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Effects of α-tocopherol on the *in vivo* antitrypanosomal effects of phenolics-rich fraction of *Khaya senegalensis* stem bark

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ABSTRACT

Objective: To investigate the effects of combined administration of a low dose of a phenolics-rich fraction of *Khaya senegalensis* (PFKS) stem bark with α-tocopherol on *Trypanosoma brucei brucei* (*T. brucei brucei*) infection.

Methods: Rats were divided into five groups of six animals, namely, normal control, uninfected but treated with PFKS and α-tocopherol, infected control, infected and treated with PFKS and α-tocopherol (ITTF) and infected treated with diminazine aceturate. Rats in infected control, ITTF and infected treated with diminazine aceturate were infected with *T. brucei brucei* while the animals in uninfected but treated with PFKS and α-tocopherol and ITTF were treated with a combination of PFKS (100 mg/kg body weight) and α-tocopherol (100 mg/kg body weight) for 8 days. At the end of the experiment, indices of anemia as well as hepatic and renal functions were analysed.

Results: The combined treatment significantly ($P < 0.05$) retarded the proliferation of *T. brucei brucei* in the infected animals compared to the infected group but could not completely eliminate the parasites from the bloodstream of infected animals. Furthermore, the trypanosome-associated pathological changes such as anemia, hepatic and renal damages were significantly ($P < 0.05$) alleviated by the combination of PFKS and α-tocopherol.

Conclusions: Combination of a low dose of PFKS stem bark and α-tocopherol could be a therapeutically active regimen against animal trypanosomiasis.

1. Introduction

Animal trypanosomiasis is a neglected tropical disease that still retards the growth of livestock industry in Africa. Currently, the prevalence of the disease is strongly dependent on control measures, which are often neglected leading to resurgence and, on the other hand, the hope for the development of vaccine against the infection is almost lost[1]. Thus, in the absence of either effective vector control or a vaccine, chemotherapy remains the only available option. Unfortunately, the chemotherapeutic strategies are beset with several problems, which include drug toxicity, parasite resistance, high cost and poor availability[2]. Hence, our interest is to develop an effective anti-trypanosome remedy, especially from plant sources, that is devoid of the aforementioned limitations.

Khaya senegalensis A. Juss (Meliaceae) (*K. senegalensis*) is highly reputed from numerous medicinal uses and has been reported to be the most commonly used medicinal plant for the indigenous treatment of animal trypanosomiasis in Northern

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Nigeria[3]. The *in vitro* antitrypanosomal activity of this plant crude extracts against *Trypanosoma brucei brucei* (*T. brucei brucei*) has been demonstrated[4]. Subsequently, we reported the *in vivo* antitrypanosomal activity of the stem bark crude extracts against *T. brucei brucei* and *Trypanosoma evansi*[5,6]. In a more recent study, we reported the *in vivo* antitrypanosomal activity of a phloroglucinol and 3,4-(dihydroxyphenyl) acetic acid rich fraction of *K. senegalensis* stem bark where the fraction was found to completely eliminate *T. brucei brucei* from the bloodstream of infected animals only at a high dose of 300 mg/kg body weight[7]. However, the administration of such a high dose of the fraction did not completely reverse the trypanosome-induced anemia and organ damage in addition to being slightly hepatotoxic to uninfected animals[7]. The severity of these trypanosome-associated pathological alterations are vital indicators of the disease status and their control is an integral part of the disease management. Interestingly, we have previously observed the complete amelioration of trypanosome-induced anemia and organ damage by antioxidant vitamins such as α-tocopherol (vitamin E)[8]. In our quest to further improve the ameliorative effects of phenolics-rich fraction of *K. senegalensis* (PFKS) on the trypanosome-induced pathological alterations and to minimize its toxic effects whilst achieving similar parasite clearance ability, we investigated the effects of combined administration of a low dose (100 mg/kg body weight) of PFKS with α-tocopherol on the pathogenesis of trypanosome infection in rats.

