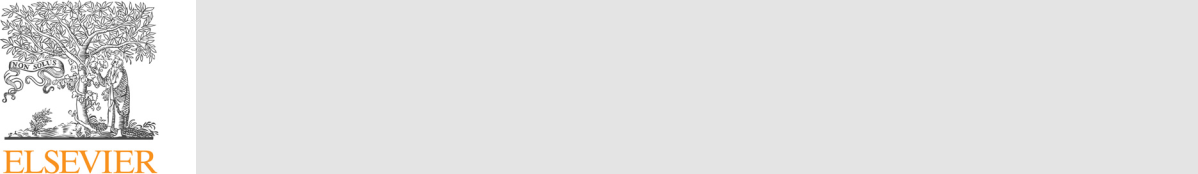
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In vitro and in vivo antitrypanosomal eﬃcacy of combination therapy of Anogeissus leiocarpus, Khaya senegalensis and potash

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ABSTRACT

Ethnopharmacological relevance: Pastoralists in Nigeria mix barks of Anogeissus leiocarpus (AL) Khaya senegalensis (KS) and potash (Pt) to treat animal African trypanosomosis.

Aim: To evaluate antitrypanosomal potential of A. leiocarpus, K. senegalensis and potash for insights into the traditional claim of antitrypanosomal combination therapy (ATCT).

Materials and methods: Fifty microliter each of six diﬀerent concentrations of AL, KS, Pt, AL + KS, AL + KS + Pt and diminazene aceturate (DA, positive control) was incubated with 50 μL of parasite-laden blood containing 108 Trypanosoma congolense cells in a 96-well microtitre plate. Negative control wells were devoid of the extracts and drug but supplemented with phosphate-buﬀered saline (PBS). Eﬃcacy of treatment was observed at 1 h interval for complete immobilisation or reduced motility of the parasites. Each incubated mixture was inoculated into mouse at the point of complete immobilisation of parasite motility or at the end of 6-h observation period for concentrations that did not immobilise the parasites completely. For in vivo assessment, thirty-five para-sitaemic rats were randomly allocated into seven groups of 5 rats each. Each rat in groups I–V was treated with 500 mg/kg of AL, KS, Pt, AL + KS and AL + KS + Pt, respectively, for 7 days. Rats in groups VI and VII were treated with diminazene aceturate 3.5 mg/kg once and PBS 2 mL/kg (7 days), which served as positive and negative controls, respectively. Daily monitoring of parasitaemia through the tail vein, packed cell volume and malondialdehyde were used to assess eﬃcacy of the treatments.

Results: The AL + KS + Pt group significantly (p < 0.05) and dose-dependently reduced parasite motility and completely immobilized the parasites at 10, 5 and 2.5 μg/μL with an IC50 of 9.1×10-4 µg/µL. All the mice with conditions that produced complete cessation of parasite motility did not develop parasitaemia within one month of observation. The AL + KS group significantly (p < 0.05) lowered the level of parasitaemia and MDA, and significantly (p < 0.05) maintained higher PCV than PBS group.

Conclusion: The combination of A. leiocarpus and K. senegalensis showed better antitrypanosomal eﬀects than single drug treatment and oﬀers prospects for ATCT. Our findings support ethnopharmacological use of com-bined barks of A. leiocarpus and K. senegalensis by pastoralist in the treatment of animal African trypanosomosis in Nigeria.

1. Introduction

African trypanosomosis is a chronic debilitating disease of animals and man ravaging sub-Saharan Africa. Animal African trypanosomosis (AAT) is caused primarily by Trypanosoma congolense and T. vivax ([Giordani et al., 2016](#page6)); while human African trypanosomosis is caused



by T. brucei gambianse and T. brucei rhodensiase. The disease is a great threat to sustainable agricultural and economic prosperity of sub-Sa-haran Africa ([Okello et al., 2015](#page6)). It renders vast areas of arable land inhabitable and unsuitable for agriculture ([Alsan, 2015](#page6)). Over 12.3 million of human ([Kato et al., 2015](#page6)) and 50 million heads of cattle are constantly exposed to the infection ([Tchmdja et al., 2017](#page6)).

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Trypanosomosis has been reclassified by the World Organisation for Animal Health (OIE) as notifiable disease because of its huge economic significance ([Diall et al., 2017](#page6)). Prevalence of AAT in an endemic area is a good indicator of potential threat to human health living in that community and may necessitate urgent medical intervention ([Grant](#page6) [et al., 2016](#page6)).

