period of three days. The groups were treated intraperitoneally with the extract at 200, 300, and 500mg/kg/body weight respectively. There were also the positive (Berenil®), negative, not infected and not treated control groups. Parasitemia was monitored throughout the study ( $P \leq 0.05$ ). It was observed that the extract did not clear the animals of infection. It however slightly improved the PCV in the 500mg/kg/bw group as it similarly improved the weights in the 500mg/kg/bw dose group as well as increasing survival time compared with other lower dose groups. Conclusively, the seed-extract, showed no significant curative effect against *T. brucei brucei* infection in wistar rats. Further studies using other plant parts in combination with other plant materials is recommended.

**Keywords:** Crude-extract, Phytochemicals, *Parkia biglobosa*, Trypanosomiasis, in vivo

### INTRODUCTION

Trypanosomiasis, also known as sleeping sickness is a significant health concern in sub-Saharan Africa. Tsetse flies (*Glossina* spp.) are blood feeding dipteran insects that transmit African Trypanosomes, which are responsible for sleeping sickness which has considerable impact on public health and economy in sub-Saharan Africa. In Nigeria, eleven of the twenty-two species of tsetse flies are recorded to infest over 80% of the 928,300km<sup>2</sup> landmass, and are widely distributed in the country (Omonona *et al.*, 2020; De Gier *et al.*, 2020). The disease in animals is commonly called *Nagana* (in domestic cattle) and sleeping sickness in humans and can be fatal if untreated (Stijlemons *et al.*,

2024). Tsetse fly bite erupts into a red sore and within a few weeks, the person can experience fever, swollen lymph glands, aching muscles and joints, headaches and irritability. In advanced stages, the disease attacks the Central Nervous System (CNS), causing changes in personality, confusion, slurred speech, seizures, and difficulty walking and talking and eventually death may occur (Morrison *et al.*, 2023). In 2023, there were 546 reported cases of Human African Trypanosomiasis with *T. b. gambiense* being responsible for 94% of reported cases. *Trypanosoma brucei gambiense* is endemic in 24 countries in West and Central Africa. *Trypanosoma brucei rhodesiense* on the other hand, is responsible 6% of reported cases and is endemic in 13 countries in East and South Africa. In 2023, 24 cases of *rhodesiense* Human African Trypanosomiasis (HAT) were reported in four countries (Wendi *et al.*, 2025). In a study reported by Odebunmi (2024), Animal African Trypanosomiasis (AAT), was reported to have an overall prevalence rate of 27.3% in Nigeria with cattle being the most infected.

The African locust bean tree, *Parkia biglobosa* (Jacq.) is a perennial tree legume, belonging to the sub-family Mimosoideae and family Leguminosae. It is called *Dorowa* by the Hausa people of Northern Nigeria and *Igba* or *Irugba* by the Yoruba of Western Nigeria. It is known to occur in a diversity of agro-ecological zones ranging from tropical rain forests to arid zones in many countries of West Africa. It is a popular food plant that has been used as a source of food, medicinal agent, timber and is of high commercial value. The seed of *P. biglobosa* has been found to be rich in protein between 34 - 35% and Carbohydrates to be 20.70% (Alabi *et al.*, 2005). It was reported that the stem bark of *P. biglobosa* was successfully used in the treatment of many infectious diseases like diarrhea, severe cough, stomach ache, bilharziasis, etc. The seeds have been used in the treatment of arterial hypertension, the leaves against piles, amoebiasis, cough, etc. *Parkia biglobosa* has shown promise in treating trypanosomiasis. Studies have found that the methanolic leaf extract of *P. biglobosa* exhibits antitrypanosomal activity, killing *Trypanosoma evansi* at concentration of 500 µgml<sup>-1</sup> within nine hours (Shaba *et al.*, 2021). In vitro studies demonstrate the extract's ability to immobilize, reduce and kill trypanosomes in a concentration and time dependent manner. The extracts of *P. biglobosa* have also shown antimicrobial activity against various pathogens including *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* (Ajiboye and Hameed 2020). The methanolic extract and aqueous fraction of the stem bark of *P. biglobosa* has shown antibacterial potentials (Udobi *et al.*, 2010). It has also been reported to have anti-snake bite activities as well. *Parkia biglobosa* leaf extracts have been reported to have antiplasmodial activity thus giving an insight into its antitrypanosomal efficacy (Modupe *et al.*, 2011). Global usage of herbal preparations for treatment of ailments shows that approximately 80% of the world's population which is around 4 billion people use herbal medicine for some aspects of primary health care, In Nigeria, 85% of respondents have used herbal medicines in the last 2 years with middle income users being the highest (88.3%). Herbal medicines have effectiveness of 39.6% and affordability of 31.9% hence being major reasons for using herbal medicines in Nigeria. Safety concerns indicated that 57% of respondents believe herbal medicines is unsafe while 37% continue to take uncertified herbal medicines (Aina *et al.*, 2020). In this study, the therapeutic potentials of the aqueous seed extract of *P. biglobosa* in experimental trypanosomiasis is explored.

