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Original article

Effect of *Alstonia boonei* and *Morinda lucida* on Some Haematological Parameters of Wistar Rats Infected with *Trypanosoma brucei brucei*

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ABSTRACT

Trypanosomiasis is a neglected tropical disease which affects the well-being of both humans and animals. The socioeconomic effect of the disease cannot be overemphasized and drugs used for the treatment of the disease are faced with several challenges ranging from resistance to the parasite to high level of toxicity. Our study aimed at determining the effect of *Alstonia boonei* and *Morinda lucida* on some haematological parameters of wistar rats infected with *Trypanosoma brucei brucei*. Phytochemical screening of the methanol extract indicated the presence of alkaloids, saponins, tannins, flavonoids, carbohydrates, phenols and steroids/terpenes, while glycosides were not detected. Only four of the eight constituents tested for were detected in the chloroform leaf extract of *A. boonei*, namely alkaloids, tannins, flavonoids, and carbohydrates. Alkaloids, tannins falvonoids, and carbohydratses were detected in both the chloroform and methanol extract of *Morinda lucida*. Acute toxicity studies revealed that the extract is relatively non-toxic from the Packed cell volume indices and all the wistar rats used for the study survived. In infected rats treated with 500 and 1000 mg/kg b.wt methanol extract of *A. boonei*, there was significant decrease in packed cell volume. For the 500 mg/kg mg/kg b. wt group, the PCV values before infection with *T. brucei* were 21.4±0.23 %, and 22.8±1.21 %. However, by the second and third day of post infection, the respective PCV values were 19.4±0.15% and 17.1±1.26 % respectively. In wistar rats treated with 1000 mg/kg methanol extract of *A. boonei*, the PCV prior to infection was 20.8±1.46 %; on the first day of infection, the PCV was 21.5±0.39 %; while on the second and third day post infection with *T. brucei*, the PCV values were 19.6±1.72 and 18.9±0.28 %, respectively. Therefore, the extracts of *Astonia boonei* should be characterized and active components responsible for this ameliorative effect be detected and elucidated structurally.

Keywords: Trypanosomiasis, Parasitaemia, Phytochemicals, Toxicity

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INTRODUCTION

Trypanosomiasis is a neglected tropical disease caused by a protozoan parasite of the genus *Trypanosoma*. The species responsible for Animal African Trpanosomiasis, which causes nagana in west Africa, are *Trypanosoma vivax*, *Trypanosoma congolense* and to a lesser extent *Trypanosoma brucei brucei* while *T. brucei rhodesiense* and *T. brucei gambiense* cause sleeping sickness Human African Trypanosomiasis [1]. The disease is transmitted through the bite of an infected tsetse fly (*Glossina* species). Surra and Dourine are caused by *T. evansi* and *T. equiperdum* respectively [1].

In 1995, World Health Organization Expert Committee estimated that 60 million people were at risk with an estimated 300,000 new cases per year in Africa, with fewer than 30,000 cases diagnosed and treated. In 2004, the number of new reported cases fell to 17,616 and WHO considered that it is due to increase in the control measure [2]. In 2009, the number of new cases reported dropped below 10,000 (9878) for the first time in 50 years and the estimated number of actual cases is currently 30,000. This trend has been maintained in 2012, with 7,216 cases reported [3].

In Nigeria, studies of ethno--medicinal plants used in the traditional management of trypanosomiasis indicated both significant *in vitro* and *in vivo* anti-trypanosomal activities [4]. Nigeria is richly endowed with indigenous plants, which are used in herbal medicine to cure diseases and heal injuries;; some of these plants are used as food or medicine [5]. These indigenous plants exhibit a wide range of biological and pharmacological activities such as anticancer, anti-inflammatory, diuretic, laxative, antispasmodic, antihypertensive, antidiabetic and antimicrobial functions. It is generally assumed that the active medicinal constituents contributing to these protective effects are the phytochemicals, vitamins and minerals [6]. Phytochemicals, which possess many ecological and physiological roles are widely distributed as plants constituents [5]. Woody plants and herbs can synthesize and accumulate in their cells a large reservoir of phytochemicals including flavonoids, alkaloids, terpenoids, phenolic compounds, tannins, cynogenic glycosodes, saponins, lignins and lignans. It has been highlighted that the additional role of flavornoids and other polyphenolic compounds of higher plants may act as antioxidants or agents of other mechanisms that contribute in their anticarcinogenic or cardioprotective actions [7].

In Nigeria, the majority of citizens still use medicinal plants and visit traditional medicine practitioners for their health care need [5]. The current chemotherapy of human African trypanosomiasis (HAT) relies on only six drugs (suramin, pentamidine, melarsoprol, eflorinithine, al ob rsobal and mel B), five of which were developed more than 30 years ago [7]. Others such as homidium, isometamidium and diminazene aceturate are used in animal infections. Each of these drugs has one or more of these challenges: expensive, highly toxic, need parenteral administration and parasites increasing resistance. The need for alternative new molecules that are safe, effective and affordable is urgent [4].

It has been observed that natural products derived from plants offer novel possibilities to obtain new drugs that are

active against trypanosomes and investigation of antitrypanosomal activity of traditionally used plants has been a major area of contemporary research focus. There is a need for the development of new agents to complement the existing drugs for the treatment of African trypanosomiasis. *Alstonia boonei* and *Morinda lucida* are used as traditional remedy for several infectious and noninfectious diseases including antirheumatic anti-inflammatory, analgesic/pain-killing, antimalaria/antipyretic, antidiabetic (mild hypoglycaemic), antihelminthic, antimicrobial and antibiotic properties [4]. Hence, the need to explore these plants for antitrypanosomal activity.