Chemotherapy and chemoprophylaxis of AAT depends largely on diminazene aceturate, isometamidium and homidium ([Holmes, et al.,](#page6) [2004](#page6); [Tauheed et al., 2016](#page6)). Suramin is being used to treat T. b. evansi in camel ([Giordani et al., 2016](#page6)). Treatment and control of trypanoso-mosis has been faced with high reported cases of treatment failure caused by resistance to the available antitrypanosomal drugs and high toxicity associated with the drugs ([Giordani et al., 2016](#page6)). Even the recently repurposed oral antitrypanosomal drug, fexinidazole for the treatment of second stage HAT is associated with severe treatment-emergent and serious adverse eﬀects; notably, neuropsychiatric adverse eﬀects manifested as headache, insomnia and anxiety ([Mesu et al.,](#page6) [2018](#page6)). Control of trypanosomosis is aimed at the destruction of vectors (Glossina species) and the elimination of parasite in infected hosts through the use of insecticides and chemotherapeutic agents, respec-tively.

Medicinal plants have been excellent sources of naturally produced compounds which account for a significant proportion of modern therapeutic agents ([Harvey et al., 2015](#page6)). It is estimated that 85% of the world population depends directly on plants as medicines ([Ganesan,](#page6) [2008](#page6)). Plants have a long history in medicine with a number of re-corded successes, the most recent one being the anti-malarial, artemi-simin which is obtained from the plant, Artemisia annua ([Ogbadoyi](#page6) [et al., 2007](#page6)). Anogeissus leiocarpus ([Singh et al., 2016](#page6)) and Khaya se-negalensis ([Takin et al., 2013](#page6)) are important medicinal plants with wide ethnomedicinal applications and some scientifically validated medical uses. A. leiocarpus is used in African and Asian traditional medicine to treat myriad of diseases/conditions, including, protozoan diseases (eg, trypanosomiasis, malaria, leshmaniasis) helminthosis, tuberculosis, diabetes, diarrhea, skin diseases, wound healing, febrile conditions, snake bite and scorpion sting, etc ([Arbab, 2014](#page6); [Shuaibu et al., 2008](#page6); [Shuaibu et al., 2008](#page6); [Singh et al., 2016](#page6)). Furthermore, K. senegalensis is used to treat plethora of diseases, eg, helminthosis ([Suleiman et al.,](#page6) [2013](#page6); [Suleiman et al., 2019](#page6)), cancer ([Zhang et al., 2007](#page6)), trypanoso-miasis ([Ibrahim et al., 2008](#page6)) bacterial infections ([Konaté et al., 2011](#page6)) and diabetes ([Kolawole et al., 2012](#page6)).

High prevalence of African trypanosomosis in the 21st century ([Tchmdja et al., 2017](#page6)), coupled with daunting challenges to large-scale Glossina spp. control and eradication programmes ([Percoma et al.,](#page6) [2018](#page6)) and bleak prospect for vaccine development ([Black and](#page6) [Mansfield, 2016](#page6)) has made chemotherapy and chemoprophylaxis as the only available option for the management of the disease. Improvement in trypanosomosis control in Africa will be of great economic benefits, especially in animal production in areas where there is poverty and chronic malnutrition. Interestingly, livestock, particularly cattle remain the important target for possible eradication of both AAT and zoonotic fatal HAT ([Hamill et al., 2017](#page6)).

Single drug treatment has been the gold standard treatment protocol for the treatment of trypanosomosis. Wide spread treatment failures and dose-limiting toxicities with some of these conventional single drug treatments call for research into antitrypanosomal combination therapy (ATCT). A survey conducted by [Atawodi et al. (2002)](#page6) showed that pastoralists in Nigeria mix barks of A. leiocarpus, K. senegalensis and potash to treat AAT. Therefore, the aim of the present work is to sci-entifically evaluate this claim in the laboratory for possible break-through in the chemotherapy of African trypanosomosis.

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2. Materials and methods

2.1. Plant materials, identification and extraction

Fresh stem barks of Anogeissus leicarpus Guill & Perr (called marke in Hausa speaking communities of Nigeria and African birch in English) and Khaya senegalensis (Desv.) A. Juss (called madaci in Hausa speaking communities of Nigeria and mahogany in English) were harvested in Area BZ (11°9ʹ48.21048ʺN 7°38ʹ5.91828ʺE) main campus of Ahmadu Bello University (ABU), Zaria, Nigeria on November, 2018. The leaves, flowers and seeds of the plants were sent to the Herbarium Unit of the Department of Biological Sciences, ABU Zaria, Nigeria for identifica-tion. Voucher Numbers 1738 and 900181were assigned to A. leiocarpus and K. senegalensis, respectively. The barks were dried to a constant weight at room temperature in the laboratory. Each bark was ground into powder using mortar and pestle and kept in a polythene bag before extraction. Two hundred grams each of A. leiocarpus and K. senegalensis was percolated in 900 ml of absolute methanol for 72 h. Thereafter, the liquid extract was drained into a clean bottle. The marc was rinsed oﬀ with 200 ml of fresh solvent and added to the initial solution collected. The liquid extract was concentrated with rotary evaporator and the extract was kept in a bottle.

