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# Efficacy of the Whole Plant Ethanol Extract of *Phyllanthus Amarus* on *Trypanosoma Brucei*-Induced Pathology in Wistar Rat

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**Abstract:** African animal trypanosomiasis is one of the most important protozoal diseases affecting animal health and production. This study was conducted to assess the phytochemical composition of *Phyllanthus amarus* ethanol extract and to assess its safety and efficacy on *Trypanosoma brucei*-induced pathology in Wistar rats. The plant sample was obtained from around the residential area in Ahmadu Bello University, main campus, Zaria, Nigeria, and subjected to phytochemical screening following standard procedures. Sixteen Wistar rats were divided into five groups, each rat in the groups receiving 0.1 mL of $10^6$ *T. brucei* trypomastigotes. After three days of patency, rats in groups III to V were given different plant extract treatments for four days, while rats in groups I and II served as negative and positive controls. Following treatment, clinical parameters, parasitaemia, gross pathology and biochemical analysis were observed and recorded. The results showed that the ethanol extract contained primary constituents such as alkaloids, phenols, cardiac glycosides, saponins, carbohydrates, triterpenes, anthraquinones, tannins, and steroids. The extract was found to be non-toxic and orally safe for Wistar rats. It showed moderate suppressive ability against parasitaemia, ameliorating fever, weight loss, and anaemia in *Trypanosoma brucei*-infected rats. It also demonstrated significant modulatory activity in reducing internal organ pathologies, liver enzyme pathology, and oxidative stress in the heart, kidney, and spleen. The study highlights the safety and efficacy of *Phyllanthus amarus* ethanol extract in managing African animal trypanosomiasis.

**Keywords:** *Phyllanthus amarus*, Phytoconstituents, African Animal Trypanosomiasis, Wistar Rat Model, Ameliorative and Modulatory Activities, Anti-Oxidative Stress Prospect.

**RESEARCH PAPER**

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## INTRODUCTION

Many species of the *Trypanosoma* parasite are responsible for the parasitic disease known as African trypanosomiasis, also called sleeping sickness. According to the Food and Agricultural Organisation of the United Nations (FAO, 2008; Steverding, 2008, 2010), this disease poses a significant health burden in sub-Saharan Africa. The disease has a detrimental impact on human and animal populations, resulting in substantial livestock losses and food insecurity. Infected individuals experience the onset of neurological and behavioural symptoms, which can lead to sleep disturbances and, ultimately, mortality (FAO, 2008; Steverding, 2008, 2010; WHO, 2023).

The condition is characterised by a range of pathogenic clinical manifestations, such as increasing anaemia, elevated body temperature, the accumulation of fluid in tissues (oedema), severe weight loss (emaciation), tissue impairment, and ultimately, mortality if left untreated (Toya 2010; Chamond *et al*., 2010; CDC, 2023). Several recent investigations have demonstrated that infection with the parasite is associated with several reproductive abnormalities in animals (Okubanjo *et al.*, 2014; Wada *et al*., 2016). In the female population, various reproductive issues can occur, including irregular estrous cycles, abortion, stillbirth, neonatal mortality, cystic degeneration of the ovaries, decreased conception rate, and irregularities in the synthesis of reproductive hormones (Chamond *et al*., 2010; Rodrigues *et al*., 2013; Silva *et al*., 2013). In males, the following manifestations may occur: delayed onset of puberty, decreased libido, swelling of the scrotum, deterioration of testicular function, reduced quality of semen, low sperm count, absence of

ejaculation, and depletion of sperm reserves in the epididymis and gonads, resulting in infertility and sterility (Wada *et al*., 2016).

To treat African trypanosomiasis, chemoprophylactic or chemotherapeutic medicines are commonly utilised. However, drug scarcity, high costs, side effects, and toxicity make treating African trypanosomiasis difficult (Wurochekke *et al*., 2004, 2005; Ayawa *et al*., 2021). Furthermore, drug resistance is a significant concern that threatens to undermine global efforts to control or eradicate African Trypanosomiasis. Considering this growing problem in treating African trypanosomiasis, it is crucial to search for readily available, less toxic, and cheaper chemotherapeutic agents for treating African trypanosomiasis (Atawodi *et al*., 2003; Mann *et al*., 2009; Ayawa *et al*., 2021).

*Phyllanthus amarus*, a widely distributed leafy herbal plant, has garnered attention for its therapeutic properties in several infectious and non-infectious disorders throughout different regions worldwide (Mazumdar *et al*., 2022).

**Figure 1: *Phyllanthus amarus***

The efficacy of Phyllanthus amarus has been thoroughly examined in several studies, indicating its potential therapeutic use in the management of *Plasmodium* infection, antimicrobial, dropsy, diabetes, cancer, HIV, jaundice, diarrhoea, dysentery, intermittent fevers, urinogenital disorders, scabies, ulcers, and COVID-19 (Adegoke *et al*., 2010; Ajala *et al*., 2011; Mazumdar *et al*., 2022). However, to our knowledge, no published work on the anti-trypanosomal properties of *Phyllanthus amarus* is available. Therefore, this study was designed to assess the phytochemical constituents and to investigate the pharmacological prospect of the whole plant ethanol extracts of *Phyllanthus amarus* in the Wistar rat model to achieve the following sub-objectives: i) to determine the qualitative and quantitative phytochemical constituents of the whole plant extracts of *Phyllanthus amarus*, ii) to determine the pharmacological activities against *Trypanosoma brucei*-induced parasitaemia and clinical pathology in the Wistar rat model, and iii) to determine pharmacological activities against *Trypanosoma brucei*-induced oxidative stress and liver pathology in the Wistar rat model.

# METHODOLOGY

### Ethical Statement

The experimental methodology and animal care were done per the guidelines of the Animal Welfare Committee on Animal Use and Care at Ahmadu Bello University (ABU) in Zaria, Nigeria.

### Collection and Authentication of Plant Material

The plant was collected from around the University staff quarters, Ahmadu Bello University (ABU), Zaria. Identification was done at the Herbarium Unit, Department of Botany, ABU. Zaria

### Extraction of Plant Material

The leaves were cleaned and taken to the laboratory, cut into pieces, air-dried at room temperature for 14 days, and ground into a fine powder using laboratory mortar and pestle. The powder of *Phyllanthus amarus* was cold macerated with ethanol (absolute) at room temperature for 48 hours. This was then filtered using filter paper (Whatman size No. 1), and the filtrate was evaporated to dryness in the water bath at $30^0$ C to a dry extract of 43.49g. The portion of the extract was used for phytochemical screening, and the remaining was kept in an air-tight bottle until it was reconstituted for administration.

### Phytochemical Analysis

The Phytochemical screening was performed using established methods as described by Harborne (1973), Trease and Evans (1989), and Sofowora (1993) to identify the presence of bioactive constituents such as

alkaloids, flavonoids, anthraquinone, carbohydrates, tannins, unsaturated steroids and triterpenes, cardiac glycosides, and saponin. Quantitative determination of phytoconstituents was carried out as per standard procedures (Solich, 1992; Zhishen *et al.*,1999; Marinova *et al*., 2005; Ainsworth and Gillespie, 2007; Makkar *et al.*, 2007; Shamsa *et al*., 2007; Sakulpanich and Gritsanapan, 2009; Sharief *et al*., 2014**).** The assay was conducted at the Department of Pharmacognosy and Drug Development in the Faculty of Pharmaceutical Sciences at ABU, Zaria.

**Source of Wistar Rats and *Trypanosoma Brucei***

Twenty healthy Wistar rats aged (both sexes) between 7-8 weeks and weighing between 100-120g were purchased and housed at the animal house, faculty of Pharmaceutical Science, Ahmadu Bello University. Rats were housed under standard laboratory conditions. Rats were allowed to acclimatise for one week before the commencement of the experiment. The animals were fed with grower's marsh; water was supplied in plastic drinkers.

*Trypanosoma brucei* (Federe strain) was obtained from the Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna State, Nigeria.

**Acute Toxicity of the Extract**

The lethal dose (LD50) of the ethanol leaf extract of *Phyllanthus amarus* was determined in rats using the method described by OECD (2002) guidelines. Four Wistar rats weighing between 93g and 100g were used for the $LD_{50}$.

**Experimental Design**

Sixteen Wistar rats were divided into five groups, I, II, III, IV, and V, in a simple, complete, randomized design (Figure 2). Each rat belonging to subgroups I through V received an inoculum of 0.1 mL containing $10^6$ *T. brucei* trypomastigotes. After three days of patency, the rats in groups III to V were subjected to different treatments, 200, 400, and 800 mg/kg/day of the plant extract for four days, while rats in groups I and II served as the negative and positive control, respectively.