MATERIALS AND METHODS

Plants Materials

The plants were harvested in the morning in Sakaru village, Zaria Local Government Area of Kaduna State with the coordinate ($11^0$5'7.9476'N; $7^0$43'11.8020'E). It was authenticated in the herbarium, Department of Biological Science, Kaduna State University and given the voucher number (A765 and Q567) for *Alstonia boonei* and *Morinda locida* respectively. It was air dried at room temperature, pulverized in a mortal using pestle to obtained fine powder.

Experimental Animals

Sixty (60) wistar rats weighing between 200g to 250g were used for this study. The animals were purchased from the animal house of Nigerian Institute for Trypanomiasis Research, Kaduna. Wistar rats were kept in a group of five rats per cage in an environment with adequate light and were allowed to acclimatized for 30 days. They were fed with standard pellet diet with water *ad libitum.*

Infection of Animals with Trypanosomes

The parasite *Trypanosoma brucei brucei* was obtained from Nigerian Institute for Trypanosomiasis Research, Kaduna. The animals were inoculated with 1000 parasite per meal of blood intraperitoneally. The number of parasite was compared with Harbert and Lumsden rapid matching method [8].

Extraction from Plant Materials

Extraction of plant material was done using Soxhlet apparatus. One thousand grams (1000 g) of the powdered plant was divided into five hundred (500 g) each. 2,000 ml of each of methanol and chloroform were used for the extraction of each of the powdered plant. The solvent was recovered and concentrated in vacuo to obtain the extract.

Phytochemical Analysis of the Plant Parts

The phytochemical analysis was carried out according to the method of [9,10]. The presence of tannins, saponins, flavonoids, glycosides, anthraquinones, carbohydrate, phenols, terpens and alkaloids was tested qualitatively.

Test for Tannins

A portion of the plant sample was separately boiled with 20 ml distilled water for five minutes in a water bath and was filtered while hot.1 ml of cool filtrate was distilled to 5 ml with distilled water and a few drops (2-3) of 10 % ferric chloride was observed for any formation of precipitates and any colour change. A bluish-black or brownish-green precipitate indicates the presence of tannins.

Test for Saponins

A portion of each plant extract was separately boiled with 10ml of distilled water in a water bath for 10minutes. The mixture was filtered while hot and allowed

to cool. Frothing indicated the presence of saponin in the filtrate.

Test for Flavonoids

A portion of each specimen was boiled with 10 ml of distilled water for 5 minutes and filtered while hot. Few drops of 20 % sodium hydroxide solution were added to 1 ml of the cooled filtrate. A change to yellow colour which on addition of acid changed to colourless solution was depict the presence of flavonoids.

Test for Glycosides

5 ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicates the deoxysugar characteristics of cardenolides.

Test for Anthraquinones

0.2g of extract sample of each specimen was boiled with 2 ml of 10 % hydrochloric acid for 5 mins. The mixture was filtered while hot and filtrate was allowed to cool. The cooled filtrate was partitioned against equal volume of chloroform and the chloroform layer was transferred into a clean dry test tube using a clean pipette. Equal volume of 10% ammonia solution was added into the chloroform layer, shaken and allowed to separate. The separated aqueous layer was observed for any colour change; delicate rose pink colour showed the presence of an anthraquinone.

Test for Alkaloids

A portion of the extract sample of each specimen was separately boiled with water and 10 ml hydrochloric acid on a water bath and filtered. The pH of the filtrate was adjusted with ammonia to about 6-7. A very small quantity of the following reagents was added separately to about 0.5 ml of the filtrate in a different test tube and observed. The test tubes were observed for colored precipitates or turbidity.

Test for Terpens

About 0.5g of the extracts was diluted with 5 ml of distilled water, it was mixed in 2 ml of chloroform and 3 ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration at the interface showed positive results for the presence or absence of terpenoids.

Phenols

The quantity of phenols is determined using the spectrophotometer method. The fat free plant sample was boiled with 50 ml of Ether [$(CH_3CH_2)2O$] for 15min. 5 ml of the boiled sample was then pipetted into 50 ml flask, and 10 ml of distilled water was added. After the addition of distilled water, 2 ml of NH4OH solution and 5 ml of concentrated $CH_3(CH_2)3CH_2OH$ is added to the mixture (Adeneye and Agbaje, 2008). The sample is made up to the mark and left for 30 min to react for colour development and measured at 505 nm wavelength using a spectrophotometer.

Carbohydrate

Ten percent 10 % of α-naphthol alcoholic solution was prepared by adding α-naphthol in 10% alcoholic solution of 1ml each of Fehling's A and Fehling's B. Copper sulfate was dissolved in distilled water and few drops sulfuric acid was added. Sodium potassium tartrate and sodium hydroxide were dissolved in 150ml of distilled water. Sodium citrate 0.25g of anhydrous sodium carbonate was added in distilled water and copper sulfate solution indicating red precipitate which shows the presence of carbohydrate.

Determination of Weight and Temperature of the Experimental Wistar Rats

The weight and temperature of the rats were determined before commencement and at the end of acute toxicity studies. The net change in body weight (difference between final body weight and initial body weight) and change in temperature (difference between final and initial temperature) were determined for all the animals [11].

Acute Toxicity Study of the Plant Extract on Wistar Rats

The toxicity study was conducted using Lorke method as described by [12].

This method has two phases w hich are phases 1 and 2 respectively.

Phase 1

This phase requires nine animals. The nine animals were divided into three groups of three animals each. Each group of animals are administered different doses (10, 100 and 1000 mg/kg b. wt) of test substance. The animals were placed under observation for 24 hours to monitor their behavior as well as if mortality was occurred.