## **MATERIALS AND METHODS**

### **Sample Collection and Preparation**

The seeds of *P. biglobosa* (Jacq.) were obtained within Samaru Zaria and were identified in the Department of Biological Sciences, Ahmadu Bello University, Zaria by Mr. Umar Gallah with identification No. 2846 in the Departmental herbarium. Maceration method of extraction was employed. The seeds (600g) were pounded by pestle and mortar after which it was soaked in separating funnel using distilled water for 24hrs and then filtered. After filtration, the filtrate was poured into an evaporating dish to concentrate to dryness inside a water bath at 45°C– 50°C. The strain of *Trypanosoma brucei brucei* (Federer strain) was obtained from the Nigerian Institute of Trypanosomiasis Research (NITR) Kaduna. It was maintained by sub-passaging into a clean uninfected wistar (*Rattus norvegicus*) rat when established that parasitemia was high. The parasites in the blood were estimated according to the rapid “matching” method of Herbert and Lumsden (1976). A total of 30 Wistar rats of weights 170 – 230g were used for the study. They were purchased from NITR, Kaduna, Nigeria. They were housed in appropriate cages and were fed with grower’s mash (vital feeds) and were watered *ad libitum*.

### **Phytochemical Screening**

The aqueous seed extract was screened for nine classes of phytochemicals as described by Trease and Evans (1985). These included carbohydrates, cardiac glycosides, anthraquinones, saponins, steroids, triterpenes, flavonoids, tannins and alkaloids. Carbohydrates were screened using Molisch test, cardiac glycosides by Kella-killiani test using glacia acetate with traces of ferric chloride. Anthraquinones were tested using Bontrager’s test while frothing test was used for saponins; Shinoda test for flavonoids; lead sub-acetate test for tannins; alkaloids by Mayer’s test, Dragendorff’s and Wagner’s (Sofowora, 1993).

### **Acute Toxicity Test**

Nine rats were separated into three groups of three rats each. The three groups were administered the seed extract intraperitoneally with graded doses of 10, 100, 1000mg/kg/bw. The rats were observed for number of deaths and behavioral changes for seventy-two hours. Based on percentage survival rates, the second phase was carried out after 72hrs of the first phase. In the second phase, another set of three rats were divided into three groups with one rat per group and administered graded doses of the seed extract in 1,600, 2,900 and 5,000 mg/kg/bw. The LD<sub>50</sub> was calculated as the geometric mean of the highest non-lethal and the lowest lethal doses (Lorke, 1983).