2.2. Phytochemical screening

The thin-layer chromatography (TLC) profile of each extract was obtained by spotting each extract on silica gel 60 TLC254 plate (Merck KGaA, Germany) and the plate developed in predetermined solvent system. The plate was sprayed with anisaldehyde/sulphuric acid, Drandendoﬀ (alkaloids), Bontragers (anthraquinones), ferric chloride (phenolic compounds) and Liebermann-Burchard (steroids and tri-terpines) as detecting reagents followed by heating at 110 °C.

2.3. Experimental animals

The rats were obtained from the Animal House of the Department of Veterinary Pharmacology and Toxicology, ABU, Zaria. They were kept for 2 weeks in the laboratory for acclimatisation. They were housed in clean plastic cages with wood shavings as beddings. The beddings were changed every three days. The rats were fed on standard rat feed and given access to clean water ad libitum. The ethical approval for the use of rats was obtained from Ethical Committee on Animal Use and Care, Ahmadu Bello University, Zaria; with reference number: ABUCAUC/ 2019/005.

2.4. Test organism

T. congolense was obtained from the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, ABU, Zaria. The parasite was maintained in the laboratory by continuous passage in rats ([Tauheed et al., 2016](#page6)).

2.5. Determination of median lethal dose of the extracts and potassium (LD50)

The LD50 of extracts of A. leiocarpus and K. senegalensis, and po-tassium was determined according to the [OECD guide line (2008)](#page6). Limit doses of 5000 mg/kg was used for each extract and potassium. Healthy young adult non-pregnant nulliparous female rats weighing between 120 and 150 g and aged 8–12 weeks were used. Feed was withheld overnight and for additional 4 h after administration of treatments. One rat was dosed with 5000 mg/kg and observed for a period of 48 h. Four additional rats were dosed sequentially and the rats were observed daily for any sign of toxicity for 14 days.

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Table 1

Scoring of trypanosome viability for assessment of eﬃcacy of treatment for in vitro antitrypanosomal study.

|  |  |  |
| --- | --- | --- |
| Score | Number of trypanosomes per microscopic field | Inference |
|  |  |  |
| 0 | 0 | No viable (motile) parasite in ≥10 fields |
| 1 | 1–2 | Sluggish parasites in ≥10 fields |
| 2 | 3–5 | Active parasites in at least 3 fields |
| 3 | 6–10 | Active parasites in at least 3 fields |
| 4 | 11–20 | Active parasites in at least 3 fields |
| 5 | 21–40 | Active parasites in at least 3 fields |
| 6 | > 40 | Active parasites in at least 3 fields |
|  |  |  |

2.6. Experimental design

2.6.1. In vitro study

Fifty microliter each, of 0.1, 0.2, 2, 5, 10 and 20 μg/μL of A. leio-carpus (AL), K. senegalensis (KS), potash (Pt), AL + KS (1:1), AL + KS + Pt (1:1:1) and diminazene aceturate (DA; postive control) was pipetted into pre-labeled wells of 96-well round bottom microtire plate. Fifty microliter of parasite-laden blood (2.5 × 108 parasite/ml of blood) was added to each concentration to give final concentrations of 0.05, 0.1, 1, 2.5, 5 and 10 μg/μL, respectively. The experiment was carried out in triplicate. Similarly, well with 50 μL of parasite-laden blood and 50 μL of phosphate-buﬀered saline (PBS) served as negative control; while three additional wells with 50 μL of parasite-laden blood only served as untreated control. The plate was incubated at 25 °C and the eﬃcacy of treatment was observed at 1 h interval for 6 h. Scoring of complete cessation or reduction of motility of the parasite relative to negative control was used to rate the eﬃcacy of the treatments ([Table 1](#page6)).

2.6.2. Drug-incubation infectivity test

Any concentration that completely immobilized the parasites was immediately inoculated into mouse and observed daily for development of parasitaemia. At the end of the 6-h observation period, the con-centrations that did not immobilise the parasite were also individually inoculated into one mouse each and observed daily for development of parasitaemia.