Phase 2

This phase involves the use of three animals, which are distributed into three groups of one animal each. The animals are administered higher doses (1600, 2900 and 5000 mg/kg) of test substance and then observed for 24 hours for behavior as well as mortality [12].

Then the LD_{50} is calculated using Lork formula as described by [12].

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

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

Determination of Packed Cell Volume of the Wistar Rats

The PCV is the volume of red blood cells (RBCs) expressed as a fraction of the total volume of the blood. The microhaematocrit method was used. Two meals (2ml) of blood from EDTA bottle was allowed by capillary action to flow through the capillary tube and one end of the tube were sealed using plaster seal. It was then centrifuged at a speed of 3000rpm for 10 minutes. The PCV was estimated using a microheamatocrit reader and expressed as percentage erythrocytes in the blood [13].

Determination of Hemoglobin Concentration of the Wistar Rats

The level of Heamoglobin concentration was determined according to the method of [14], Sample solutions and standard solutions were prepared as follows.

Random sample: Approximately 5000 µl of Drabkin reagent was mixed with 20 µl of distilled water standard: Exactly 5000 µl of Drabkin reagent was mixed with 20 µl of sample solution of haemoglobin Target sample: About 5000 µl of Drabkin reagent + 20 µl of blood. The concentration of haemoglobin was marked with Drabkin‟s method, with the use of a spectrophotometer, at 540 nm wavelength. Drabkin reagent was mixed with the blood, the solution will be incubated at room temperature for the duration of 5 mins and absorbance was measured. The spectrophotometer was set to zero using distilled water. Measurement formula: Haemoglobin and its derivatives affected by Drabkin reagent transform into cyanmethemoglobin, whose optical density may be measured photometrically.

The concentration of hemoglobin was calculated according to the following formula:

$$\text{Hb concentration (g/dl)} = \frac{\text{absorbance of tested sample}}{\text{absorbance of standard}} \times \text{concentration of standard in g/dl}$$

In vivo Antitrypanosomal Test

The *in vivo* antitrypanosomal activity of the plant extract was carried out according to Conti method as described by [15]. Thirty-five (35) wistar rats grouped into seven groups of five rats each was used for the study. The animals were inoculated with approximately 10^4 trypanosomes/ml by serial dilution of the infected blood and infection was allowed to establish. Four groups served as extract test (A: Methanolic extract, B: Methanolic extract, A: chloroform extract and B: chloroform extract) and fifth as positive control (treated with reference drug Diminazene aceturate). A sixth group was serve as negative control (untreated) and the seventh was served as uninfected and untreated. The extract was dissolved in distilled water then Tween 20 and administered orally. Treatment with extract and reference drug was commenced on 3th day of post infection.

Therapeutic Monitoring of Wistar Rats during Treatment with Extract

Parasitaemia of each rat was monitored every forty-eight hours during the administration of the extracts and reference drug (Diaminazene diaceturate) and the levels were estimated by Rapid Matching Method of Herbert and Lumsden as described by [16]. Parasitaemia levels were compared with untreated control animals. Treatments with extracts and reference drug was last for seven days.

Ethical Clearance

Ethical clearance for the use of laboratory animals was obtained from Kaduna State Ministry of Agriculture and Forestry, Kaduna.

Data Analysis

Values of the data obtained from the study was summarized and expressed as mean $\pm$ standard deviation. Data analysis was performed using Statistical Package for Social Science (SPSS) 2018 version 23.0. To compare the results obtained from different groups, one-way ANOVA followed by Duncan multiple comparison tests was performed to determine statistical significance. P values less than 0.05 was considered significant.

RESULTS

Phytochemical Constituents of Extracts of *Alstonia boonei* and *Morinda lucida* Using Methanol and Chloroform as Solvents

The results of the phytochemical constituents and screening of the methanolic leaf extract of *Alstonia boonei* indicated the presence of seven constituents, namely, alkaloids, saponins, tannins, flavonoids, carbohydrates, phenols and steroids/terpenes, while glycosides were not detected. In terms of relative abundance, carbohydrates were detected in lesser quantities in the methanol extract of *A. boonei*, steroids and terpenes were present in moderate quantities, while alkaloids, saponins, tannins, flavonoids, and phenols were detected in relatively higher quantities (Table 1).

Only four of the eight constituents, tested for, were detected in the chloroform leaf extract of *A. boonei*, namely, alkaloids, tannins, flavonoids, and carbohydrates; carbohydrates were detected in lesser quantities while alkaloids, tannins, and flavonoids were detected in a very good quantities in the chloroform extract of *A. boonei*, in quantities comparable to those seen in the methanol extract of the plant.

Four phytoconstituents namely, alkaloids, tannins, flavonoids, and carbohydrates were detected in the methanol extract of *Morinda lucida*. Alkaloids, tannins, and flavonoids were highly present in the extract while only minute quantities of carbohydrates were detected in the methanol extract of *M. lucida*. In the chloroform extract of *M. lucida*, only three of the eight phytoconstituents were detected namely, alkaloids, tannins, and flavonoids; saponins, glycosides, carbohydrates, phenols, and steroids were not detected. All three constituents that were present in the chloroform extract of *M. lucida* were detected in relatively high quantities (Table 1).