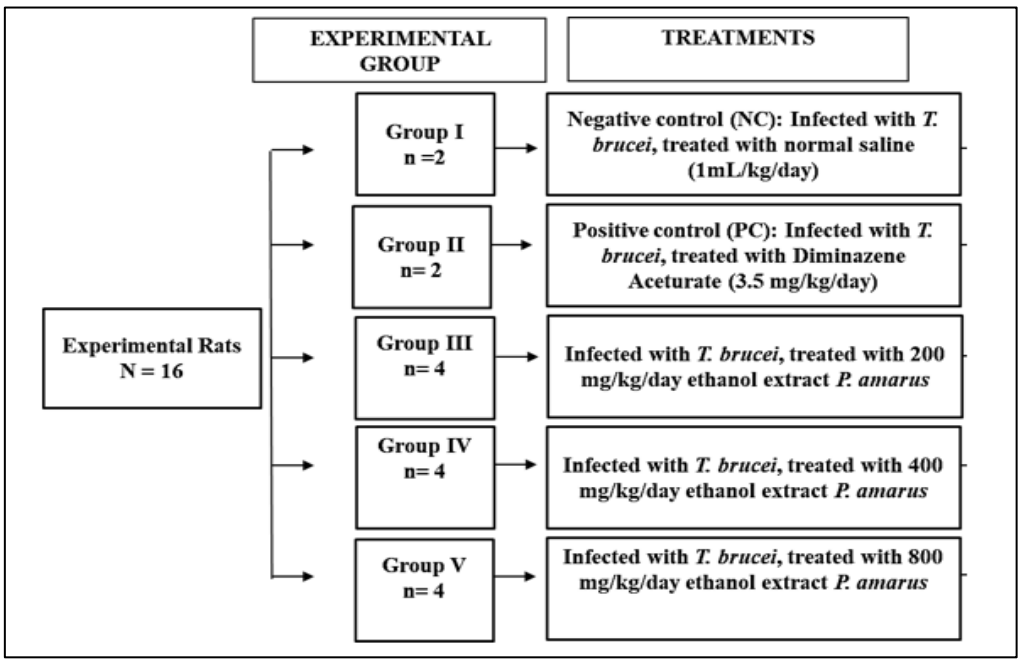


**Figure 2: A simple CRD Experimental Design**

**Observation of Clinical Signs**

Clinical signs investigated during the study included rectal temperature, body weight, condition, weakness, dullness, rough hair coat, packed cell volume, and leucocyte count.

**Determination of Rectal Temperature**

The rectal temperature for each experimental animal was assessed daily between 8:00 a.m. and 9:00 a.m. using a digital thermometer. The thermometer was inserted into the rectum and tilted to an angle to touch the rectal mucosa. After a beep sound, the thermometer was removed, and body temperature changes were read and recorded in degrees centigrade (°C).

**Determination of Weight**

A sensitive weighing balance was used to determine the weights of the animals daily, which were recorded in grams (g).

**Determination of the Level of Parasitaemia**

The parasitemia level was estimated using a wet mount or direct smear method (Herbert and Lumsden, 1976). The procedure involved pricking the tip of the animal's tail using a lancelet or surgical scissors. Then, a drop of the blood was obtained on a clean glass slide and covered using a cover slip, and then it was viewed using a binocular microscope under an objective lens (×40).

**Determination of Packed Cell Volume (PCV)**

The PCV was determined using a standard microhaematocrit centrifugation technique, and the values were read by a Hawksley microhaematocrit reader (Gellman Hawksley Ltd, (England). The packed cell volume was determined on the 3rd day after injection.

**Determination of Haemoglobin Concentration**

Haemoglobin (g/dl) was estimated by calculation. Its value approximates one-third (1/3) of the PCV value.

**Determination of Total Plasma Protein**

The protein level was determined by using a refractometer. The procedure involved dabbing the unbroken end of the fragment containing the plasma onto the refractometer surface and looking through the eyepiece to read the result in g/dL.

**Organ Collection and Homogenisation**

The organs (Kidney, Liver, Spleen, Heart) of experimental Wistar rats were harvested and processed separately to determine their oxidative status. They were prepared independently, weighed, and placed in a buffered solution (pH 7.4). Each organ was homogenised using a mortar and pestle and centrifuged at 800 x g for 10 minutes. The supernatant was dispensed into plain bottles and stored at -80°C before analysis.

**Determination of Malonaldehyde Level (MDA)**

Lipid peroxidation, as evidenced by the formation of TBARS, was measured using the MDA Assay kit. The method is based on the principle of lipid peroxidation generating peroxide intermediates, which, upon cleavage, release malondialdehyde, a product that reacts with thiobarbituric acid. The product of the reaction is a complex colour which absorbs light at 535nm and can hence be measured.

**Determination of Aspartate Transaminase (AST) and Alanine Transaminase (ALT)**

Aspartate Transaminase (AST) and Alanine Transaminase (ALT) were determined using commercial Randox kit chemicals in the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria.

**Data Analysis**

Multiple line graphs compared the mean daily rectal temperature and parasitaemia level. Way Analysis of Variance (ANOVA) was used to compare inter-group mean values of clinical, haematological, and oxidative stress parameters. LSD post hoc test was used to correlate between mean haematologic values, parasitaemia, and oxidative stress parameters. Data analysis was carried out using IBM SPSS statistics version 20. The values of $p \leq 0.05$ were considered statistically significant.

## RESULTS AND DISCUSSION

**Phytochemical Constituents**

Table 1 shows the qualitative phytochemical constituents of *Phyllanthus amarus* using ethanol extract. The results show that the ethanol extract of *Phyllanthus amarus* contains alkaloids, phenols, cardiac glycosides, saponins, carbohydrates, triterpenes, anthraquinones, flavonoids, tannins, and steroids (Table 1).

**Table 1: Qualitative phytochemical constituents of *Phyllanthus amarus* using ethanol extract**

| S/no | Phytoconstituents | Test | Inferences |
|---|---|---|---|
| 1 | Alkaloids | Dragendorff test | + |
| 2 | Cardiac Glycosides | Keller-Kiliani test | + |
| 3 | Saponins | Frothing test | + |
| 4 | Phenolic compounds | Lead acetate test | + |
| 5 | Tannins | Ferric Chloride test | + |
| 6 | Steroids | Salkowiski test | + |
| 7 | Carbohydrates | Molisch test | + |
| 8 | Flavonoids | Shinoda test | + |
| 9 | Terpenoids | Liebermann Burchard test | + |
| 10 | Anthraquinones | Bontrager's test | + |
| | + = Present | - = Absent | |

Quantitatively, the constituents include Alkaloids (33.87±0.48 mg/g), phenols (29.37±0.06 mg/g), cardiac glycosides (28.10±0.01 mg/g), saponins (16.37±0.03 mg/g), carbohydrates (15.87±0.06 mg/g), triterpenes (14.33±0.03 mg/g), anthraquinones (10.86±0.02 mg/g), tannins (5.78±0.17 mg/g), and steroids (5.53±0.04 mg/g) (Figure 3).