2.6.3. In vivo

Thirty-five male Wistar rats were infected intraperitoneally with 0.2 mL of blood containing T. congolense at 106 per mL of blood. Seven days later when the rats were patent, they were randomly divided into seven groups of five rats each. Rats in groups I–V were treated with AL (500 mg/kg), KS (500 mg/kg) Pt (500 mg/kg), AL + KS (250 + 250 mg/kg) and AL + KS + Pt (166.67 + 166.67 + 166.67 mg/kg), respectively. Rats in groups VI and VII were treated with diminazene aceturate 3.5 mg/kg and phos-phate-buﬀered saline 2 mL/kg and served as positive and negative controls, respectively. All the treatments were given for 7 days except diminazene which was given once. The eﬀect of treatments was eval-uated daily by taking a drop of blood from the tail of each rat and observed under light microscope at × 400 magnification. Complete elimination of parasites from systemic circulation, reduction of parasite multiplication and survival of rats were taken as measures of eﬃcacy of treatments. The rats were observed for three weeks. At the end of the experiment, the rats were euthanized and 3 mL of blood from each rat was collected into EDTA sample bottle for determination of packed cell volume and erythrocyte malondialdehyde.

2.7. Determination of packed cell volume (PCV)

At the end of the experiment, the animals were sacrificed and blood was collected from each rat into EDTA bottle for determination of PCV using haematocrit reader as described by [Bain et al. (2016)](#page6).

2.8. Determination of malondialdehyde (MDA)

The MDA of erythrocyte was determined to evaluate eﬀects of treatment on the level of oxidative stress. The method of [Draper and](#page6) [Hadley (1999)](#page6) was used with little modification. Lipid peroxidation of red blood cells (RBCs) was measured by the thiobarturic acid reaction with MDA. In this assay, 0.5 ml of washed RBCs was added to 2.5 ml of 100 g/L trichloroacetic acid (TCA) solution and placed in boiling water bath for 15 min. It was removed from the water bath, cooled in tap water and centrifuged at 1000 g for 10 min. Then 2 ml of the super-natant was taken and added to 1 ml of 6.7 g/L thiobarbituric acid (TBA) in a test tube and placed in boiling water bath for another 15 min. It was then cooled in tap water and its absorbance (TBA reactive sub-stances) was measured at 532 nm with spectrophotometer (Spec-trumlab 23A China). One ml of 10% TCA and 1 ml of 0.67% TBA served as the blank.

2.9. Data analysis

Data were expressed as mean ± standard error of mean and sub-jected to one-way analysis of variance followed by Tukey post-test using GraphPad prism version 5.0. Values of P < 0.05 were considered statistically significant.

3. Results

3.1. Phytochemical screening of the plants showed the presence of phenolic compounds, alkaloids, steroids and triterpines. Anthraquinones were however, present in K. senegalensis only.

3.1. Acute toxicity study

None of the rats administered extract of A. leiocarpus and potash showed any apparent sign of toxicity at the limit dose of 5000 mg/kg evaluated. However, two rats in K. senegalensis treated group showed mild lethargy and depression within 24 h of administration but re-covered fully throughout 14-day observation with no morbidity and mortality.

3.2. In vitro

The combination group AL + KS + Pt significantly and dose-de-pendently reduced motility of the parasite when compared to PBS group ([Fig. 1](#page6)). The highest concentration 10 μg/μL and the two lower concentrations 5 and 2.5 μg/μL of AL + KS + Pt completely im-mobilized the parasites within 3 and 6 h, respectively, with an IC50 of 9.1 × 10-4 µg/µl compare to an IC50 of 0.998 µg/µl of PBS group. Furthermore, 10 μg/μL of AL + KS, AL and KS groups completely im-mobilized the parasites within 4 h while 5, 2.5, 1 and 0.1 μg/μL sig-nificantly (p < 0.05) reduced parasite motility when compared to PBS group. Whereas Pt group significantly reduced (p < 0.05) parasite motility only at 10 and 5 μg/μL with an IC50 of 1.27 × 10-3 µg/µl, DA group significantly reduced motility of the parasites at 10, 5 and 2.5 μg/ μL with an IC50 of 2.10 compared to 0.998 µg/µl of PBS group ([Table 2](#page6)).

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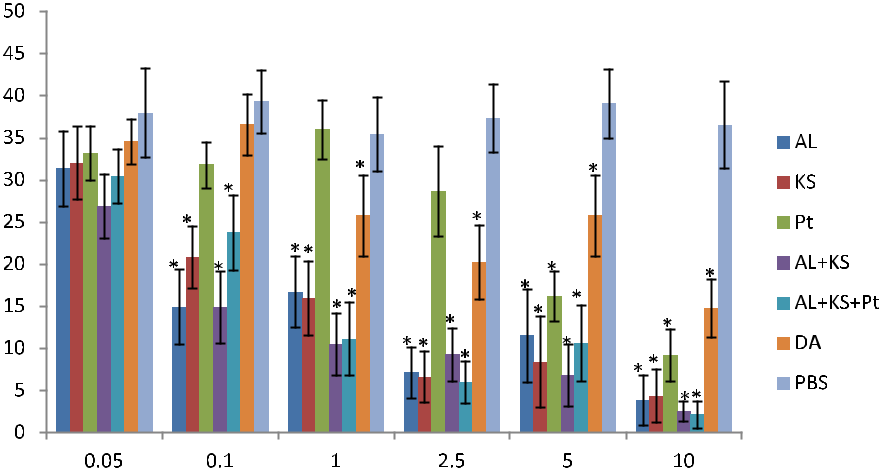