Table 1. Phytochemical Constiyuents of the Methanol and Chloroform Leaf Extracts of *Alstonia boonei* and *Morinda lucida*

Phytochemical Constituents	ABME	ABCE	MLME	MLCE
Alkaloids	+++	+++	+++	+++
Saponins	+++	---	---	---
Tannins	+++	+++	+++	+++
Flavonoids	+++	+++	+++	+++
Glycosides	---	---	---	---
Carbohydrates	+	+	+	---
Phenolic compounds	+++	---	---	---
Terpenes	++	---	---	---

ABME: *Alstonia boonei* Methanol Extract; ABCE: *Alstonia boonei* Chloroform Extract; MLME: *Morinda lucida* Methanol Extract; MLCE: *Morinda lucida* Chloroform Extract

+++: highly present; ++: moderately present; +: present; ---: undetected

Acute Toxicity Effect of *Alstonia boonei* and *Morinda lucida* on Body Mass (g) of Wistar Rats

In all treatment groups (except in group G) the body masses of the albino rats treated with the extract were lower than pre-treatment values, implying a loss in body mass. Significant losses in body masses were observed in treatment groups A, B, C, and F; in Groups D (100 mg/kg methanol extract of *M. lucida*) and E (500 mg/kg methanol extract of *M. lucida*), the loss in body masses were not statistically significant ($p > 0.05$). In group A (100 mg/kg methanol extract of *A. boonei*), a 33.5% reduction in post-treatment body mass was recorded as 54.6 g was lost from a pre-treatment value of 162.8±22.3g. In group B (500 mg/kg methanol extract of *A. boonei*), the body masses of the rats reduced from 158.6±18.7g before treatment to 101.8±28.5g after treatment, a loss in body mass which corresponded to a 35.8% (56.8g). A 20.2% (34.6g) loss in body mass was recorded in group C (3000 mg/kg methanol extract of *A. boonei*) as body mass changed from 171.6±24.4g prior to treatment to 137.0±18.9g after treatment. The mass of rats in group D (100 mg/kg methanol extract of *M. lucida*) decreased from 119.8±44.3g before treatment to 103.3±10.7g post-treatment, signifying a 13.8% (16.5g) loss in body mass. In group E (500 mg/kg methanol extract of *M. lucida*), a 16.7% (26.1g) loss in body mass was recorded in the rats as body mass declined from 156.0±46.2g prior treatment to 129.9±16.6g post-treatment. The post-treatment mass of rats in group F (3000 mg/kg methanol extract of *M. lucida*) was 30.3% (58.0g) lesser

than pre-treatment values as the mean body mass decreased from 191.6±30.4 to 133.6±30.2g. However, in group G (Control) there was a significant increase ($p < 0.05$) in post-treatment body mass as the mean body mass changed by 35.9% (33.7g) from 94.0±12.3g to 127.7±9.29g. In groups where there were significant losses in body masses, the highest loss in mass was observed in group B with a loss of 35.8%, while the least was recorded in group D with a 13.8% loss in body mass.

there were significant gain in body mass in the control group (Group G) while in groups that received either of the extracts there were losses in body mass: the loss in body mass in rats treated with the methanol extract of *M. lucida* was dose dependent while in rats treated with *A. boonei* the higher loss in body mass was recorded in the 500 mg/kg group with the least seen at 3000 mg/kg dose (Table 2).

Table 2: Acute Toxicity Effect of Methanol Leaf Extracts of *Alstonia boonei* and *Morinda lucida* on body Weight (g) of Wistar rats

Treatment groups	Body mass (g)				*p* value
	BeforeTest	After Test	Change (g)	Percent change	
Group A	162.8±22.3	108.2±13.2	- 54.6	- 33.5	0.002
Group B	158.6±18.7	101.8±28.5	- 56.8	- 35.8	0.006
Group C	171.6±24.4	137.0±18.9	- 34.6	- 20.2	0.037
Group D	119.8±44.3	103.3±10.7	- 16.5	- 13.8	0.458
Group E	156.0±46.2	129.9±16.6	- 26.1	- 16.7	0.288
Group F	191.6±30.4	133.6±30.2	- 58.0	- 30.3	0.016
Group G	94.0±12.3	127.7±9.29	+ 33.7	+ 35.9	0.004

Group A = 100 mg/kg methanol extract of *A. boonei*; Group B = 500 mg/kg methanol extract of *A. boonei*; Group C = 3000 mg/kg methanol extract of *A. boonei*; Group D = 100 mg/kg methanol extract of *M. lucida*; Group E = 500 mg/kg methanol extract of *M. lucida*; Group F = 3000 mg/kg methanol extract of *M. lucida*; Group G = Control group

Acute Toxicity Effect of *Alstonia boonei* and *Morinda lucida* on Packed Cell Volume (%) of Wistar rats

The post-treatment values for packed cell volume were lesser than pre-treatment values in all groups except in groups C and G where gains in packed cell volumes were recorded. In group A (100 mg/kg methanol extract of *A. boonei*), PCV values declined from 49.6±4.51 to 42.0±2.55% signifying a 15.3% decrease in PCV after treatment. Similarly, a significantly lower PCV value was recorded in group B (500 mg/kg methanol extract of *A. boonei*) after treatment with the extract as values declined from 46.0±11.3 to 28.2±3.19% which corresponded to a 38.7% decrease. Non-significant increase of 6.0% was recorded in group C (3000 mg/kg methanol extract of *A. boonei*) as PCV changed from 40.0±13.1 to 42.4±4.45%. In group D (100 mg/kg methanol extract of *M. lucida*), a non-significant reduction ($p > 0.05$) of 7.28% in PCV was recorded as mean value changed from 41.2±4.21% prior to treatment to 38.2±2.39% post-treatment. A 6.44% decrease in PCV was recorded in group E (500 mg/kg methanol extract of *M. lucida*) from a pre-treatment value of 46.6±7.60% to 43.6±6.43% after treatment. In group F (3000 mg/kg methanol extract of *M. lucida*), PCV prior to treatment was 50.6±3.78% and declined by 7.91% to a mean value of 46.6±3.44 after treatment. A significant increase in PCV was recorded in group G (Control) with PCV value increasing by 66.7% from a pre-treatment value of 39.6±3.28% to a post-treatment value of 66.0±4.18%. When changes in PCV values

were compared between groups, the highest decrease in PCV was recorded in group B (500 mg/kg methanol extract of *A. boonei*) with a 38.7% decline in PCV value when compared with the pre-treatment values, while the least decline in PCV was recorded in group E (500 mg/kg methanol extract of *M. lucida*) with a 6.77% decrease in PCV value (Table 3).