### **Experimental Design**

The Wistar rats for the trial were separated into five groups of five rats each. The groups were administered with the extracts at 200, 300, and 500mg/kg/bw. There were the negative control group (Infected and not treated) and the positive control group (infected and treated with Diminazen acetate (Berenil®). There was the uninfected control group to monitor course of disease. Treatments commenced on the 3<sup>rd</sup> day post inoculation and administration was for five days (five doses) intraperitoneally except for the berenil® that was treated once intramuscularly at 3.5mg/kg/bw. Parasitemia was monitored. A matching technique in which microscopic fields were compared with a range of standard logarithmic values was employed (Herbert & Lumsden 1976). To

count the number of parasites in blood, a drop of blood was obtained on slide from animal's tail and covered with a cover slip. The wet mount on the slide was observed under x400 magnification. The number of trypanosomes per microscopic field was compared with those of the standard logarithmic table. The values which matched the microscopic observation were then converted to antilogarithm, from where the absolute number of trypanosomes per ml of blood was obtained. Blood samples from an animal, were collected into heparinized capillary tubes. One end of each tube was sealed with plasticine and spun at 2000g for five minutes in a microhematocrit centrifuge. The Packed Cell Volumes (PCVs) were determined with the aid of a micro-hematocrit reader, which gave the values as percentages (Barbara, 1980).

### Statistical Analysis

The statistical analysis was carried out using SPSS statistical package version 25.0. Mean values in all groups were compared using the two-way analysis of variance (ANOVA). Level of significance was set at 0.05 (Mukhtar, 2003).

### RESULTS

The phytochemical screening of the aqueous seed extract of *P. biglobosa* showed that the extract contains carbohydrate, cardiac glycosides, triterpenes, tannins and flavonoids. Anthraquinones, saponins, steroids and alkaloids were absent.

**Table 1.** Phytochemical compounds present in the aqueous seed extract of *P. biglobosa*

Phyto-compound	Remark
Carbohydrate	+
Cardiac glycosides	+
Anthraquinones	—
Saponins	—
Steroids	—
Triterpenes	+
Flavonoids	+
Tannins	+
Alkaloids	—

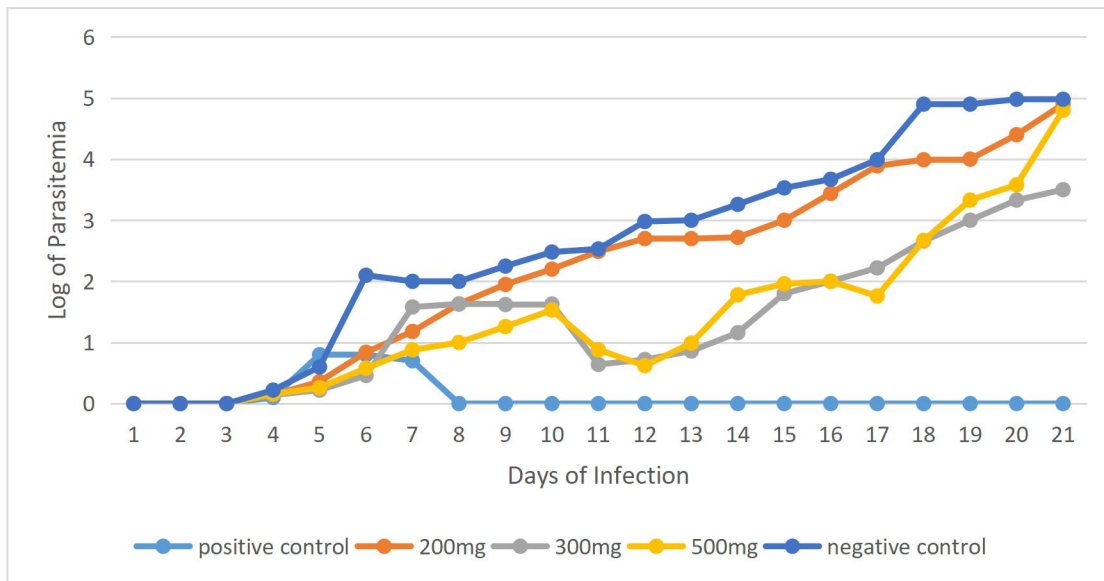
+ = Test Compound Present; - = Test Compound Absent

The acute toxicity (LD<sub>50</sub>) tests by intraperitoneal routes (ip) showed that the aqueous seed extract of *P. biglobosa* had an LD<sub>50</sub> of >5000mg/kg/ bw as shown in Table 2.