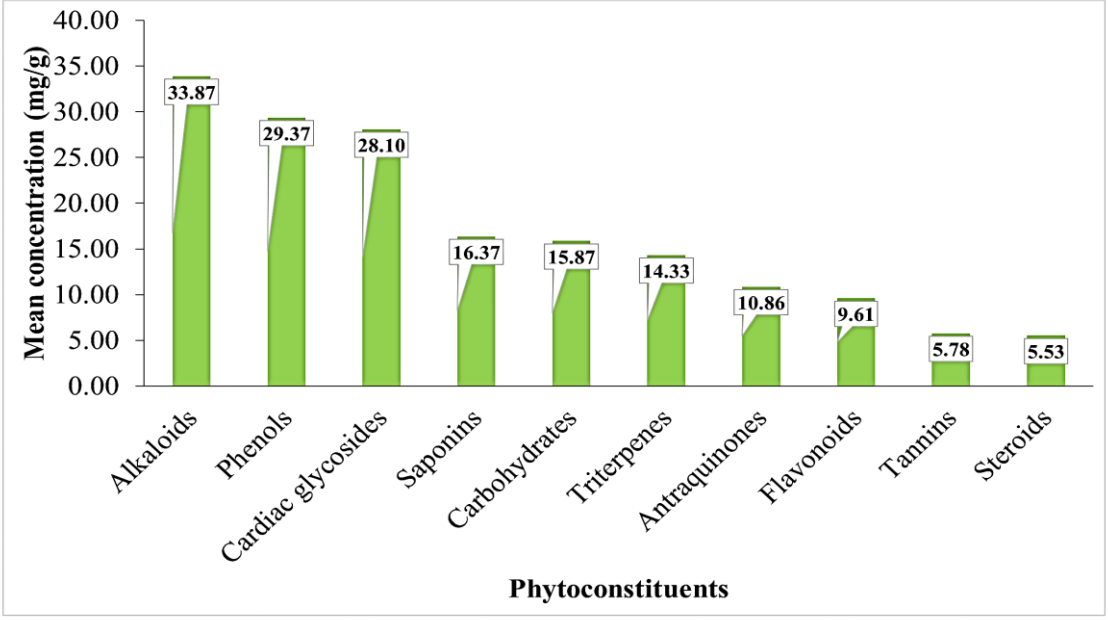


**Figure 3: Quantitative phytochemical analysis of the ethanol extract of *Phyllanthus amarus* depicting the concentration of most important phytoconstituents detected using a UV-visible-light spectrophotometer**

**Oral Toxicity Studies of *Phyllanthus Amarus* in Wistar Rat**

The clinical and behavioural observation of the acute toxicity studies of ethanol extract of *Phyllanthus amarus* in Wistar rats is shown in Table 4.2. All rats that received lower doses (2000 mg/kg body weight) and higher doses (5000 mg/kg body weight) of whole plant ethanol extracts of *Phyllanthus amarus* showed mild signs of toxicity, mild agitations, piloerections, reduced water intake, increased feed intake, urination, and sleepiness characterised their behavioural responses at 24 hours, 48 hours and 168 hours. There was no mortality recorded. There were no signs of diarrhoea, tremor, and abdominal writhing observed. The median lethal dose was calculated to be greater than 5000 mg/kg (Table 2).

**Table 2: Clinical observation and behavioural response of Wistar rat subjected to toxicity studies of the ethanol extract of *Phyllanthus amarus***

| | First group, 2000 mg/kg body weight (n=2) | | | | Second group, 5000 mg/kg body weight (n=2) | | | |
|---|---|---|---|---|---|---|---|---|
| **Parameters** | **24 hours** | **48 hours** | **72 hours** | **168 hours** | **24 hours** | **48 hours** | **72 hours** | **168 hours** |
| **Agitation** | + | + | + | + | + | + | + | + |
| **Convulsion** | × | × | × | × | × | × | × | × |
| **Piloerection** | + | + | + | + | + | + | + | + |
| **Stretch** | × | × | × | × | × | × | × | × |
| **Reduced water intake** | + | + | × | × | + | + | + | × |
| **Defecation** | + | + | + | + | + | + | + | + |
| **Diarrhoea** | × | × | × | × | × | × | × | × |
| **Urination** | + | + | + | + | + | + | + | + |
| **Abdominal writhing** | × | × | × | × | × | × | × | × |
| **Tearing** | × | × | × | × | × | × | × | × |
| **Reduced feed intake** | + | + | × | × | + | + | × | × |
| **Sleepiness** | + | + | + | + | + | + | + | x |
| **Tremors** | × | × | × | × | × | × | × | × |
| **Ptosis** | × | × | × | × | × | × | × | × |
| **Mortality** | × | × | × | × | × | × | × | × |

**+ Observed, × Not observed**

**The Pharmacological Activity of Ethanol Extract of *Phyllanthus Amarus* on the Mean Parasitaemia Counts in *T. Brucei*-Induced Pathology in Wistar Rat**

Following patency of 3 days after inoculation, all rats were either non-treated, treated with standard drug, and or treated at varying dosages of ethanol extracts of *Phyllanthus amarus* for four days. The group administered an extract dose of 400 mg/kg, which resulted in a significant ($p < 0.05$) suppression in parasitaemia by three days post-treatment (day 7) in comparison with the positive control and the negative control. However, by day four post-treatment (day eight post-infection), the parasitaemia level resurges significantly ($p < 0.05$) in the negative control but at a moderate level in the positive control and those treated with the plant extract. (Figure 4).

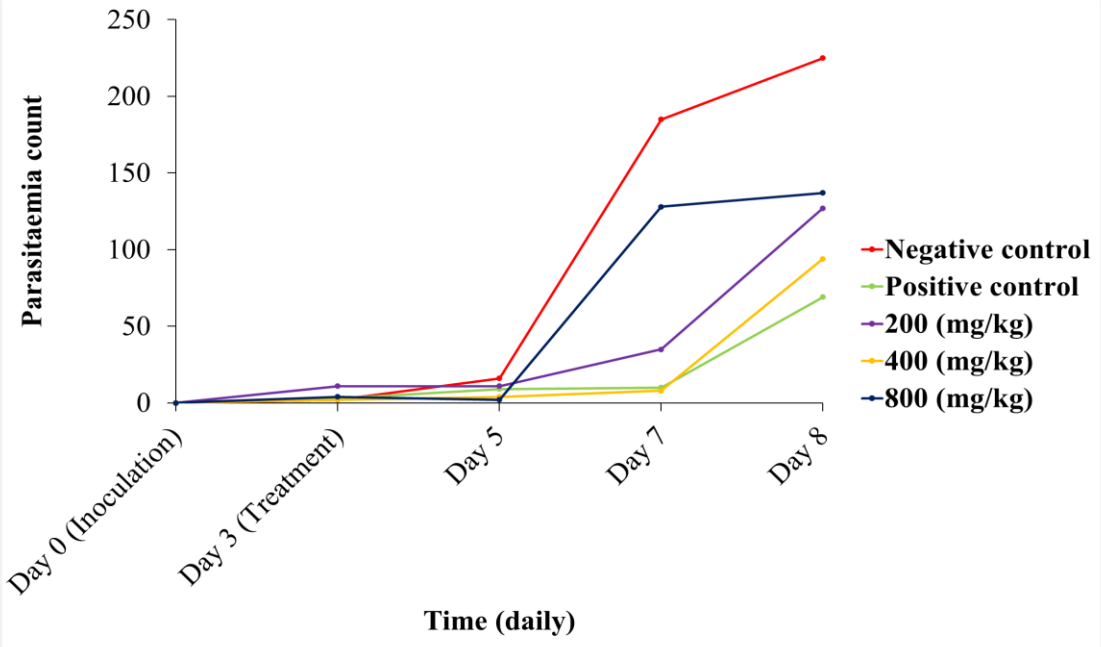


**Figure 4: Activity of *Phyllanthus amarus* ethanol extract on the parasitemia level against *Trypanosoma brucei*-induced parasitaemia in the Wistar rat model. Negative Control (Normal Saline), Positive Control (Diminazene Aceturate 3.5 mg/kg)**

**Pharmacological Activities of Ethanol Extract of *Phyllanthus Amarus* on the Clinical and Haematological Symptoms in *T. Brucei*-Induced Pathology in Wistar Rat**

Figure 5 (a-b) is a radar chart showing the activities of *Phyllanthus amarus* ethanol extract on the rectal temperature and live weight of *Trypanosoma brucei*-infected Wistar rat three days pre-treatment and four days post- treatment. There was a significant variation in the mean rectal temperature across all the treatments. The rectal temperature of the extract-administered groups, including the positive control, significantly reduced ($p < 0.05$) from their baseline 3-day pre-treatment values. However, only the negative control showed a significant rise in rectal temperature compared to the treated groups (Figure 5a). Furthermore, mean live body weights in the positive control and all groups administered the plant extracts did not differ significantly in live body weights from their baseline pre-treatment values. Only the negative control significantly reduced ($p < 0.05$) body weights compared to the other experimental groups (Figure 5b).