Table 3: Acute Toxicity Effect of Methanol Leaf Extracts of *Alstonia boonei* and *Morinda lucida* on Packed Cell Volume of Wistar Rats

Treatment groups	Packed cell volume (%)				*p* value
	Before Test	After Test	Change	Percent change	
Group A	49.6±4.51	42.0±2.55	- 7.6	- 15.3	0.011
Group B	46.0±11.3	28.2±3.19	- 17.8	- 38.7	0.009
Group C	40.0±13.1	42.4±4.45	+ 2.4	+ 6.0	0.714
Group D	41.2±4.21	38.2±2.39	-3.0	- 7.28	0.203
Group E	46.6±7.60	43.6±6.43	- 3.0	- 6.44	0.519
Group F	50.6±3.78	46.6±3.44	- 4.0	- 7.91	0.188
Group G	39.6±3.28	66.0±4.18	+ 26.4	+ 66.7	< 0.001

Group A = 100 mg/kg methanol extract of *A. boonei*; Group B = 500 mg/kg methanol extract of *A. boonei*; Group C = 3000 mg/kg methanol extract of *A. boonei*; Group D = 100 mg/kg methanol extract of *M. lucida*; Group E = 500 mg/kg methanol extract of *M. lucida*; Group F = 3000 mg/kg methanol extract of *M. lucida*; Group G = Normal Control

Acute Toxicity Effect of *Alstonia boonei* and *Morinda lucida* on Body Temperature (°C) of Wistar Rats

Although body temperature variations were recorded in all groups when pre-treatment and post-treatment values were compared with each other, the differences were not statistically significant (P-value>0.05). In groups A, B, E and G, there were decreases in body temperature post-treatment while in groups C and D, elevations in body temperatures were recorded post-treatment. The highest decrease in body temperature (1.91% decrease) was recorded in group E (500 mg/kg methanol extract of *M. lucida*) while the most gain in body temperature (1.64% increase) was recorded in group D 100 mg/kg methanol extract of *M. lucida* (Table 4).

Table 4: Acute Toxicity Effect of Methanol Extracts of *Alstonia boonei* and *Morinda lucida* on Body Temperature (°C) of Wistar Rats

Treatment groups	Body temperature (°C)				*p* value
	Before Test	After Test	Change (g)	Percent change	
Group A	38.5±0.26	38.2±0.33	- 0.3	- 0.78	0.077
Group B	36.4±1.95	38.2±0.67	- 1.8	- 1.25	0.110
Group C	36.6±1.57	36.9±0.56	+ 0.3	+ 0.82	0.717
Group D	36.6±0.39	36.0±0.73	+ 0.6	+ 1.64	0.199
Group E	36.6±1.18	35.9±0.45	- 0.7	- 1.91	0.194
Group F	37.2±0.88	37.2±0.62	0.00	0.00	1.000
Group G	35.4±1.18	35.3±1.19	- 0.1	- 0.28	0.866

Values are given as mean ± standard deviation.
Key: Group A = 100 mg/kg methanol extract of *A. boonei*; Group B = 500 mg/kg methanol extract of *A. boonei*; Group C = 3000 mg/kg methanol extract of *A. boonei*; Group D = 100 mg/kg methanol extract of *M. lucida*; Group E = 500 mg/kg methanol extract of *M. lucida*; Group F = 3000 mg/kg methanol extract of *M. lucida*; Group G = Normal Control

Effect of Methanol and Chloroform Leaf Extracts of *Alstonia boonei* and *Morinda lucida* on Body Mass of Wistar Rats Infected with *Trypanosoma brucei brucei*

Among rats in Group A (500 mg/kg methanol extract of *A. boonei*), there was no significant difference ($p > 0.05$) in the body mass before and after infection. However, the following trend was observed: an increase (0.71%) in body mass on day 1 post-infection when compared to pre-infection values, then followed by a steady decline in body mass between days 2 and 4 after infection; by the fourth day post-infection, the decline of 6.68% in body mass was recorded in infected rats treated with (Group A) when compared to pre-infection value.

In Group B (1000 mg/kg methanol extract of *A. boonei*), the mean body mass of the rats was 116.0g followed by a 4.39% (110.9g) decline by day 1 post-infection, after which the body mass of the infected rats recorded a steady increase from day 2 to day 4 after infection with masses of 115.7 and 118.4g, respectively.

In Group C (500 mg/kg chloroform extract of *M. lucida*), the mean body mass of rats prior to being inoculated was 142.2g. By day 1 after inoculation, the mean mass of the rats was 151.9g (6.68% increase), followed by a 7.44% decrease to 140.6g on day 2 post-infection, after which an increase of 9.74% was recorded by the 4th day post-infection.

In Group D (1000 mg/kg chloroform extract of *M. lucida*), there was significant difference ($p < 0.05$) in the body mass of the rats before and after infection, with the least mass (92.6g) recorded prior to infection: increases in body mass were recorded by day 1 (168.3g) and 2 (168.1g) post-infection, followed by a decrease (144.4g) recorded by the 4th day post-infection (Table 4.5).