**Table 2.** Overall Acute Toxicity of Animals Tested with *Parkia biglobosa* Seed Extract

Experiment	Dose (mg/kg/BW)	Proportion of animals dead		
		After 24hrs	After 48hrs	After 2 weeks
Phase 1	10	0/3	0/3	0/3
	100	0/3	0/3	0/3
	1000	0/3	0/3	0/3
Phase 2	1600	0/1	0/1	0/1
	2900	0/1	0/1	0/1
	5000	0/1	0/1	0/1

Figure 1. demonstrates parasitemia pattern among various dose groups treated with the aqueous extract as well as the controls. The white albino rats were susceptible to infection with the strain of *Trypanosoma brucei* injected into them and were parasitaemic by the third day after inoculation. Following infection, parasite counts rose throughout the study period in all the groups, except the positive control group (diminazen) ( $P < 0.05$ ). Relapse of infection was not observed in any of the animals in this group.



**Figure 1.** Parasitemia Pattern after Treatment with Aqueous Crude Seed Extract of *Parkia biglobosa* and the Controls

The mean values for PCV between day one to fourteen is presented in Figure 2. The drop was significant ( $P \geq 0.05$ ) in all the groups except the positive control group. This trend in counts remained significantly lower than the first day of infection. This gave an inverse relationship between parasitemia and PCV. Treatment with the extract only improved the PCV slightly in the 500mg/kg/bw group as compared to the other groups. Animals given 3.5mg/kg/bw dose of the standard drug however were better off as their PCV remained higher.