Fig. 1. In vitro eﬀects of the treatments on parasite motility. \* indicates significant reduction of parasite motility compare to PBS group. AL = Anogeissus leiocarpus, KS = Khaya senegalensis, Pt = Potash, DA = Diminazene aceturate and PBS = Phosphate-buﬀered saline.



Table 2

Median inhibitory concentrations (IC50) of the treatment groups.

|  |  |  |
| --- | --- | --- |
| Group | IC50 μg/μL (10-3) | R2 |
|  |  |  |
| AL | 1.16 | 0.9309 |
| KS | 1.54 | 0.9608 |
| Pt | 1.27 | 0.9772 |
| AL+KS | 0.23 | 0.9783 |
| AL + KS + Pt | 0.91 | 0.9773 |
| DA | 2.1 | 0.9602 |
| PBS | 998 | 0.9995 |
|  |  |  |
| AL = Anogeissus | leiocarpus, KS = Khaya senegalensis, Pt | = Potash, |

DA = Diminazene aceturate and PBS = Phosphate-buﬀered saline.

Surprisingly, AL + KS + Pt, KS + KS, AL and KS groups exhibited better (p < 0.05) in vitro antitrypanosomal eﬀect than diminazene aceturate at all the concentrations tested.

3.3. Drug incubation infectivity test

All the concentrations that completely immobilized the parasite motility and inoculated into mice did not develop infection throughout 4 weeks of observation. In addition, AL + KS + Pt and AL + KS groups at 1 μg/μL concentration inoculated into the mice with 1–2 sluggish parasites per microscopic field (7,943,000 trypanosome cells/mL of blood) at the end of 6-h observation did not develop parasitaemia (infection). However, one mouse and 2 mice inoculated with 1–2 moribund parasites at the end of 6-h observation period for AL and KS developed parasitaemia and succumbed to the infection ([Table 3](#page6)).

Table 3

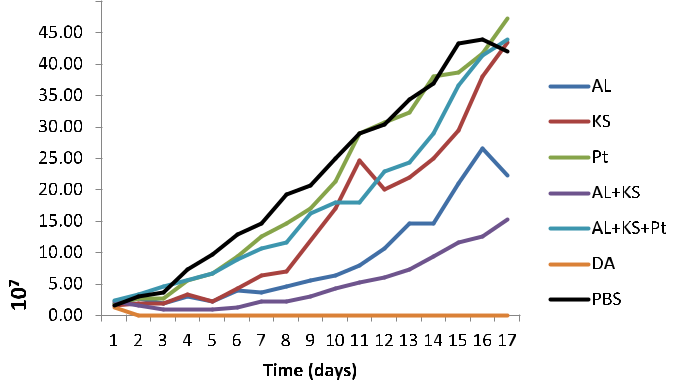
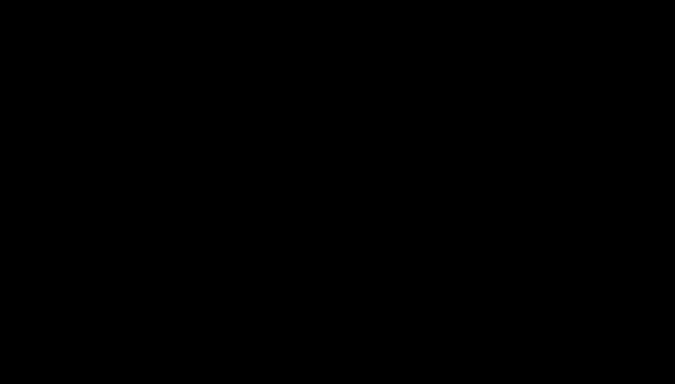
Ratio of survived to inoculated mice in drug incubation infectivity test.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Concentrations (μg/ | AL | KS | Pt | AL + KS AL + KS + Pt | | DA | PBS |
| μL) |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
| 0.05 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 |
| 0.1 | 0/3 | 0/3 | 0/3 | 0/3 | 1/3 | 0/3 | 0/3 |
| 1 | 1/3[a](#page6) | 2/3[a](#page6) | 0/3 | 3/3[a](#page6) | 3/3[a](#page6) | 0/3 | 0/3 |
| 2.5 | 3/3[a](#page6) | 2/3[a](#page6) | 0/3 | 3/3a | 3/3 | 0/3 | 0/3 |
| 5 | 3/3 | 3/3 | 0/3 | 3/3 | 3/3 | 0/3 | 0/3 |
| 10 | 3/3 | 3/3 | 3/3 | 3/3 | 3/3 | 2/3 | 0/3 |
|  |  | |  |  |  |  |  |
| AL = Anogeissus | leiocarpus, KS | | = | Khaya | senegalensis, Pt | = | Potash, |