The mean body mass of rats in Group E (3.5 mg/kg Diminazine aceturate) from day 1 to day 4 post-infection were higher than the value recorded before inoculation with the parasite. However, the difference was not significant ($p < 0.05$) (Table 5).

Before infection with *T. brucei*, the mean mass of rats in Group F (Infected untreated) was 140.2g; by the first day after infection with the protozoa, the mean body mass had increased to 183.5g.

however, by the second and fourth day of infection body mass had declined to 166.2g and 118.7g, respectively. In the uninfected control (Group F), there were steady increase ($p < 0.05$) in body mass throughout the duration of the observation: baseline body weight for that treatment group was 143.8±4.23g, and the fourth day of observation, the mean body mass was 152.6±8.06g (Table 5).

Table 5. The Effect of Methanol Leaf Extracts of *Alstonia boonei* and *Morinda lucida* on Body mass (g) of Wistar Rats Infected with *Trypanosoma brucei brucei*

Treatment groups	Bogy mass (g)				*p* value
	Before Test	Day 1	Day 2	Day 3	
Group A	155.6±15.4[a]	156.7±17.9[a]	146.7±16.9[a]	145.2±29.1[a]	0.740
Group B	116.0±33.2[a]	110.9±29.9[a]	115.7±31.4[a]	118.4±28.9[a]	0.984
Group C	142.2±23.3[a]	151.9±19.1[a]	140.6±20.0[a]	154.3±21.7[a]	0.669
Group D	92.6±9.94[a]	168.3±48.6[b]	168.1±47.8[b]	144.4±0.00[b]	0.034
Group E	135.0±18.2[a]	149.6±19.9[a]	163.7±22.2[a]	157.2±19.5[a]	0.254
Group F	140.2±32.3[b]	183.5±25.2[c]	166.2±21.6[c]	118.7±0.00[a]	< 0.001
Group G	143.8±4.23[a]	146.9±4.17[ab]	148.3±2.51[b]	152.6±8.06[c]	< 0.001

Values are given as mean ± standard deviation. In each row, mean values with different superscripts have statistically significant difference ($p < 0.05$).

Key: Group A: 500 mg/kg methanolic extract of *A. boonie*; Group B: 1000 mg/kg methanolic extract of *A. boonie*; Group C: 500 mg/kg chloroform extract of *M. lucida*; Group D: 1000 mg/kg chloroform extract *M. lucida*; Group E: 3.5 mg/kg Diminazine; Group F: infect and untreated control; Group G: uninfected-untreated control

Effect of Methanol and Chloroform Leaf Extracts of *Alstonia boonei* and *Morinda lucida* on Temperature of Wistar Rats Infected with *Trypanosoma brucei brucei*

Prior to infection with trypanosomes, the mean body temperature of rats in all seven experimental groups ranged between 33.00±0.46 and 34.4±1.65°C. However, by the day 1 post-infection, there were significant ($p < 0.05$) elevations in the mean body temperature of rats (relative to the pre-infection values) across all experimental groups except in the control group where the difference in temperature was not significant ($p > 0.05$). Body temperatures remained significantly elevated (compared to pre-infection values) by the second day of infection, before a generalized non-significant decline by the third day of infection. When compared with pre-infection values, the body temperatures recorded in the infected rats by the third day of infection were significantly higher. In the control group, there were no significant variations in mean body temperatures throughout the duration of observation (Table 6).

Table 6. The Effect of Methanol and Chloroform Leaf Extracts of *Alatonia boonei* and *Morinda lucida* on Temperature (°C) of Wistar Rats Infected with *Trypanosoma brucei brucei*

Treatment groups	Body temperature (°C)				*p* value
	BeforeTest	Day 1	Day 2	Day 3	
Group A	33.8±0.47[a]	37.4±1.09[b]	37.5±0.28[b]	36.9±0.70[b]	< 0.001
Group B	34.4±1.65[a]	37.9±0.58[c]	37.7±0.96[bc]	36.4±0.54[b]	< 0.001
Group C	34.0±1.22[a]	38.0±0.72[b]	37.9±0.67[b]	37.3±0.47[b]	< 0.001
Group D	33.9±0.74	35.7±1.12	35.6±0.68	36.2±0.00	0.018
Group E	33.1±0.63[a]	38.9±0.80[c]	38.3±0.21[c]	36.7±0.93[b]	< 0.001
Group F	33.0±0.46	35.7±1.12	37.8±0.69	36.6±0.00	< 0.001
Group G	34.2±0.15	34.6±0.02	33.8±1.94	33.5±0.28	

Values are given as mean ± standard deviation. In each row, mean values with different superscripts have statistically significant difference ($p < 0.05$).

Key: Group A: 500 mg/kg methanolic extract of *A. boonie*; Group B: 1000 mg/kg methanolic extract of *A. boonie*; Group C: 500 mg/kg chloroform extract of *M. lucida*; Group D: 1000 mg/kg chloroform extract *M. lucida*; Group E: 3.5 mg/kg Diminazine; Group F: infect and untreated control; Group G: uninfected-untreated control

Effect of methanol and Chloroform Leaf Extract of *Alstonia boonei* and *Morinda lucida* on the Packed Cell Volume of Wistar Rats Infected with *Trypanosoma brucei brucei*

In untreated-infected rats (Group F), there was significant decrease in packed cell volume throughout the course of observation. Prior to infection with the parasite, the PCV was 22.5±0.19%; by day 1 of infection, the PCV was 22.1±1.13%, and 20.3±0.86% by the second day of infection, while on the third day of infection, the PCV in the infected-untreated rat was 16.4±2.01%.