2. Materials and methods

2.1. Plant material and preparation of PFKS

The stem bark of *K. senegalensis* was collected from the Samaru campus of Ahmadu Bello University Zaria (ABUZ), Nigeria and the species was identified at the herbarium unit of Biological Sciences Department of the same university. The voucher herbarium specimen was deposited with number 900081. The stem bark samples were washed and air-dried for four weeks to a constant weight and then processed to fine powder before storage in air-tight dry containers until needed. The PFKS was prepared as previously described by Ibrahim *et al*[7]. Briefly, the powdered plant material (2 kg) was extracted with 12 L of 96% ethanol (maceration) for a week. The extract was filtered using Whatman filter paper (No. 1) and concentrated on a Buchi Rotary Evaporator (Buchi rota vapor R-124) at 40 ˚C. The concentrated extract was finally evaporated to dryness on a water bath which afforded 410 g of crude extract. The crude ethanol extract (100 g) was suspended in 300 mL of distilled and successively partitioned with diethyl ether (2 × 300 mL) and ethyl acetate (2 × 300 mL). The ethyl acetate fraction was concentrated under reduced pressure to yield a fraction (19 g) which was considered as the PFKS. The identity of the bioactive components of the fraction was determined by gas chromatography–mass spectrometry analysis where phenolics [phloroglucinol and 3,4-(dihydroxyphenyl) acetic acid] were found to be the most abundant phytochemical components of the fraction[7].

2.2. Experimental animals and trypanosomes parasites

The protocol was employed by the guidelines of the Good Laboratory Practice regulations of World Health Organization and the rules and regulations of experimental animal ethics committee of ABUZ were duly followed. Apparently healthy white albino rats (Wistar strain) weighing 140-200 g were obtained from the animal house of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, ABUZ, Nigeria. The animals were maintained in polycarbonated laboratory cages [(23 ± 2) ˚C, 12 h light–dark cycle] and fed on a commercial rat chow (Vital Feeds, Jos, Nigeria) with drinking water *ad libitum*. The *T. brucei brucei* parasite (Federe strain) used for the study was obtained from the Protozoology Laboratory, Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, ABUZ, Nigeria.

2.3. Effects of combined administration of PFKS and α-tocopherol on T. brucei brucei infection

Thirty Wistar rats (140-200 g) of both sexes were randomly allocated into five groups of six rats each in order to investigate the effects of α-tocopherol on the *in vivo* antitrypanosomal effects of PFKS. The rats in each group were received the following treatments: Normal control (NC): neither infected with the parasites nor treated with PFKS or α-tocopherol, Non-infected + PFKS and α-tocopherol (NTTF): uninfected but orally treated with PFKS (100 mg/kg body weight) and α-tocopherol (100 mg/kg body weight), Infected control (IC): infected and not treated, Infected + PFKS and α-tocopherol (ITTF): infected and orally treated PFKS (100 mg/kg body weight) and α-tocopherol (100 mg/kg body weight), and Infected + diminazine aceturate (ITS): infected and treated with 100 mg/kg body weight of diminazine aceturate.

The rats in three of the groups (IC, ITTF and ITS) were infected by intraperitoneal injection of 10^4 *T. brucei brucei* per 100 g body weight and were daily treated with the respective doses of PFKS and α-tocopherol or diminazine aceturate, beginning on Day 3 post-infection (PI) when parasitemia approximately reached 10^8 trypanosomes/mL of blood. The level of parasitemia was monitored daily using the rapid matching counting method and the experiment was terminated on Day 8 PI[9]. The pre-infection and terminal (on Day 8 PI) packed cell volumes (PCV) of all groups of rats were determined by the microhematocrit method from which the percentage change in PCV was computed. In order to assess organ damage in all groups of animals, serum harvested from the blood of all animals after humane decapitation on Day 8 PI was used to measure alanine transaminase (ALT) and aspartate aminotransferase (AST) activities as well as urea concentrations using commercial reagent kits (Randox Laboratories, Ireland). Liver, and kidney of all rats were also collected and weighed to ascertain the relative organ weight for all groups of animals.

Table 1
Effects of combined administration of a low dose of PFKS and α-tocopherol on the severity of *T. brucei brucei* infection in rats.