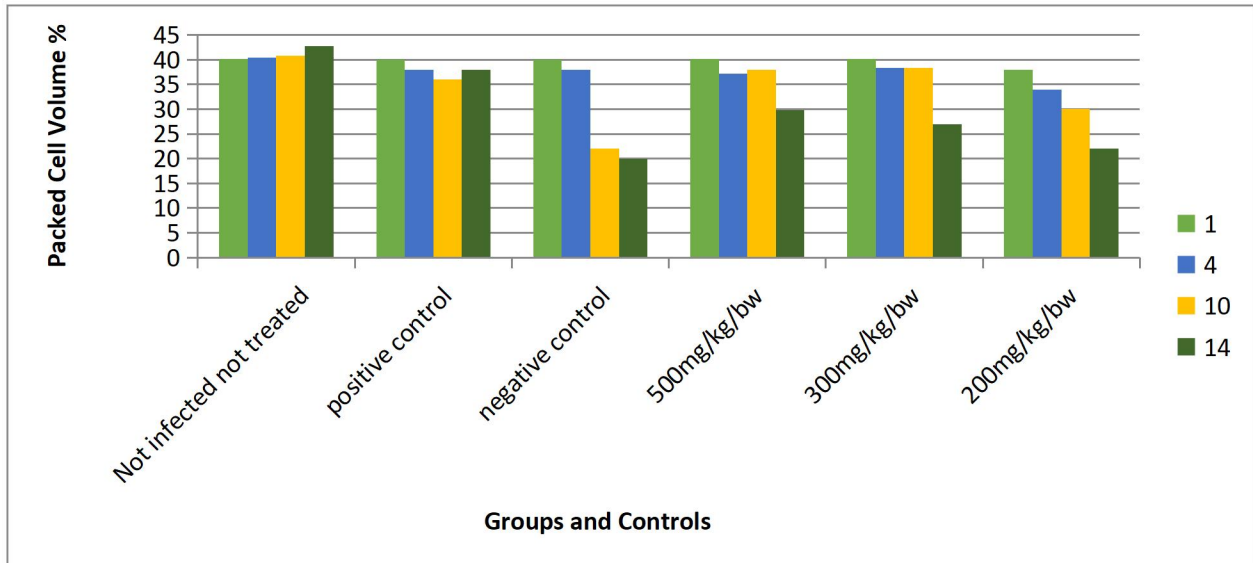
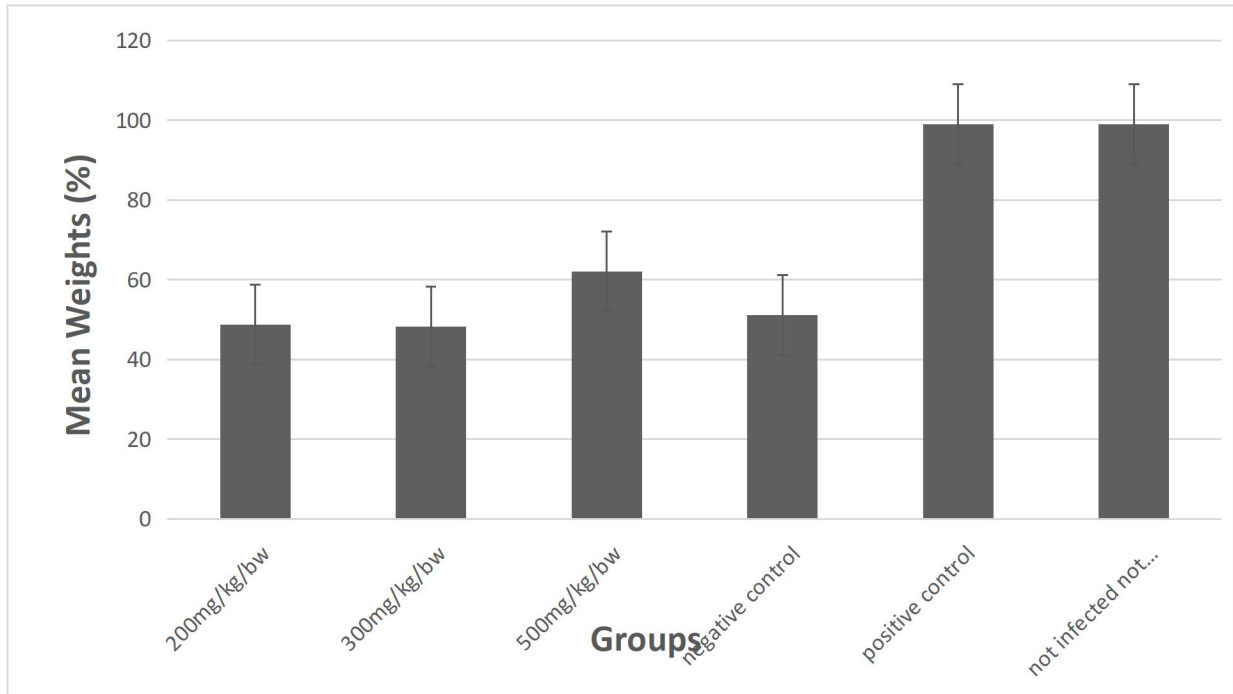
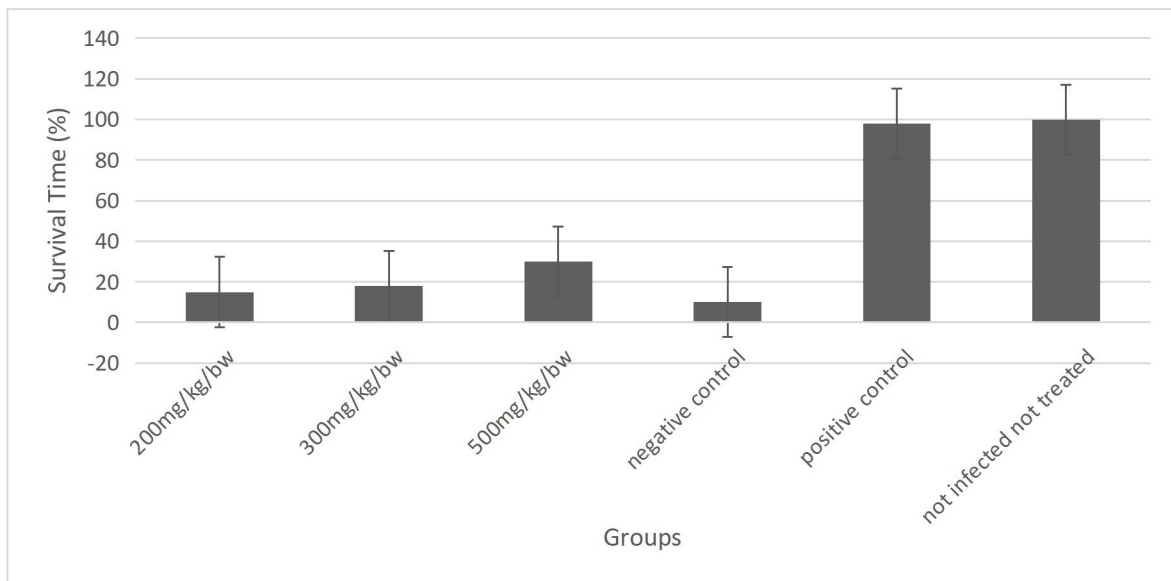