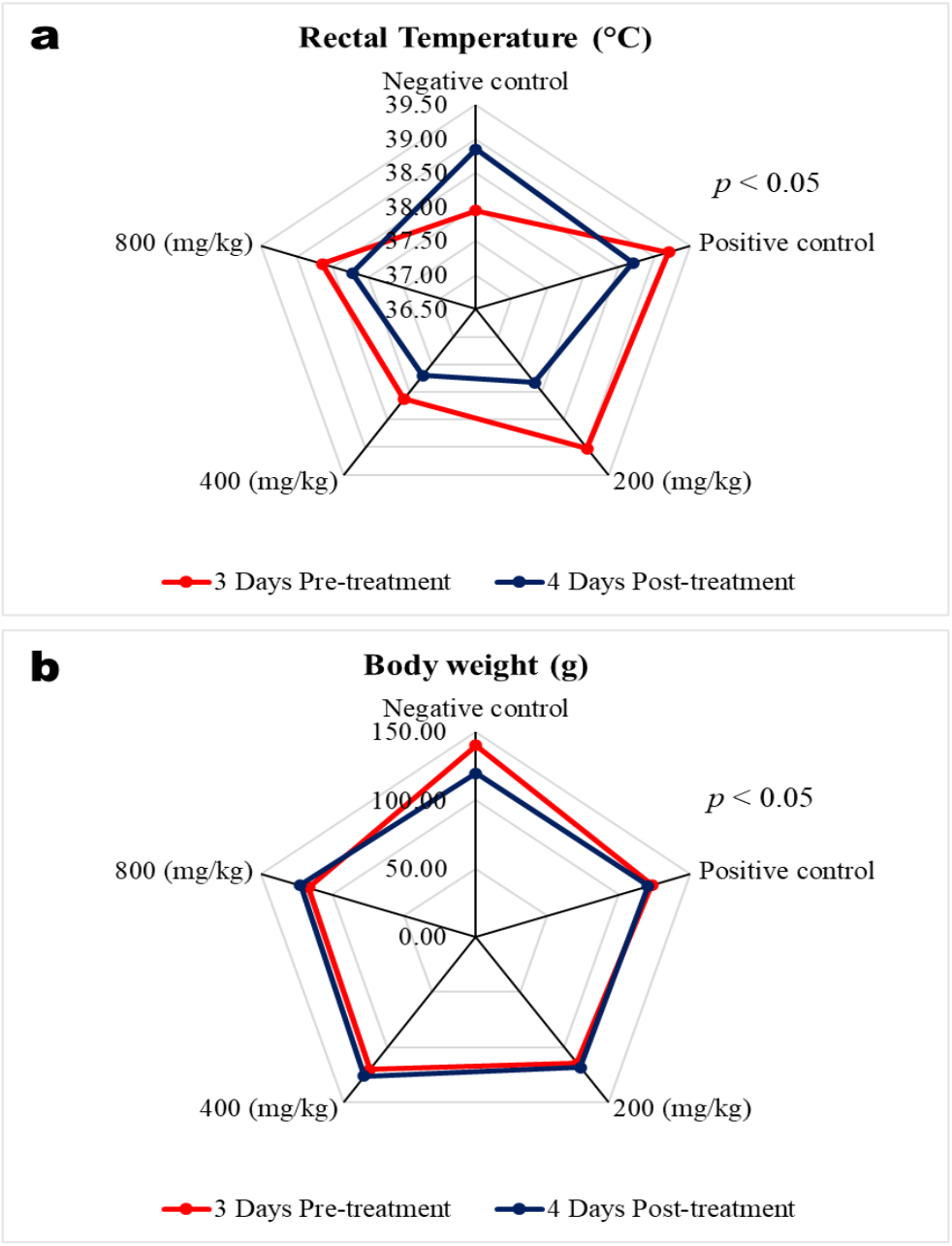


**Figure 5: Activity of ethanol extract of *Phyllanthus amarus* on the rectal temperature (a) and live body weight changes against *Trypanosoma brucei*-induced clinical pathology in the Wistar rat model. Negative Control (Normal Saline), Positive Control (Diminazene Aceturate 3.5 mg/kg)**

The mean change in haematological indices of *Trypanosoma brucei*-induced pathology in rats treated daily for four days with ethanol extract of *Phyllanthus amarus* is also shown in Figure 6 (a-c).

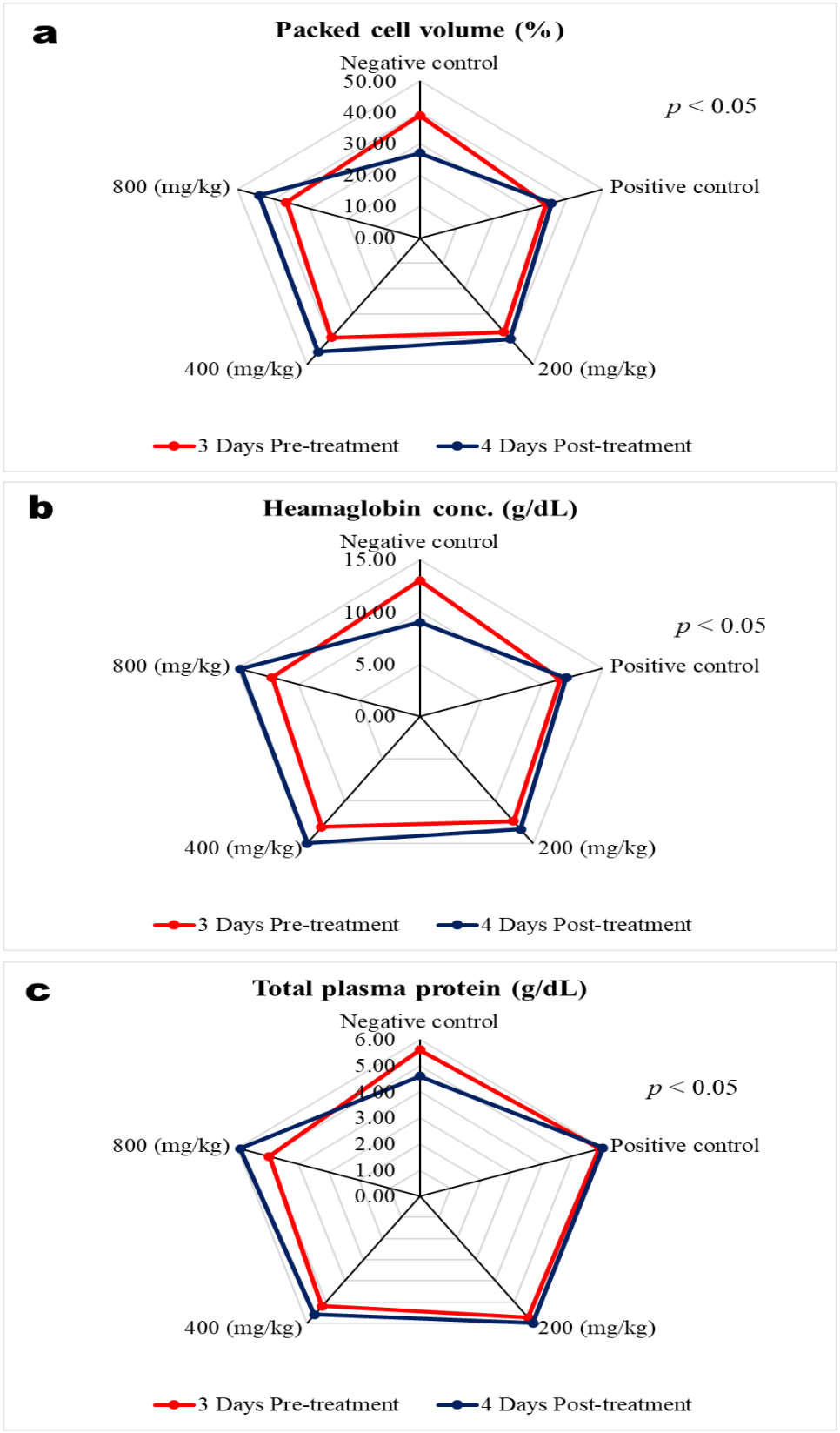


**Figure 6 (a-c): Activity of ethanol extract of *Phyllanthus amarus* on the packed cell volume (a), haemaglobin concentration (b), and total plasma protein (c) against *Trypanosoma brucei*-induced haematological changes in the Wistar rat model Negative Control (Normal Saline), Positive Control (Diminazene Aceturate 3.5 mg/kg)**

Following a four-day treatment at 200, 400, and 800 mg/kg/day, there was a significant increase in the PCV of rats administered 400 and 800 mg/kg/day of the extract, and this differed significantly ($p < 0.05$) from those of the positive and the negative controls and also when compared with their pre-treatment baseline values (Figure 6a). A similar trend was observed for the haemoglobin concentration (Figure 6b).