In infected rats treated with (500 or 1000 mg/kg) methanol extract of *A. boonei*, there was significant decrease in packed cell volume: In the 500 mg/kg group, the PCV value before infection with *T. brucei* was 21.4±0.23%, and 22.8±1.21%. However, by the second and third day after infection, the respective PCV values were 19.4±0.15 and 17.1±1.26. In rats treated with 1000 mg/kg methanol extract of *A. boonei*, the PCV prior to infection was 20.8±1.46%; on the first day of infection, the PCV was 21.5±0.39 while on the second and third days after infection with

T. brucei, the PCV values were 19.6±1.72 and 18.9±0.28%, respectively.

In *T. brucei* infected rats treated with 500 or 1000 mg/kg chloroform extract of *M. lucida* there were significant difference in PCV during the course of infection. In the 500 mg/kg group, the PCV values began to decline by the second day through the third day of infection: prior to infection, the PCV was 22.1±0.52%, and 22.9±1.62% by the first day of infection. However, by the second and third day of infection, the PCV values were 20.6±1.89 and 17.7±2.19%, respectively (Table 4.7).

However, in *T. brucei* infected rats treated with 1000 mg/kg chloroform extract of *M. lucida*, there was significant increase in packed cell volumes of the animals throughout the duration of observation: prior to infection, the PCV was 23.7±1.16%, and 25.1±1.07% by the first day of infection; by the second and third days of infection, the PCV values were 28.9±2.16 and 32.4±0.84%, respectively (Table 7).

Table 7: The Effect of Methanol and Chloroform Extracts of *Alstonia boonei* and *Morinda lucida* on Packed Cell Volume (%) of *Trypanosoma brucei brucei* Infected Wistar Rats

Treatment groups	Packed cell volume (%)				*p* value
	Before Test	Day 1	Day 2	Day 3	
Group A	21.4 ± 0.23^{c}	22.8 ± 1.21^{c}	19.4 ± 0.15^{b}	17.1 ± 1.26^{a}	0.017
Group B	20.8 ± 1.46^{b}	21.5 ± 0.39^{c}	19.6 ± 1.72^{ab}	18.9 ± 0.28^{a}	0.028
Group C	22.1 ± 0.52^{c}	22.9 ± 1.62^{c}	20.6 ± 1.89^{b}	17.7 ± 2.19^{a}	0.014
Group D	23.7 ± 1.16^{a}	25.1 ± 1.07^{b}	28.9 ± 2.16^{c}	32.4 ± 0.84^{d}	0.006
Group E	21.9 ± 0.18^{a}	22.7 ± 1.54^{a}	24.9 ± 1.48^{b}	26.1 ± 1.53^{c}	0.004
Group F	22.5 ± 0.19^{c}	22.1 ± 1.13^{c}	20.3 ± 0.86^{b}	16.4 ± 2.01^{a}	< 0.001
Group G	22.1 ± 2.21^{a}	22.6 ± 0.41^{b}	23.9 ± 2.16^{bc}	25.5 ± 0.31^{c}	< 0.001

Values are given as mean $\pm$ standard deviation. In each row, mean values with different superscripts have statistically significant difference ($p < 0.05$).
Key: Group A: 500 mg/kg methanolic extract of *A. boonie*; Group B: 1000 mg/kg methanolic extract of *A. boonie*; Group C: 500 mg/kg chloroform extract of *M. lucida*; Group D: 1000 mg/kg chloroform extract *M. lucida*; Group E: 3.5 mg/kg Diminazine; Group F: infect and untreated control; Group G: uninfected-untreated control

DISCUSSION

The presence of bioactive components in the aqueous and chloroform extracts of the leaves of *A. boonei* agreed with the reports of [17] who noted the presence of phytochemicals in the water and methanol leaf extracts of *A. boonei*. [18] had also reported the presence of some bioactive agents in the methanol stem bark and leaf extracts of the plant extracts, a finding that had been corroborated by a more recent work by [19]. These reports indicated that *A. boonei* is an excellent source of chemical moieties that could find important medicinal and pharmacological applications.

Similarly, extracts of *Morinda lucida* showed an array of phytoconstituents including alkaloids, tannins, flavonoids, and carbohydrates. This was in consonance with the reports of [20,21] who had previously reported the presence of bioactive agents in solvent extracts of the stem bark and root of *M. lucida* (saponins, tannins, anthracenes, flavonoids, alkaloids, carbohydrate and protein). The present results indicated that the class of phytochemicals varied between the two plants (*A. boonei* and *M. lucida*) and between the solvent used for extracting a particular plant. According to [22] the metabolic profiles of medicinal plants are mainly controlled by genetic factors, adding that variations in the levels of phytochemicals may represent long-term ecological and evolutionary interactions. Thus, the genetic differences between *A. boonei* and *M. lucida* may account for the differences in the number of phytochemical classes detected in each plant extract [23].

Variations in phytochemical constituents between the methanol and chloroform extracts of either *A. boonei* and *M. lucida* could be attributed to differences in the polarity of the two solvents. had Phytochemical composition of extracts significantly varied with the solvent used for extraction [24]. Other factors such as solvent to plant material ratio, particle size of plant material, temperature, extraction method, seasons, geographical location and soil type also influence the phytochemical constituents of an extract [25]. The influence of solvent polarity on

phytochemical content and bioactivity of plant extracts have been investigated. Also, antioxidant activity was associated with the use of polar solvents to extract plant materials [26].