Figure 2. Packed Cell Volume for Groups and their Controls after treatment with Crude Seed Extract of *Parkia biglobosa*

Figure 3. Illustrates the mean weights of the rats during the study period. Infection and treatment with the crude aqueous seed extract of *P. biglobosa* did not grossly affect the weights of the rats as only slight decrease was observed within the various groups. The positive control group and the not infected and not treated groups however, had the highest mean weights followed by the 500mg/kg/bw. Furthermore, the extract did not clear rats of infection but prolonged the survival time in the 500mg/kg/bw dose group compared to the lower dose groups. The untreated group however had the least survival time. This is illustrated in Figure 4.



**Figure 3.** Mean Weights of Rats in the Various Treatment groups and the Controls during the study Period



**Figure 4.** Mean life Span of Rats in the Various Treatment groups and the Controls during the study Period

## DISCUSSION

The result of the acute intraperitoneal toxicity of the crude aqueous seed extract of *P. biglobosa* was found to be 5000mg/kg/bw as no mortality was recorded in any group of the experimental rats. In an acute toxicity test of *P. biglobosa* seed extract, Abdullahi *et al.*, (2025), documented it to be non-lethal at 5000mg/kg/bw. However, LD<sub>50</sub> should not be viewed as a fixed biological constant, because results can vary when the test is repeated or conducted in different laboratories due to various factors (Lorke, 1983). The preliminary phytochemical screening of the freeze-dried decoction of the aqueous extract of *P. biglobosa* revealed the presence of carbohydrate, cardiac glycosides, triterpenes, tannins and flavonoids. The therapeutic benefits of traditional remedies are often attributed to the presence of bioactive constituents present in the crude materials. Many secondary metabolites of plants' origin have been shown to have anti plasmodial activity hence may also have anti trypanosomal activities (Modupe *et al.*, 2011). Secondary metabolites are known to be effective anti plasmodials by a number of mechanisms such as elevating red blood cell oxidation and inhibiting the parasite's protein synthesis. Studies have shown that the leaves of *P. biglobosa* possess antioxidant activities which have been related to the presence of flavonoids and other phenolic compounds; these are known to counteract the oxidative damage induced by parasites. While it might be suggestive that this could be one of the mechanisms by which the aqueous seed extract of *P. biglobosa* exerts some of its antitrypanosomal activity, it is not possible to make such conclusions from this study. This issue is pursued in further studies. The white albino rats were susceptible to infection with the strain of *Trypanosoma brucei* injected into them and had shown infection on day three post infection. The infection was acute and terminated with 100% mortality except in the positive control group that recovered completely without relapse of infection. The extract did not clear rats of infection but prolonged the survival time in the 500mg/kg/bw dose group compared to the lower dose groups and the untreated groups. Similar findings were recorded by Adamu *et al.*, (2009) where it was recorded that the aqueous leaf extract of *Ocimum gratissimum* prolonged survival time in rats infected with *T. brucei brucei*. The inability of the extract to achieve an effective therapy might be as a result of inactivation of the active compounds *in vivo*. Infection did not grossly affect the weights of the rats as only slight decrease was observed this could be attributed to the components of the extract which is rich in proteins and carbohydrates. Anemia depicted by drop in PCV was found to accompany rise in parasitemia during course of infection. The negative correlation indicates an inverse relationship between PCV and parasitemia. This indicates that increase in parasitemia decreases the Packed Cell Volume of the infected rats. Several theories have been postulated to elucidate this inverse relationship. Among such postulations is that dead and dying trypanosomes release hemolytic factors into the animal's blood. As the parasites divide and increase in the blood and die, more of the hemolytic factors are produced leading to increased destruction of erythrocytes, hence the reduction in PCV (Morrison *et al.*, 2023). This agrees with data observed in rats experimentally infected with *T. brucei* (Timothy, 2004, Adamu *et al.*, 2009, Vilar-Pereira *et al.*, 2025). The weights of the rats was only slightly decreased this could be attributed to the components of the extract which is rich in proteins and carbohydrates similar observations was also corroborated by Abubakar and Dabo (2023) where the body weight of infected rats decreased compared to the control.