DA = Diminazene aceturate and PBS = Phosphate-buﬀered saline.

1. Contained 1–2 sluggish parasites per microscopic field at the point of in-oculation into the mice.

Fig. 2. Mean daily parasitaemia of treated rats. AL = Anogeissus leiocarpus, KS = Khaya senegalensis, Pt = Potash, DA = Diminazene aceturate and PBS = Phosphate-buﬀered saline.



3.4. In vivo

There was significant reduction in the levels of parasitaemia in KS + AL (p < 0.001), KS (p < 0.001) and AL (p < 0.05) groups when compared with PBS group ([Fig. 2](#page6)). The KS + AL group sig-nificantly reduced (p < 0.001) parasitaemia when compared with KS + AL + Pt. Furthermore, KS + AL showed significant (p < 0.001) reduction in the levels of parasitaemia when compared to KS and AL groups. Diminazene aceturate cleared the parasites from systemic cir-culation of the treated rats within 24 h and did not show relapse parasitaemia.

3.5. Packed cell volume

[Fig. 3](#page6) shows the eﬀect of treatments on packed cell volume (PCV). The PCV of rats treated with AL + KS + Pt was significantly (p < 0.001) higher than the PCV of PBS group. Furthermore, AL and Pt groups had significantly (p < 0.01) higher PCV than the PBS treated group. PCV of rats treated with AL + KS was significantly (p < 0.05) higher than the PCV of rats administered PBS. Considering individual treatment groups, Pt group exhibited non-significantly higher (p > 0.05) PCV compared to AL and KS groups. Paradoxically, the PCV of rats in the AL + KS + Pt group was comparable with the PCV of rats treated with the standard antitrypanosomal drug, diminazene. The DA group had the highest PCV which is significantly higher than PBS group

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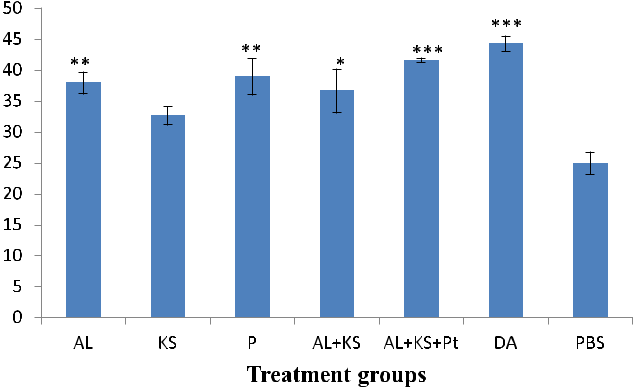


Fig. 3. Eﬀects of treatments on packed cell volume. Asterisks indicate sig-nificant levels of AL = Anogeissus leiocarpus, KS = Khaya senegalensis, Pt = Potash, DA = Diminazene aceturate and PBS = Phosphate-buﬀered saline. \* (p < 0.05), \*\* (p < 0.01) and \*\*\* (p < 0.001).

(p < 0.001).

3.6. Malondialdehyde of washed red blood cells

The eﬀect of treatments on lipid peroxidation of erythrocyte is shown in [Fig. 4](#page6). There was significant reduction (p < 0.001) in MDA of rats treated with AL + KS group when compared with PBS treated group. More so, AL + KS + Pt group had significantly lower (p < 0.001) MDA compared to PBS group. With the exception of KS group, there was significant reduction (p < 0.001) in MDA of rats in all the treated groups when compared to PBS group. Rats in the KS group showed significantly higher (p < 0.05) MDA than the rats in PBS group though not statistically significant.

