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Short communication

Combination therapy of vitamin C and phenolics-rich fraction of *Khaya senegalensis* stem bark extract against *Trypanosoma brucei brucei* infection

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ABSTRACT

The phenolics-rich fraction of *Khaya senegalensis* A. Juss (Meliaceae) stem bark extract (pfks) has been previously reported to possess potent antitrypanosomal activity at 300 mg/kg body weight (bw) but could not completely prevent disease-induced anemia and organ damage in addition to being slightly hepatotoxic at the same dose. Therefore, the effects of a combined administration of low dose pfks and vitamin C on the severity of *Trypanosoma brucei brucei* infection in rats were investigated. Daily oral administration of a combined treatment of pfks (100 mg/kg bw) and vitamin C (100 mg/kg bw) for seven days significantly ($p < 0.05$) reduced the number of *T. brucei brucei* in the bloodstream compared to infected untreated control with an ED_{50} of 51.15 mg/kg bw. Also, the trypanosome-induced pathological alterations such as anemia, hepatic and renal damages were significantly ($p < 0.05$) prevented by the same dosage of the combined treatment. Thus, the results obtained suggest that the combined treatment of a low dose of pfks and vitamin C is therapeutically potent as antitrypanosomal regimen and could effectively ameliorate the trypanosome-induced anemia and organ damage.

1. Introduction

African animal trypanosomiasis is one of the neglected tropical parasitic diseases that still wreak havoc in the livestock industry of the continent [1]. The disease is associated with anemia and organ damage which are considered as important pathological features for the disease [2]. The mechanism of the trypanosome-induced anemia has been attributed to the activities of sialidase and phospholipase A_2 released by the parasites [3]. Also, large amounts of free radicals and superoxides generated by the trypanosomes make an enormous contribution to the development of anemia as well as organ damage [3]. Hence, the severity of these pathological alterations is a vital indicator of the disease status and their control is an integral part of the disease management. Consequently, any agent used for the treatment of African trypanosomiasis should also be effective in alleviating the associated pathological changes. At present, the chemotherapy of African trypanosomiasis remains far from satisfactory because the clinically available drugs have limitations such as toxicity, parasite resistance, high cost, and poor availability [4]. Hence, our interest is to develop an effective anti-trypanosome remedy, especially from plant sources, that is devoid of the aforementioned limitations and could ameliorate the trypanosome-associated pathological changes.

Khaya senegalensis A. Juss (Meliaceae) is highly reputable for numerous medicinal uses and has been reported to be the most commonly used medicinal plant for the indigenous treatment of animal trypanosomiasis in northern Nigeria [5]. The *in vitro* antitrypanosomal activity of the plant crude extract against *Trypanosoma brucei brucei* has been demonstrated [6,7]. Subsequently, we reported the *in vivo* antitrypanosomal activity of the stem bark crude extracts against *T. brucei brucei* [8] and *T. evansi*

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[9]. In more recent studies, we reported the potent antioxidative effects of *K. senegalensis* stem bark [10] as well as an *in vivo* antitrypanosomal activity of a phloroglucinol and 3,4-(dihydroxyphenyl) acetic acid rich fraction of *K. senegalensis* stem bark (pfks) 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 [11]. Unfortunately however, administration of such a high dose of the fraction did not completely prevent the trypanosome-induced anemia and organ damage in addition to being slightly hepatotoxic to uninfected animals [11]. In order to further improve the ameliorative effects of the fraction on the disease-induced pathological alterations and to minimize its toxic effects whilst maintaining similar parasite clearance ability, we investigated the effects of combined administration of a low dose (100 mg/kg BW) of pfks with vitamin C on the pathogenesis of trypanosome infection in rats using the same experimental conditions and set up as Ibrahim et al. [11] to enable good comparison.

2. Materials and methods

2.1. Plant material

The stem bark of *K. senegalensis* was collected in May 2010 from the Samaru campus of Ahmadu Bello University, Zaria (ABUZ), Nigeria and the species were identified by Mr. Umar Gallah 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.