## CONCLUSION

Conclusively, the fact that the extract did not achieve an effective all-round therapy might be as a result of inactivation of the active compounds *in vivo*. While the results from this study are promising, more research is needed to confirm the efficacy and safety of *P. biglobosa* extract in treating trypanosomiasis in humans and animals. Further studies using other parts of *P. biglobosa* in combination with other plant materials and methods is recommended.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## REFERENCES

- Abdullahi, R. A., Muhammad B.F., and Abubakar, A (2025). Acute liver impact of aqueous seed extract of *Parkia biglobosa* in Experimental Rats. Sahel Journal of Life Sciences FUDMA 3(3):68- 70 Doi <https://doi.org/10.33003/Sajols-22025-030-09>.
- Abubakar, Z and Dabo N.T (2023). Erythrocytic, Enzymatic and Histological markers of oxidative stress in subacute and chronic stage infections in wistar rats (*Rattus norvegicus*) infected with *Trypanosoma brucei brucei*, Wiley Issue1 3590893.
- Adamu, M, Agbede,R and Nwosu (2009) Anti trypanosomal effects of aqueous extract Of *Ocimum gratissimum* (Lamiaceae) leaf in Rats infected wit *Trypanosoma brucei brucei*. African Journal of Traditional Complementary and Alternative Medicine 6(3): 262-267.
- Aina, O., Gautam, L., Simkhada, P., Hall, S, (2020). Prevalence determinants and Knowledge about herbal medicine and Non hospital utilization in South West Nigeria: A cross- Sectional Study. BMJ Open 10: e040769 doi 10:1136/bmj open
- Ajiboye, A.E and Hamed B.A (2020). Anti-microbial Activity of the Crude Extracts of *Parkia biglobosa* (jacq) Seeds in Selected Clinical Isolates. Journal of Biological Research and Biotechnology (Bio- Research) Vol 18. No 2 pp 1135-1146 ISSN (Print): 1595-7409.
- Alabi, D.A, Akinsulire, O.R and Sanyaolu, M.A (2005) Qualitative determination of Chemical and nutritional composition of *Parkia biglobosa* (jacq). Beth. African Journal of Biotechnology Vol 4(8) Pp 812-815.
- Barbara, A. B (1980). Hematology: principles and procedures. Henry Kempton publishers London.71- 83.
- De Geir, J., Cechi, G., Paone, M., Dede, P., Zhao, W (2020). The continental Atlas of tsetse and African Trypanosomiasis in Nigeria. Acta tropica 204: 105328.doi 10/1016/J. acta tropica 2020105328
- Herbert and Lumsden, W.H.R. (1976) A rapid 'matching' method for estimating the host's parasitemia. *Experimental Parasitology*, 40:427-431.
- Lorke, D (1983) A new approach to practical toxicity testing. Archives of toxicology 54: 257-287.