Similarly, the total plasma protein values of the following 4-day treatment did not significantly differ from the 3-day pre-treatment values for the groups treated with 200 and 400 mg/kg/day of the plant extract. However, the group treated with 800 mg/kg/day showed a significant increase ($p < 0.05$) in plasma protein. At the same time, in the negative control, there was a significant reduction ($p < 0.05$) in the mean plasm protein compared to all the other experimental groups following the four-day treatment period (Figure 6c).

**Pharmacological Activities of Ethanol Extract of *Phyllanthus Amarus* on the Weights of Internal Organs of *T. Brucei* Induced Pathology in Wistar Rat**

The pathological changes in the weights of internal organs of Wistar rats following treatment with *Phyllanthus amarus* plant ethanol extracts are presented in Table 3. The data show significant changes in the weights of kidneys, lungs, liver, heart, and spleen following *T. brucei*-induced pathology. A significant increase ($p < 0.05$) was observed in the weight of the kidney of the negative control and 400 mg/kg treated group. However, there was a significant reduction ($p < 0.05$) in the weight of kidney-positive control and 200 and 800 mg/kg treated groups (Table 3). For the lung, post-treatment, and post-mortem findings showed a significant reduction ($p < 0.05$) in the weight of the heart in the negative and positive control groups when compared with the *Phyllanthus amarus* ethanol extract treated groups (Table 3). A significant reduction ($p < 0.05$) in the weight of the liver was observed in the group treated with 200 and 800 mg/kg *Phyllanthus amarus* ethanol extract when compared with the negative and positive controls (Table 3). Based on the weight of the heart, a significant reduction ($p < 0.05$) was observed in the positive control, 200, 400, and 800 mg/kg *Phyllanthus amarus* ethanol extract treated groups (Table 3). A significant reduction ($p < 0.05$) in the weight of the spleen was observed in the positive control group treated with 200 and 800 mg/kg *Phyllanthus amarus* ethanol extract when compared with the negative and 400 mg/kg treated group (Table 3).

**Table 3: Pharmacological activities of ethanol extract of *Phyllanthus amarus* on the weight changes in internal organs of *T. brucei*-induced pathology in Wistar rat**

| | Kidneys | Lungs | Liver | Heart | Spleen |
|---|---|---|---|---|---|
| **Negative control** | $1.30\pm0.10^{a}$ | $1.60\pm0.30^{b}$ | $10.05\pm1.05^{a}$ | $0.85\pm0.35^{a}$ | $2.40\pm0.20^{a}$ |
| **Positive control** | $1.00\pm0.40^{b}$ | $1.50\pm0.20^{c}$ | $8.75\pm0.75^{b}$ | $0.70\pm0.00^{b}$ | $1.75\pm0.15^{c}$ |
| **200 (mg/kg)** | $1.00\pm0.15^{b}$ | $1.80\pm0.26^{b}$ | $7.77\pm0.38^{c}$ | $0.73\pm0.12^{a}$ | $1.87\pm0.54^{bc}$ |
| **400 (mg/kg)** | $1.20\pm0.07^{a}$ | $2.15\pm0.15^{a}$ | $9.08\pm0.34^{ab}$ | $0.75\pm0.09^{a}$ | $2.48\pm0.25^{a}$ |
| **800 (mg/kg)** | $0.98\pm0.08^{b}$ | $1.70\pm0.28^{b}$ | $7.33\pm0.15^{c}$ | $0.78\pm0.09^{a}$ | $2.00\pm0.12^{b}$ |
| ***P*-values** | 0.047 | 0.043 | 0.012 | 0.048 | 0.043 |

**Means (with standard error) with different superscripts along the columns differed significantly ($P < 0.05$)**

Furthermore, a descriptive line chart showing the significant changes in the mean weight changes of internal organs of rats following treatment with *Phyllanthus amarus* plant ethanol extracts, as explained in Table 3, is shown in Figure 7.

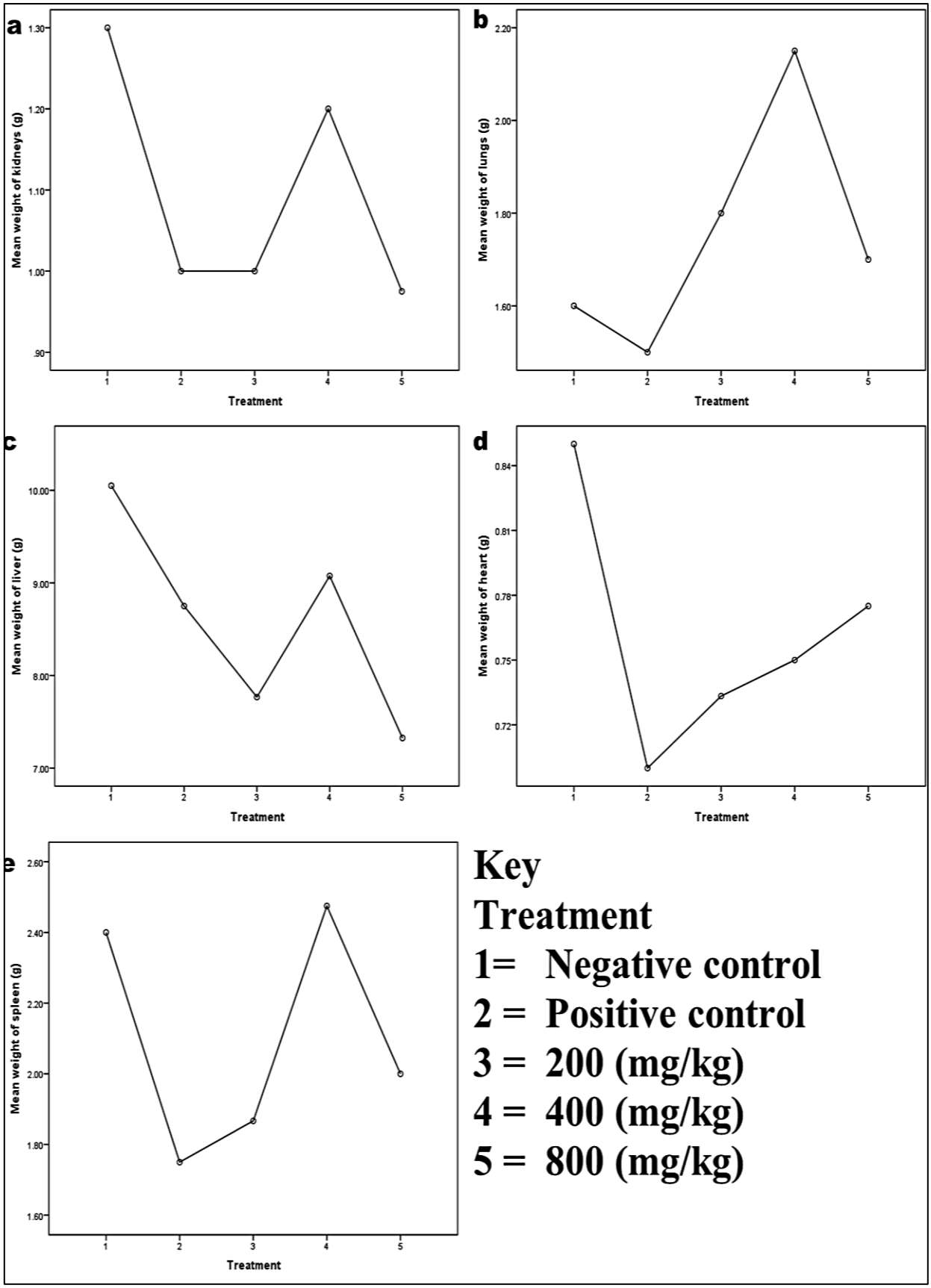


**Figure 7: Pharmacological activities of ethanol extract of *Phyllanthus amarus* on the weight changes in internal organs of *T. brucei*-induced pathology in Wistar rat: (a) kidney, (b) lungs, (c) liver, (d) heart, and (e) Spleen. Negative Control (Normal Saline), Positive Control (Diminazene Aceturate 3.5 mg/kg)**

**Pharmacological Activities of Ethanol Extract of *Phyllanthus Amarus* on Liver Pathophysiological Enzymes in *T. Brucei*-Induced Pathology in Wistar Rat**

The liver tissue pathophysiological enzymes of aspartate transaminase (AST) and alanine transaminase (ALT) following the four days of treatment of *T. brucei*-induced liver pathology in Wistar rats were significantly increased in the negative and positive control groups in comparison to the experimental *Phyllanthus amarus* treated groups, with a lower level of the enzymes observed in rats administered 200, 400 and 800 mg/kg/day of the extracts. The ratio of AST to ALT was significantly lower ($p < 0.05$) in rats administered 200, 400, and 800 mg/kg of plant extract compared to the higher ratios observed in the negative and positive controls (Figure 8).