The present results indicated that the extracts of *A. boonei* exerted negative effects on body weight, packed cell volume and temperature of rats that received acute doses of the extract. The decrease in body weight recorded in the rats agreed with the reports of [27] who reported that *A. boonei* given at 10% of the body weight resulted in significant decrease in body mass of experimental rats. Similarly, [28] had reported the dose-dependent loss in body weight in rats treated with 200 and 400 mg/kgb.wt of *S. dulcis*, adducing appetite suppression as possible cause of the decrease in body mass of the experimental animals. Also, posited that extract-related nutrient malabsorption may account for weight losses in experimental animals [29].

The decrease in packed cell volume (PCV) recorded in this study was in line with the reports of [30] who also reported a loss in PCV values in rats that receive graded doses of the stem bark extract of *A. boonei*. However, these results differ from those of [31] who reported on the red cell protective effect of ethanol stem bark extract of *Alstonia boonei*. Similarly, [32] had reported on the ameliorative effects of *A. boonei* against *Plasmodium berghei* induced decrease in packed cell volume in mice. Packed cell volume determination has been variously adopted as a means to indirect assess the red cell population and hence the oxygen carrying capacity of an organism. Decline in packed cell volume, thus, alludes to reduced red cell population and ultimately an impaired oxygen carrying capacity [33]. Decline in red cell counts may be mediated via two mechanisms, namely, active destruction of red cells and/or depressed red cell production by hemopoiesis [34]. The former mechanism is usually the immediate outcome of toxic insults as may be accompanied with administration of plant materials or other chemicals, and could thus account for the decrease in packed cell volume recorded in the experimental animals.

The temperature lowering effect of *Alstonia boonei* extracts recorded in this study may be associated with it anti-inflammatory and antipyretic properties. [35] reported on the anti-inflammatory effects of the n-hexane fraction of *A. boonei* stem bark in lipopolysaccharide-induced inflammation in Wistar rats, and noted that the n-hexane fraction of the plant decreased the concentration of tumor necrosis factor alpha (TNF-α), C-reactive protein (CRP) and creatinine kinase (CK) in circulation.

The results indicated that in the *T. brucei brucei* infected untreated control (Group F), there were a significant net loss in body mass of the experimental rats with body weight value at day four were compared with pre-inoculation value. This agreed with the observations of [36] who had reported decrease in body weight in untreated trypanosome infections. Weight loss has been noted as a major clinical sign of trypanosomiasis, as it is being associated with trypanosome induced loss of appetite [37]. In groups of *T. brucei brucei* infected rats, it was observed that the methanol extract of *A. boonei* and the chloroform extract of *M. lucida* ameliorated the trypanosome-associated loss in body weight, as seen in the infected untreated control group; however, the effect was not dose dependent. This effect

of both extracts on the body weights of the infected Wistar rats may be associated with probable appetite stimulatory effect of the extracts. Increased food intake makes for increased supply of nutrient needed for growth and maintenance of the body [37].

Our findings indicated that in untreated rats infected with *T. brucei brucei,* there was significant decrease in packed cell volume. This was consistent with the reports of [35] who reported that anemia is a principal clinical symptom of trypanosome infection. Packed cell volume has been variously utilized as an indirect measure of red blood cell count and, thus, as an index for the anemic status of an individual. Therefore, the decreased PCV values seen in the *T. brucei* infected-untreated control were indicative of progressive anemia. Trypanosomes are reported to metabolize the sialic acids that constitute the membrane of red cells resulting in their increased susceptibility to lyses, and the attendant anemia. Severe anemia impairs oxygen transport and cellular metabolism, and could ultimately result in death [33]. The present result indicated that the methanol extract of *A. boonei* did not prevent, ameliorate or reverse the trypanosome associated decline in packed cell volume of the infected rats. This observation was consistent with the PCV lowering effect of *A. boonei* observed during the acute toxicity phase of the study in which the packed cell volumes of the experimental animals were shown to decrease with administration of acute doses of the extract. The stem bark extract of *A. boonei* was shown by [30] to be associated with decline in PCV values in experimental animals that were uninfected with trypanosomes. This find, however, contradicted the reports of other authors who reported that extracts obtained from other medicinal plants prevented decrease in PCV values in experimental trypanosome infection. The methanol extract of the roots of *Echinop kebericho* prevented *T. brucei* induced decreased in packed cell volumes in mice. Furthermore, aqueous and methanol extracts of *Brillantaisia owariensis* ameliorated *T. brucei* induced decline in packed cell volume [37]. This decline in PCV values in *T. brucei* infected rats treated with *A. boonei* may be connected with the very high saponin contents recorded during the determination of phytoconstituents. Saponins have been reported to possess hemolytic properties [38].

Extracts of *Morinda lucida* was showed to inhibit trypanosome induced decrease in packed cell volume when administered at 1000 mg/kg. This observation was consistent with those of administration of 400 mg/kg aqueous extract of *M. lucida* improved the packed cell volume in *T. brucei brucei* infected mice. The anti-anemic effect of the chloroform extract of *M. lucida* may be due to its ability to prevent the lysis of red blood cells, which are reported to be severely affected in untreated animals as trypanosomes metabolize the sialic acids component of the RBC membrane, thereby resulting in increased RBC fragility and hemolysis [39].

Ethics Approval and Informed Consent

The ethical approval for the use of laboratory animals was obtained from Animal committee of Kaduna State Ministry of Agriculture and Forestry.

Authors Contribution

OYA and MSSD conceptualized the study. OYA, MSSD and MKA designed the study. OYA and MSSD participated in laboratory work and data collection. OYA performed the data analysis; OYA, MSSD and MKA

interpreted the data. OYA prepared the first draft of the manuscript, reviewed by MSSD and MKA. All authors contributed to the development of the final manuscript and approved it.

Declaration

None

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