4. Discussion

The clinically approved combination therapy for late stage human African trypanosomosis is oral nifurtimox-intravenous eflornithine. Its varied eﬃcacy, the need for it to be administered by trained personnel in hospital setting and heavy burden of transportation placed on the majority of patients who live in remote areas are the major dis-advantages of this combination therapy ([Mesu et al., 2018](#page6)). The aim of the present study was to explore possibility of synergistic or

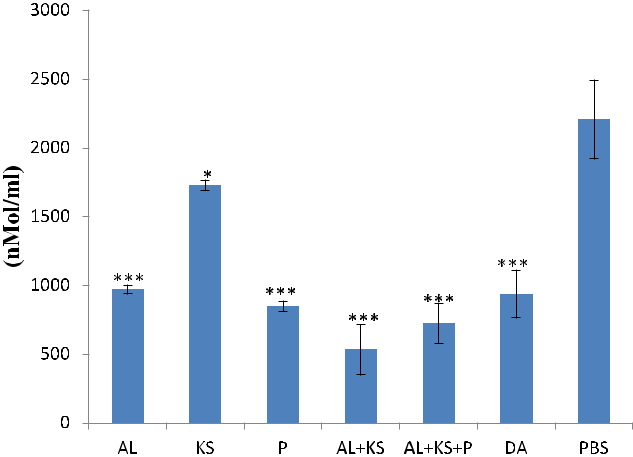


Fig. 4. Eﬀect of treatments on malondialdehyde of treated rats. Asterisks in-dicate significantly lower values of malondialdehyde compare to PBS group.

AL = Anogeissus leiocarpus, KS = Khaya senegalensis, Pt = Potash,

DA = Diminazene aceturate and PBS = Phosphate-buﬀered saline. \*

(p < 0.05) and \*\*\* (p < 0.001).

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potentiating eﬀects of Anogeissus leiocarpus, Khaya senegalensis and po-tash in the treatment of African trypanosomosis.

The results of our findings show prospect for ATCT. There were enhanced antitrypanosomal eﬀects between AL and KS in both the in vitro and in vivo studies, which could be additive or synergistic. AL + KS group rapidly reduced viability of the parasites and completely im-mobilized their motility within 2 h in the in vitro study. Furthermore, all the mice inoculated with few moribund parasites at 1 μg/μL in drug-incubation infectivity test did not develop parasitaemia. It is possible that the combined eﬀects of AL and KS in the AL + KS group aﬀected parasite viability at 1 μg/μL. On the contrary, some mice developed infection and succumbed to the infection in the AL and KS groups in-oculated at the same concentration with the same levels of parasites. This may support synergistic eﬀect between AL and KS.

The AL + KS group suppressed parasitaemia throughout the study, which surpassed the antitrypanosomal eﬀect observed in the AL + KS + Pt group. Potassium appeared to have interfered with promising in vivo antitrypanosomal eﬀects observed in AL + KS group. This is manifested with poor antitrypanosomal eﬀects in both Pt and AL + KS + Pt groups. It follows that potassium is not inert in this case. Had potassium not antagonised the antitrypanosomal eﬀects observed in the AL + KS group, AL + KS + Pt would have at least yielded similar promising antitrypanosomal eﬀects observed in the AL + KS group. Similar observation has been reported by [Rial et al. (2018)](#page6), who observed that addition of high doses of benznidazole to allopurinol antagonised good antitrypanosomal eﬀects of allopurinol with low doses of benznidazole against Trypanosoma cruzi. [Araujo-Lima et al.](#page6) [(2019)](#page6) reported that the combination of atorvastatin (a statin) and benznidazole produced better antitrypanosoml eﬀects on T. cruzi in-fection than conventional single drug therapy. Indeed, the combination of A.leiocarpus and K. senegalensis significantly suppressed T. congolense infection than the single therapy with A. leiocarpus and K. senegalensis.

Anaemia is a cardinal feature of African trypanosomosis ([Nok and](#page6) [Balogun, 2003](#page6); [Baloguna et al., 2014](#page6)) and often the major cause of death. Control of anaemia but not parasitaemia is crucial for the sur-vival and productivity of animal infected with trypanosomes ([Naessens,](#page6) [2006](#page6)). Thus, agents that can alleviate anaemia may be used as adjunct in the treatment of African trypanosomosis. Treatment with potassium was able to prevent anaemia. Pt group exhibited highest value of PCV compare to each of the two extract groups. The ability of potassium to maintain high PCV may explain the reason for its use by traditional herders in the management of animal African trypanosomosis in in-fected animals. Maintenance of adequate PCV in the face of high parasitaemia maintains the health of the parasitaemic animal and sus-tains their productivity ([Naessens, 2006](#page6); [Tauheed et al., 2016](#page6)). Therefore, it is possible that the use of potassium by pastoralists in Nigeria to treat the animals infected with trypanosomes hinges on the advantage potassium confers in maintaining adequate blood volume but not for elimination of the parasites. [Baloguna et al. (2014)](#page6) showed that de-galactosylation of erythrocyte membrane ameliorated anaemia and increased PCV in trypanosome-infected mice. Recently, [Saad et al.](#page6) [(2019)](#page6) found that T.congolese-induced anaemia was prevented by in-hibiting sialidase. Furthermore, our findings showed that addition of potassium to the two plants extract (ie, AL + KS + Pt group) translate into higher PCV than each of the three treatment groups (AL, KS and Pt). Identification of how potassium maintained higher PCV in the face of overwhelming T. congolense infection may provide insight on its use as adjunct in the management of African trypanosomosis. In another study, modulation of innate immune responses is known to control anaemia in T. congolense infected mice ([Noyes et al., 2009](#page6)).