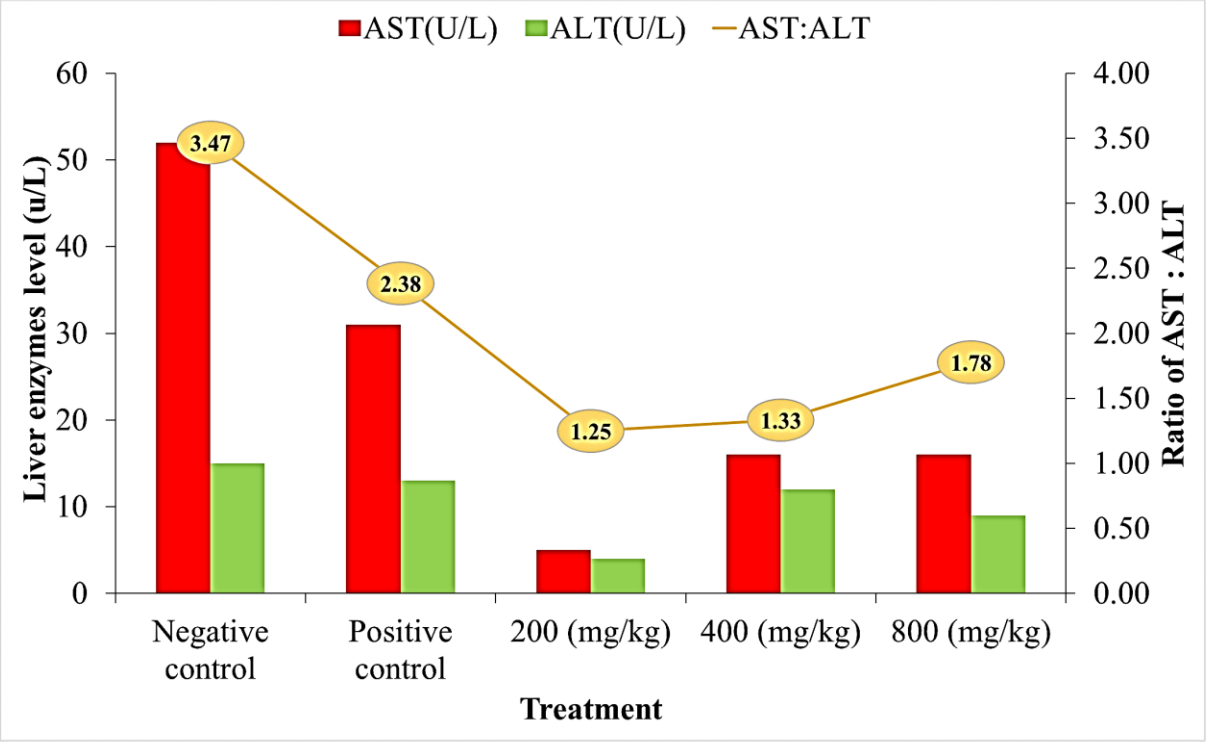


**Figure 8: Activity of ethanol extract of *Phyllanthus amarus* on the liver pathophysiological enzymes of aspartate transaminase (AST), alanine transaminase (ALT), and AST to ALT ratio of *T. brucei*-induced pathology in Wistar rat. Negative Control (Normal Saline), Positive Control (Diminazene Aceturate 3.5 mg/kg)**

**Anti-Oxidative Stress Activities of Ethanol Extract of *Phyllanthus Amarus* on the Heart, Kidney, and Spleen Tissues of *T. Brucei*-Induced Pathology in Wistar Rat**

The tissue levels of malondialdehyde in the heart, kidney, and spleen tissues were measured across the different treatments compared. In the heart tissue, the level of MDA was significantly decreased ($p < 0.05$) in the rats administered 400 and 800 mg/kg /day of the plant extract compared with the negative control, positive control, and the group administered 200 mg/kg dose of *Phyllanthus amarus* (Figure 9a). In the Kidney tissue, the levels of MDA were significantly ($p < 0.05$) decreased in the groups administered 200 and 400 mg/kg/day compared to the negative and positive controls (Figure 9b). In the spleen tissue, the level of MDA was significantly lower ($p < 0.05$) in the positive control and group administered 200, 400, and 800 mg/kg/day when compared with the negative control (Figure 9c).

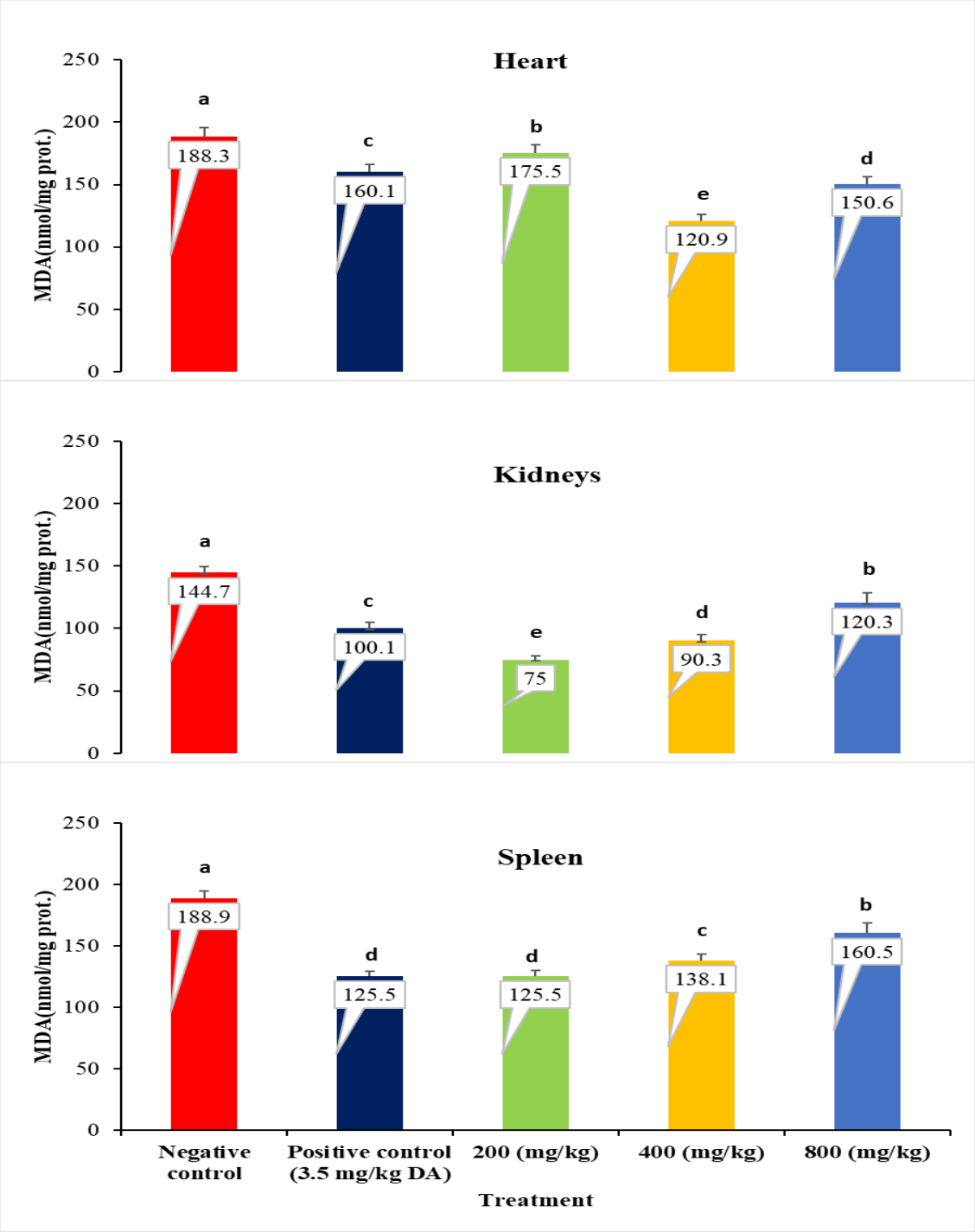


**Figure 9: Anti-oxidative stress activities of ethanol extract of *Phyllanthus amarus* on the Malondialdehyde level in the heart, kidney, and spleen tissues of *T. brucei*-induced pathology in Wistar rat. Negative Control (Normal Saline), Positive Control (Diminazene Aceturate 3.5 mg/kg)**