Groups	NC	NTTF	IC	ITTF	ITS
^ % change in PCV	+5.81 ± 1.14^{d}	+3.07 ± 0.51^{c}	-4.56 ± 0.92^{b}	+3.92 ± 1.49^{cd}	-6.99 ± 1.17^{a}
AST (IU/L)	63.97 ± 5.00^{a}	86.80 ± 5.20^{b}	119.34 ± 12.07^{d}	102.08 ± 3.01^{c}	100.02 ± 5.94^{c}
ALT (IU/L)	19.50 ± 3.43^{a}	26.80 ± 3.80^{b}	30.40 ± 4.85^{b}	28.90 ± 3.40^{b}	27.75 ± 1.66^{b}
Urea (mg/dL)	102.77 ± 15.28^{ab}	95.30 ± 17.00^{ab}	190.04 ± 23.84^{c}	86.70 ± 10.60^{a}	116.75 ± 17.69^{b}
Liver: body weight ratio ($\times 10^{-2}$)	2.90 ± 0.11^{a}	2.60 ± 0.23^{a}	5.37 ± 0.12^{d}	4.26 ± 0.31^{c}	3.40 ± 0.10^{b}
Kidney: body weight ratio ($\times 10^{-3}$)	5.60 ± 0.14^{a}	5.60 ± 0.90^{a}	7.13 ± 0.65^{b}	5.80 ± 0.34^{a}	7.03 ± 0.25^{b}

All values are presented as mean ± SD. $^{a-d}$: Values with different superscripts along a row are significantly different from each other (Tukey's multiple range *post hoc* test, $P < 0.05$). ^ These values represent the percentage differences between initial and terminal PCV values. +: Increase; -: Decrease.

2.4. Statistical analysis

All data were presented as the mean ± SD of six animals. Data was analyzed with a statistical software package (SPSS for Windows, version 18, IBM Corporation, NY, USA) using Tukey's-HSD multiple range *post hoc* test. Values were considered significantly different at $P < 0.05$.

3. Results

The parasitemia profiles indicated that the *T. brucei brucei* were first detected in the bloodstream of all infected animals on Day 3 PI. However, the ITTF group was recorded significantly ($P < 0.05$) lower *T. brucei brucei* in the bloodstream than the IC group throughout the experimental period (Figure 1). In the ITS group, the parasites were totally eliminated from the bloodstream from Day 5 to the end of the experiment on Day 8. There were no significant differences ($P > 0.05$) in the pre-infection PCV of all groups of rats but the IC and ITS groups were developed anemia as the infection progress. This was evident by the significant ($P < 0.05$) decrease in the PCV level of IC and ITS groups. Interestingly, the ITTF group had significantly ($P < 0.05$) higher final PCV levels than the initial PCV (Figure 2). In fact, the ITTF had a statistically similar percentage change in PCV with the non-infected (NC and NTTF) groups (Table 1).

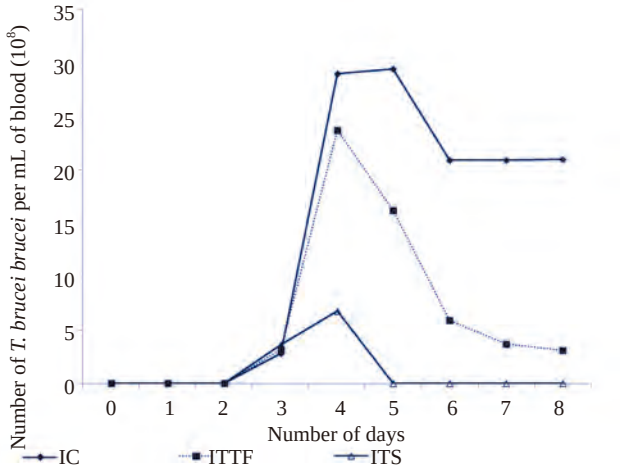

Figure 1. *In vivo* activity of a combined administration of a low dose of PFKS with α-tocopherol against *T. brucei brucei* infection in rats.