The significantly lower serum MDA concentrations seen in the combination groups (AL + KS and AL + KS + Pt) showed that A. leiocarpus, K. senegalensis and potassium acted synergistically to ame-liorate lipid peroxidation of erythrocyte membrane and thus reduced the serum concentration of MDA. Lipid maintains the integrity of cell membrane and excessive production of free radicals beyond the

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capacity of endogenous antioxidant system can lead to peroxidation of lipid membrane and overproduction of MDA. MDA is a known product of cell membrane peroxidation used to assess the degree of cellular injury to trypanosomosis resulting from lipid peroxidation ([Tauheed](#page6) [et al., 2016](#page6)). The significantly higher values of MDA in the PBS treated group imply perturbation of the rats by the T. congolense. Excessive peroxidation of lipoprotein of cell membrane alters physicochemical properties of cell membranes and cause covalent modification of pro-teins and nucleic acids ([Gaschler and Stockwell, 2017](#page6)). Recently, the combinations of two phenolic compounds (malvidin-3-O-gluco-side + Vitamin E; and quercetin-3-O-glucuronide + vitamin C) isolated from stem of grape Vitis vinifera L. have exhibited excellent antioxidant capacity and decreased lipid peroxidation ([Queiroz et al., 2017](#page6)). Thus, the combination group AL + KS could have prevented lipid peroxida-tion of cellular membrane by T. congolense, prevent permeability of cell membrane and maintain membrane fluidity.

Although mechanisms of antitrypanosomal eﬀect of drugs under in vitro study is not known, it is possible that medicinal plants with anti-trypanosomal eﬀects kill the parasite by cytotoxic eﬀect. Diminazene aceturate did not exhibit comparable in vitro antitrypanosomal activity but completely eliminated the parasites from systemic circulation of the treated rats within 24 h during in vivo study without relapse para-sitaemia. On the contrary, AL, KS, AL + KS and AL + KS + Pt groups exhibited good in vitro antitrypanosomal eﬀect but with unmatched in vivo antitrypanosomal eﬀect of diminazene aceturate. Therefore, en-hancement of host immune system may play a key role in eliminating trypanosome from infected animals. The elimination of T. congolense from infected mice treated with diminazene aceturate was reported to be due to the modulatory eﬀects of diminazene aceturate on host's cellular immune response ([Kuriakose et al., 2012](#page6)). The host kinase Akt plays key roles in inhibiting replication of T. cruzi ([Caradonna et al.,](#page6) [2013](#page6)). These underscore enhancement of host immune system by di-minazene aceturate and may explain its good in vivo antitrypanosomal eﬀect compare to plant extracts. Furthermore, the inability of some trypanosomes, notably, Trypanosoma brucei brucei to survive and infect humans and some primates is known to be a result of the trypanolytic factor, apoliporotein L1 in their sera ([Wheeler, 2010](#page6); [Pays and](#page6) [Vanhollebeke, 2008](#page6)).

5. Conclusion

Our findings demonstrate prospect for antitrypanosomal combina-tion therapy from ethnopharmacological practices. Combination of barks of Khaya senegalensis and Annogeissus leiocarpus exhibited better antitrypanosomal eﬀects than either of the plants used singly. The combined eﬀects could be described as synergistic rather potentiating; since each of the plants independently demonstrated antitrypanosomal eﬀects. With the exception of good haematopoietic eﬀect, addition of potassium did not translate into enhanced antitrypanosomal eﬀects in the in vivo study. We therefore conclude that potassium could only be used as an adjunct in the treatment of African trypanosomosis to pre-vent anaemia and thus, enhance productivity of infected animals.

List of authors and their contributions

AMT designed and conducted the experiment, analysed the results and drafted the manuscript; MM, EOB, AA and MMS designed the ex-periment and proof-read the manuscript.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at [https://](https://doi.org/10.1016/j.jep.2020.112805) [doi.org/10.1016/j.jep.2020.112805](https://doi.org/10.1016/j.jep.2020.112805).

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