## DISCUSSION

This study was designed to assess the phytochemical constituents and to investigate the pharmacological prospect of the whole plant ethanol extracts of *Phyllanthus amarus* in ameliorating parasitaemia, clinical pathology, oxidative stress, and liver damage in Wistar rats inoculated with *Trypanosoma brucei*. Interesting findings from this research indicate that *Phyllanthus amarus* is rich in various phytochemicals, including alkaloids, phenols, cardiac glycosides, saponins, carbohydrates, triterpenes, anthraquinones, flavonoids, tannins, and steroids. Quantitatively, alkaloids are the most abundant photochemical, while steroids are the least abundant. The presence of phytochemicals such as flavonoids,

alkaloids, saponins, steroids, triterpenes, and glycosides has been reported to be valuable because of their anti-trypanosomal efficacies (Oluyemi *et al*., 2020; Yun *et al*., 2021). The findings revealed that the extracts exhibited a moderate level of toxicity in animal subjects, as evidenced by observable behavioural reactions such as restlessness, raised hair, bowel movements, urine, and drowsiness. There were no instances of mortality detected, and the median lethal dose was found to be greater than 5000 mg/kg. This suggests that the extract is considered essentially non- toxic and exhibits a high level of safety when fed orally to rats, as Loomis and Hayes (1996) classified in their toxicity classification.

Following the four-day treatment against *Trypanosoma brucei*, *Phyllanthus amarus* ethanol extracts significantly suppressed parasitaemia in rats compared to the control group. However, by day 8, the parasitaemia level resurges in the negative control while at a moderate level in the positive control and those treated with the plant extract. The research revealed that the presence of parasitemias in the bloodstream was not eliminated, possibly as a result of the limited duration of treatment and the oral method of delivering the plant extract. This could also be attributed to the possibility that the active compounds may not effectively reach the target location. Furthermore, the secondary metabolites in the extracts probably underwent biotransformation within the liver and gastrointestinal system, impeding the total elimination of the parasites (Ayawa *et al*., 2021). Antigenic variation, a phenomenon characterised by the continuous transition of trypanosomes from the expression of one immunologically distinct variant surface glycoprotein (VSG) to another, enables trypanosomes to evade the immune system. This investigation represents the preliminary pharmacological examination of the *efficacy of the P. amarus whole plant extract* against *Trypanosoma brucei* in a rat model.

The study analysed the effects of *Phyllanthus amarus* ethanol extract on the rectal temperature and packed cell volume (PCV) of *Trypanosoma brucei*-infected Wistar rats. The extract-administered groups, including the positive control, significantly reduced rectal temperature from their baseline values. However, the negative control showed a significant rise in rectal temperature compared to the treated groups. Live body weights and PCV significantly increased in rats treated with the extract, while total plasma protein values did not differ substantially from pre-treatment values. Generally, the extract showed moderate suppressive ability on parasitaemia level, ameliorating fever, weight loss, and anaemia in *Trypanosoma brucei*-infected Wistar rats at an optimum dose of 200 - 400 mg/kg/day. The 400 and 800mg/kg/day also showed haematinic activity against the parasite. The increase in the values of the packed cell volumes observed for *Trypanosoma brucei*-infected rats may be due to the presence of alkaloids, terpenoids, and flavonoids (Sofowora *et al*., 2013; Ungogo *et al*., 2020) in the extract, which helps to increase blood cell count.

The negative control group significantly increased kidney weight. However, there was a decrease in the size of the kidney, liver, heart, and spleen of the group administered 200 and 800 mg/kg/day, which might be because of the ability of *P. amarus* to expel kidney stones, support the kidney, increase urination, relieve pain, protect and detoxify the liver, reduce spasms and inflammation (Chen *et al*., 2018).

At the same time, there are increases in the lungs in the group administered 400mg/kg/day due to congestion, and in the negative group, there is a shrinkage of the lung caused by the parasite. The results suggest that *Phyllanthus amarus* extracts can potentially reduce the weight of these organs.

Liver damage is one of the major causes of morbidity and mortality and has long been recognised as a clinical feature of African trypanosomiasis (Jerry, 2014; Ayawa *et al.*, 2021). *Trypanosoma*-induced hepatocyte injury may manifest significant elevated serum level enzymes of aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP). The pathogenesis due to the effect of parasitaemia or endotoxemia metabolic acidosis, apoptosis, and oxidative stress are all mechanisms involved in hepatic damage. In the present study, the liver enzymes of aspartate transaminase (AST) and alanine transaminase (ALT) in Wistar rats were significantly increased after four days of treatment with *T. brucei*-induced liver pathology, compared to the experimental *Phyllanthus amarus* treated groups, with lower levels in rats administered extracts. This demonstrates the modulatory activity of *P. amarus* in reversing liver pathology in *Trypanosoma brucei*-infected Wistar rats. The AST-ALT ratio decreased in the group administered 200 and 400 mg/kg/day. AST and ALT are liver enzymes used in evaluating liver diseases or injuries. A higher-than-normal amount of AST in the blood may indicate cirrhosis, hepatitis, or liver cell damage brought on by alcohol misuse or certain drugs. Increased ALT levels in the blood can be a sign of hepatocellular damage (Chanda *et al*., 2009). The normal range is 0.5-1.5. The group administered 200 and 400mg/kg/day were between the range compared to negative and positive control groups.

The study found that rats treated with *Phyllanthus amarus* showed significant decreases in malondialdehyde levels in the heart, kidney, and spleen tissues compared to the negative control, positive control, and 200 mg/kg dose. The levels were also lower in the spleen tissue compared to the negative control. This further demonstrates the modulatory activity in reducing oxidative stress in the heart, kidney, and spleen at 200 and 400 mg/kg body weight in *Trypanosoma brucei*-infected Wistar rats, and this might be due to the presence of saponins, flavonoids, alkaloid, terpenoids in the plant extract. Alkaloid reportedly has analgesic and anti-inflammatory activities, which helps alleviate pains

develop disease resistance, and endurance against stress (Sofowora *et al*., 2013; Ungogo *et al*., 2020).

## CONCLUSIONS

The study has established baseline information on the phytochemical constituents of ethanol extract of *Phyllanthus amarus*. The plant extract is non-toxic and orally safe in Wistar rats and showed moderate suppressive ability against parasitaemia, ameliorating fever, weight loss, and anaemia in *Trypanosoma brucei*-infected Wistar rats. It also demonstrated significant modulatory activity in reversing internal organ pathologies, liver enzyme pathology, and oxidative stress in the heart, kidney, and spleen. The present study has demonstrated the prospects and efficacy of the ethanol extract of *Phyllanthus amarus* in managing African animal trypanosomiasis.