The data for the indices of hepatic and renal functions demonstrated that the *T. brucei brucei* was able to induce hepatic and renal damages as evidenced by the significant increase ($P < 0.05$) in the serum AST, ALT and urea levels in IC group compared to the NC group (Table 1). However, the ITTF group had significantly ($P < 0.05$) lower serum AST, and not ALT, compared to the IC group which indicated mild modulation of hepatic function by the combined treatment. On the other hand, the ITTF group had significantly ($P < 0.05$) lower serum urea concentration than the IC group. Indeed, the ITTF group was recorded lower serum urea concentration than the NC group. Hepatomegaly and renal hypertrophy were observed as increase in liver: body weight ratio and kidney: body weight ratio respectively, in the infected animals (Table 1). However, the ITTF group was recorded significantly ($P < 0.05$) reduced level of hepatomegaly and renal hypertrophy.

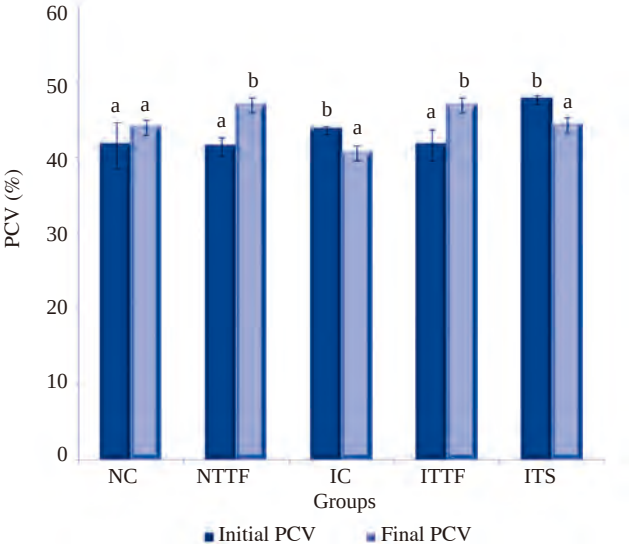

Figure 2. Effects of of a combined administration of a low dose of PFKS with α-tocopherol on the PCV levels of *T. brucei brucei* infected rats. All data are shown as mean ± SD; ^{a,b}: Values with different letters over the bars for a given group are significantly different from each other (Tukey's HSD multiple range *post hoc* test, $P < 0.05$).

4. Discussion

Previous studies have asserted that a combination of antitrypanosomal agents with antioxidant vitamins may

improve the efficacy and efficiency of treatment of trypanosome infections[8,10,11]. In the present study, the ability of α-tocopherol to improve the antitrypanosomal effects of a low dose PFKS was investigated.

The combined treatment in the ITTF group demonstrated an *in vivo* activity against the parasites. However, the observed activity was far lower than our previous observation where similar dose of PFKS was administered without α-tocopherol[7]. This observation could connote that α-tocopherol had antagonized the antitrypanosomal activity of phloroglucinol and/or 3,4-(dihydroxyphenyl) acetic acid and consequently limited the proliferation of the *T. brucei brucei*. Interestingly, α-tocopherol was also reported to possess an antagonistic effect on the efficacy of other drugs such as artemisinin-based antimalarials[12].

The combined treatment ameliorated the *T. brucei brucei*-induced anemia, hepatic and renal damages as evidenced by the improved PCV and decreased AST, ALT and urea levels in the ITTF group. This is indeed remarkable because the trypanosome-induced pathological alterations were reversed to near-normal in this study unlike the mild and/or insignificant effects observed when similar dose of PFKS was administered alone[7]. This could be linked to the scavenging action of α-tocopherol towards the *T. brucei brucei*-generated free radicals and thus spare the erythrocytes as well as hepatic and renal tissues from the oxidative damage[13].

Taking the findings of this study as a whole, it seems that α-tocopherol has a dual effect on the antitrypanosomal activity of PFKS. The vitamin possesses an antagonistic effect on the ability of phloroglucinol and/or 3,4-(dihydroxyphenyl) acetic acid to mediate the killings of *T. brucei brucei* as well as a beneficial effect towards the trypanosome-associated pathological alterations. This finding might support the conflicting outcomes on the practical application of some antioxidants treatment on the pathogenesis of plasmodium infection which is a protozoan parasite with similar pathological features as trypanosomes[14].

In conclusion, α-tocopherol is antagonistic to the parasite clearance ability of PFKS but could improve the ameliorative effects of the fraction on the trypanosome-induced pathological alterations. Hence, the therapeutic application of α-tocopherol in the management of trypanosomiasis might depend on the targeted aspect of the disease pathology.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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