- Modupe B., Noel, W and John, A (2011) Antiplasmodial activities of *Parkia biglobos* leaves in *in vivo* and *in vitro* studies. *Annals of Biological Research* 2(4):8-20.
- Morrison L.J., Stekete, P.C., Tettey, M.D., Mathew. (2023). Pathogenicity and Virulence of African Trypanosomes: from Laboratory Models to Clinically Relevant Hosts. *Virulence* Vol.14 No 12150445.
- Mukhtar, B. F (2003). *An Introduction to Biostatistics*. 1<sup>st</sup> Edition. Samarid Publishers. ISBN 9783543288.
- Odebunmi, O.E., Ibeachu, C., Chukwudi, C.U (2024). Prevalence of Human and Animal African Trypanosomiasis in Nigeria: A Scoping Review. Reprint <https://doi.org/10.21203>.
- Omonona. A.O., Abioye, S.O., Odeniran, P.O., and Ademola, I.O (2020). Distribution of Tsetse flies and its Trypanosoma species in Old Oyo National Park, Nigeria. *The Zoologist* 18: 33-38, ISSN,1596972X.
- Shaba, P., Kurade, M. P., Bhanupraksah, V., and Singh, R.K (2021). Evaluation of Methanolic leaf Extract of *P.biglobosa* (African Locust Bean, Jacq, Benth) Leaves against *Trypanosome evansi*. *Technological Innovation Pharmaceutical Research* Vol. 10, 136- 144. <https://doi/10.9734/bpi/tipr/v10/72180-2>.
- Sofowora, A (1993) *Medicine plants and traditional medicine*, Africa Spectrum Books Limited, Ibadan, Nigeria Pp. 150 – 156.
- Stijlemons, B., Andres, B., Rodriguez, A., Jin, B., Radwanska, M., Magez, S (2024). The diagnosis and treatment of Protozoan diseases Science Direct. Chapter 4pp95-148.
- Trease, G.E. Evans, W.C. (1989) *Introduction and General methods in Pharmacognosy* 12<sup>th</sup> Edition published by Alden Press Oxford London Pp 49 – 474.
- Timothy, B (2004). Trypanocidal, Phytochemical and some Toxicological Evaluation of Aqueous and metabolic Extract of *Terminalia avecinoides* and *Terminalia mollis*. *MSc Thesis Department of Biochemistry Ahmad Bello University Zaria, Nigeria*.
- Udobi, C.E. Onaolapo, J.A., Agunu, A. (2010) Antibacterial potentials of the methanolic extract and the aqueous fraction of the stem bark of the African Locust Bean (*Parkia biglobosa*). *European Journal of Scientific Research* Vol 43(4) Pp 590-597.
- Vilar-pereira, G., Eastano- Barrios, L.M., Pereira, I.R., Da Silva Pinheiro, A.P., do Espirito Santo Protasio, da Silva,T., Hernaandez-Valesco, L.L (2025). Combined benzimidazole and Pentoxifylline therapy improves behavioral cognitive changes in association with the regulation of systemic inflammatory profile in chronic experimental chagas disease. *Plos One* 20 (11): e0334708.doi: 10:1371/jor

Wendi, E.M., Tobolowsky., Priotto, G., Franco, R.J., Chancey, R (2025). Notes from the Field. Rhodesiense Human African Trypanosomiasis Sleeping sickness in a traveler returning from Zimbabwe- United States. August 2024. Morbidity and Mortality Weekly Report (MMWR) Weekly /March 20, 2025/74(9):158-159.

World Health Organization (1995) Control and Surveillance of African Trypanosomiasis. Report of The World Health Organization Expert Committee WHO Technical Report Series No. 881.