## REFERENCES


- Adegoke, A. A., Iberi, P. A., Akinpelu, D. A., Aiyegoro, O. A., & Mboto, C. I. (2010). Studies on phytochemical screening and antimicrobial potentials of Phyllanthus amarus against multiple antibiotic resistant bacteria. *International Journal of Applied Research in Natural Products*, *3*(3), 6-12.
- Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nature protocols*, *2*(4), 875-877. https://doi.org/10.1038/nprot.2007.102
- Ajala, T. O., Igwilo, C. I., Oreagba, I. A., & Odeku, O. A. (2011). The antiplasmodial effect of the extracts and formulated capsules of Phyllanthus amarus on Plasmodium yoelii infection in mice. *Asian Pacific journal of tropical medicine*, *4*(4), 283-287. https://doi.org/10.1016/S1995-7645(11)60087-4
- Atawodi, S. E., Bulus, T., Ibrahim, S., Ameh, D. A., Nok, A. J., Mamman, M., & Galadima, M. (2003). In vitro trypanocidal effect of methanolic extract of some Nigerian savannah plants. *African Journal of Biotechnology*, *2*(9), 317-321. https://doi.org/10.5897/AJB2003.000-1065
- Ayawa, N. G., Ramon-Yusuf, S. B., Wada, Y. A., Oniye, S. J., & Shehu, D. M. (2021). Toxicity study and anti-trypanosomal activities of aqueous and methanol whole plant extracts of Brillantaisia owariensis on Trypanosoma brucei-induced infection in BALB/c mice. *Clinical Phytoscience*, *7*(1), 1-11. https://doi.org/10.1186/s40816-021-00267-3
- Bose Mazumdar Ghosh, A., Banerjee, A., & Chattopadhyay, S. (2022). An insight into the potent medicinal plant Phyllanthus amarus Schum. and Thonn. *The Nucleus*, *65*(3), 437-472. https://doi.org/10.1007/s13237-022-00409-z
- Centres for Disease Control and Prevention (CDC). (2023). Parasites - African Trypanosomiasis (also known as Sleeping Sickness). Diagnosis. Page last updated: 4 February 2023.
- Chamond, N., Cosson, A., Blom-Potar, M. C., Jouvion, G., d'Archivio, S., Medina, M., ... & Minoprio, P. (2010). Trypanosoma vivax infections: pushing ahead with mouse models for the study of Nagana. I. Parasitological, hematological and pathological parameters. *PLoS neglected tropical diseases*, *4*(8), e792. https://doi.org/10.1371/journal.pntd.0000792
- Chanda, S., & Dave, R. (2009). In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. *African Journal of Microbiology Research*, *3*(13), 981-996.
- Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., ... & Zhao, L. (2018). Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*, *9*(6), 7204. https://doi.org/10.18632/oncotarget.23208
- Food and Agricultural Organisation of the United Nations (FAO). (2008). On target against poverty: Programme Against African Trypanosomosis (PAAT), 1997- 2007; *Food and Agriculture Organization, Rome*, Italy, 1-12.
- Harborne J. B. (1973). Phytochemical methods. London: *Chapman and Hall*, 52-114. https://doi.org/10.1016/0031-9422(73)80427-3
- Herbert, W. J. (1976). A rapid" matching" method for estimating host's parasitaemia. *Exp Parasitol*, *40*, 427-431. https://doi.org/10.1016/0014-4894(76)90110-7
- Jerry, N. A. (2014). A comparative pathology of Trypanosoma brucei infections. Global Advanced Research *Journal of Medicine and Medical Science*, *3*(12), 390-399.
- Loomis, T. A., & Hayes, A. W. (1996). Toxicologic testing methods. *Loomis's Essentials of Toxicology. Academic Press, Inc., San Diego, CA*, 205-248. https://doi.org/10.1016/B978-012455625-6/50014-3
- Makkar, H. P., Siddhuraju, P., & Becker, K. (2007). *Methods in molecular biology: plant secondary metabolites*, *Totowa*: Human Press https://doi.org/10.1007/978-1-59745-425-4
- Mann, A., Egwim, E. C., Banji, B., Abdukadir, N., Gbate, M., & Ekanem, J. T. (2009). Efficacy of Dissotis rotundifolia on Trypanosoma brucei brucei infection in rats. *Afr J Biochem Res*, *3*(1), 5-8.
- OECD, (2002). Guidelines for Testing Chemicals/Section 4: Health Effects Test No. 423: Acute oral toxicity- acute toxic class method. Paris: *Organization for Economic Cooperation and Development*.
- Okubanjo, O. O., Sekoni, V. O., Ajanusi, O. J., Nok, A. J., & Adeyeye, A. A. (2014). Testicular and epididymal pathology in Yankasa rams experimentally infected with Trypanosoma congolense. *Asian Pacific Journal of Tropical Disease*, *4*(3), 185-189. https://doi.org/10.1016/S2222-1808(14)60502-8
- Oluyemi, W. M., Samuel, B. B., Kaehlig, H., Zehl, M., Parapini, S., D'Alessandro, S., ... & Krenn, L. (2020). Antiplasmodial activity of triterpenes isolated from the methanolic leaf extract of Combretum racemosum P. Beauv. *Journal of ethnopharmacology*, *247*, 112203. https://doi.org/10.1016/J.JEP.2019.112203
- Rodrigues, C. M., Olinda, R. G., Silva, T. M., Vale, R. G., da Silva, A. E., Lima, G. L., ... & Batista, J.

S. (2013). Follicular degeneration in the ovaries of goats experimentally infected with Trypanosoma vivax from the Brazilian semi-arid region. *Veterinary Parasitology*, *191*(1-2), 146-153. https://doi.org/10.1016/j.vetpar.2012.08.001

- Sakulpanich, A., & Gritsanapan, W. (2009). Determination of anthraquinone glycoside content in Cassia fistula leaf extracts for alternative source of laxative drug. *Int J Biomed Pharm Sci*, *3*(1), 42-45. https://doi.org/10.1055/s-0029-1234665
- Shamsa, F., Hamidreza, M., Rouhollah, G., & Mohammadreza, V. (2008). Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. *Thailand J. Pharma. Sci*.
- Sharief, N., Srinivasulu, A., & Uma Maheshwara Rao, V. (2014). Estimation of alkaloids and total phenol in roots of Derris trifoliate and evaluation for antibacterial and antioxidant activity. *Indian J Appl Res*, *4*(5), 1-3.
- Silva, T. M., Olinda, R. G., Rodrigues, C. M., Câmara, A. C., Lopes, F. C., Coelho, W. A., ... & Batista, J. S. (2013). Pathogenesis of reproductive failure induced by Trypanosoma vivax in experimentally infected pregnant ewes. *Veterinary Research*, *44*(1), 1-9. https://doi.org/10.1186/1297-9716-44-1
- Sofowora, A. (1993). Medicinal plants and traditional medicine in Africa. Spectrum Books Limited. *Ibadan, Nigeria*, 1-153.
- Sofowora, A., Ogunbodede, E., & Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. *African journal of traditional, complementary and alternative medicines*, *10*(5), 210-229. https://doi.org/10.4314/ajtcam.v10i5.2
- Solich, P., Sedliakova, V., & Karlíček, R. (1992). Spectrophotometric determination of cardiac glycosides by flow-injection analysis. *Analytica chimica acta*, *269*(2), 199-203. https://doi.org/10.1016/0003-2670(92)85403-S
- Steverding, D. (2008). The history of African trypanosomiasis. Parasites and Vectors, 1, 3. https://doi.org/10.1186/1756-3305-1-3
- Steverding, D. (2010). The development of drug for treatment of sleeping sickness: a historical review. *Parasites and vectors*, *3*(1), 15. https://doi.org/10.1186/1756-3305-3-15
- Toya, N. B. (2010). Immunobiology of African trypanosomes: Need of alternative interventions. *Journal of Biomed Biotechnology*, Doi: 10.1155/2010/389153.https://doi.org/10.1155/2010/389153
- Trease, G. E., & Evans, W. C. (1989). *Pharmacognosy13th Edition*. London: Bailliere Tindall.
- Ungogo, M. A., Ebiloma, G. U., Ichoron, N., Igoli, J. O., De Koning, H. P., & Balogun, E. O. (2020). A review of the antimalarial, antitrypanosomal, and antileishmanial activities of natural compounds isolated from Nigerian flora. *Frontiers in Chemistry*, *8*, 617448. https://doi.org/10.3389/fchem.2020.617448
- Wada, Y. A., Oniye, S. J., Rekwot, P. I., & Okubanjo, O. O. (2016). Testicular pathology, gonadal and epididymal sperm reserves of Yankasa rams infected with experimental Trypanosoma brucei brucei and Trypanosoma evansi. *Veterinary world*, *9*(7), 759. https://doi.org/10.14202/vetworld.2016.759-765
- World Health Organisation (2023). Control and surveillance of African trypanosomosis. Technical Report Series, No. 881, Geneva, Switzerland, 284.
- Wurochekke, A. U., & Nok, A. J. (2004). In vitro anti trypanosomal activity of some medicinal plants used in the treatment of trypanosomosis in Northern Nigeria. *African Journal of Biotechnology*, *3*(9), 481-483. https://doi.org/10.5897/AJB2004.000-2094
- Wurochekke, A. U., James, D. B., Bello, M. I., & Ahmodu, A. (2005). Trypanocidal activity of the leaf of Guira senegalensis against Trypanosoma brucei brucei infection in rats. *J Med Sci*, *5*, 1-4. https://doi.org/10.3923/jms.2005.333.336
- Yun, H. S., Dinzouna-Boutamba, S. D., Lee, S., Moon, Z., Kwak, D., Rhee, M. H., ... & Goo, Y. K. (2021). Antimalarial effect of the total glycosides of the medicinal plant, Ranunculus japonicus. *Pathogens*, *10*(5), 532. https://doi.org/10.3390/pathogens10050532
- Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food chemistry*, *64*(4), 555-559. https://doi.org/10.1016/S0308-8146(98)00102-2