2.2. Preparation of phenolics-rich fraction of K. senegalensis stem bark (pfks)

The pfks was prepared as previously described by Ibrahim et al. [11]. Briefly, the powdered plant material (2 kg) was extracted by maceration in 12 L of 96% ethanol for one week. The extract was filtered using Whatman filter paper (No. 1) and concentrated on a Buchi rotary evaporator (Buchirota 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 water 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 phenolics-rich fraction (pfks). The identity of the bioactive components of the fraction was determined by gas chromatography-mass spectrometry (GC–MS) analysis where phenolics (mainly phloroglucinol and 3,4-(dihydroxyphenyl) acetic acid) were found to be the most abundant phytochemical components of the fraction [11].

2.3. Experimental animals and trypanosome parasites

The protocol employed met the guidelines of the Good Laboratory Practice (GLP) 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 Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, ABUZ, Nigeria.

2.4. Effect of combined administration of pfks and vitamin C on T. brucei brucei infected rats

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 vitamin C on the *in vivo* antitrypanosomal effects of pfks. Rats in three of the groups were each infected by intraperitoneal injection with about 10^4 *T. brucei brucei* per 100 g bw while rats in the remaining two groups were uninfected. The level of parasitemia was monitored using the rapid matching counting method [12]. On day 3 post infection (pi) when parasitemia approximately reached 10^6 trypanosomes/mL of blood, one group of infected rats was orally treated with combined administration of pfks and vitamin C (100 mg/kg bw each) whereas another group was treated with 100 mg/kg bw of diminazine aceturate and the remaining group of infected rats was left untreated (infected control). One group of uninfected rats was also treated with combined administration of pfks and vitamin C (100 mg/kg bw each) and the remaining uninfected group was left untreated (normal control). All treatments were given daily from day 3 pi to the end of the experiment on day 7 pi. The pre-infection and terminal (on day 7 pi) packed cell volumes (PCV) of all groups of rats were determined by the microheamatocrit method from where the

Table 1
Effects of combined administration of a low dose of phenolics-rich fraction of *K. senegalensis* stem bark (pfks) and vitamin C on the severity of *Trypanosoma brucei brucei* infection in rats.

	Uninfected untreated controls	Uninfected and treated with pfks and vitamin C	Infected control	Infected and treated with pfks and vitamin C	Infected and treated with diminazine aceturate
^ % change in PCV	$+5.81 \pm 1.14^{c}$	$+7.76 \pm 2.37^{c}$	-4.56 ± 0.92^{b}	$+6.90 \pm 1.97^{c}$	-6.99 ± 1.17^{a}
AST (U/L)	63.97 ± 5.00^{a}	72.96 ± 10.58^{a}	119.34 ± 12.07^{c}	81.25 ± 14.53^{a}	100.02 ± 5.94^{b}
ALT (U/L)	19.50 ± 3.43^{ab}	19.20 ± 0.92^{a}	30.40 ± 4.85^{c}	22.73 ± 2.80^{b}	27.75 ± 1.66^{c}
Urea (mg/dL)	102.77 ± 15.28^{a}	96.67 ± 14.01^{a}	190.04 ± 23.84^{b}	93.00 ± 13.11^{a}	116.75 ± 17.69^{a}
Liver: BW ratio ($\times 10^{-2}$)	2.90 ± 0.11^{a}	3.02 ± 0.14^{a}	5.37 ± 0.12^{c}	3.48 ± 0.94^{b}	3.40 ± 0.10^{b}
Kidney: BW ratio ($\times 10^{-3}$)	5.60 ± 0.14^{a}	6.20 ± 0.76^{ab}	7.13 ± 0.65^{b}	5.75 ± 1.47^{a}	7.03 ± 0.25^{b}

Data are presented as mean ± SD of six animals. [a–c]Values with different superscript letters among the experimental groups for a given parameter are significantly different from each other (Tukey's multiple range post-hoc test, $p < 0.05$). No significant difference exists between the experimental groups with the same superscript letters for a given parameter (Tukey's multiple range post-hoc test, $p < 0.05$). ^ These values represent the percentage differences between initial and terminal PCV values and positive sign (+) indicates increase while negative sign (−) indicates decrease. AST, aspartate transaminase; ALT, alanine transaminase; pfks, phenolics-rich fraction of *K. senegalensis*; BW, body weight.