academicJournals

Vol. 14(6), pp. 525-529, 11 February, 2015
DOI: 10.5897/AJB2014.14136
Article Number: 8965FED50417
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Antitrypanosomal activity of *Khaya senegalensis* and *Anogeissus leiocarpus* stem bark on *Trypanosoma brucei brucei* infected rats

Awobode, Henrietta O.*, Fagbemi, Folasade T. and Afolayan, Funmilayo I. D.

Department of Zoology, University of Ibadan, Ibadan, Oyo State, Nigeria.

Received 23 October, 2014; Accepted 12 January, 2015

***Trypanosoma brucei brucei*, a haemo-protozoan parasite causes African Animal Trypanosomiasis (AAT). *Khaya senegalensis* (KS) and *Anogeissus leiocarpus* (AL) are medicinal plants used either individually or in combination by local farmers in Northern Nigeria in the treatment of many diseases including trypanosomiasis. There is however, no information on the efficacy of the plants used in combination. In this study, the antitrypanosomal activity of combined methanolic stem bark extracts of *K. senegalensis* and *A. leiocarpus* were determined *in vivo* using suppressive and repository tests. The combined extracts were administered at 250 mg/kg to *T. b. brucei* infected rats in ratios 1:4, 2:3, 1:1, 3:2 and 4:1 (*K. senegalensis* to *A. leiocarpus*). Diminor® (3.5 mg/kg) was positive control and Tween-80 the negative control. Trypanocidal activity was recorded in all four ratios with the highest in the 4:1 ratio for both tests. All ratios in repository test had varying levels of prophylactic activity which were significantly higher ($p<0.05$) than the negative control group. Chemo-prophylactic activity in the 4:1 ratio compared ($p>0.05$) favorably with the positive control. The extracts however had significantly lower ($p<0.05$) parasite suppressive activity compared to Diminor® (100%). The 1:4 combinations had the lowest activity (4.35%). In the repository test, packed cell volume (PCV) levels varied in the groups with an increase as the quantity of *K. senegalensis* in the dose increased. The results therefore show that the antitrypanosomal activity and haemolytic effects of the extracts was dependent on the ratio of *K. senegalensis* to *A. leiocarpus*. A higher quantity of *K. senegalensis* provided a more effective prophylaxis and normal PCV. The use of a threefold quantity of *K. senegalensis* to *A. leiocarpus* in the local management of animal trypanosomiasis is therefore suggested.**

Key words: Antitrypanosoma, suppressive test, repository test, *Khaya senegalensis, Anogeissus leiocarpus.*

INTRODUCTION

Trypanosoma species are blood or tissue parasites of all classes of vertebrates. In some hosts particularly man and domestic animals, they are highly pathogenic causing diseases generally known as trypanosomiasis. The most important species causing African animal trypanosomiasis (AAT) are *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei brucei* (CFSPH, 2009). In wild animals, these parasites cause relatively mild infections

*Corresponding author. E mail: awobodet@yahoo.com. Tel: +234 802 682 0100.

while in domestic animals they cause a severe, often fatal disease. African animal trypanosomiasis is most pronounced in cattle but can cause serious losses in pigs, camels, goats, and sheep. Despite the attempts at control, trypanosomiasis is still one of the limiting factors to livestock industry in sub-Saharan Africa (Kamuanga, 2003).

Currently, trypanocidal drugs constitute principal method of control of trypanosomiasis. Poor clinical efficiency, drug resistance and toxicity are some of the limitations facing programmes targeted at controlling trypanosomiasis (Onyeyili and Egwu, 1995; Legros et al., 2002). This emphasizes the need for the identification of new therapeutic and prophylactic agents for controlling the disease. Resistance associated with chemotherapeutic agents has necessitated the development of potent drugs with antitrypanosomal activity from plant source. Plants have provided the basis for traditional treatment for different types of diseases and still always offer enormous potential for new chemotherapeutic agents.

Khaya senegalensis also known as African mahogany has been documented to be active *in vitro* against *T. b. brucei* (Wurochekke and Nok, 2004; Atawodi, 2005). Many prescribed recipes for local treatment of trypanosomiasis contained *K. senegalensis* (Atawodi et al., 2002). Traditional healers in North Eastern Nigeria, also believe that the bark of *Anogeissus leiocarpus* also known as African Birch is very effective in the treatment of African trypanosomiasis (Bizimana, 1994). In Northern Nigeria, *K. senegalensis* and *A. leiocarpus* are listed among the plants used in combination as treatment for animal trypanosomosis by local farmers and herdsmen (Atawodi, 2002) but there is no information on the appropriate combination ratios and activity of the plants in combination. The study was therefore designed to ascertain the efficacy of *K. senegalensis* and *A. leiocarpus* when used in varying combination ratios.

MATERIALS AND METHODS

Plant materials

The stem bark of *K. senegalensis* and *A. leiocarpus* were collected from the Botanical Garden University of Ibadan, Ibadan, Nigeria. Plants were identified and authenticated at the Herbarium of Forestry Research Institute of Nigeria (FRIN), Ibadan. Samples deposited in the herbarium were assigned voucher numbers 109535 (*Anogeissus leiocarpus*) and 109536 (*Khaya senegalensis*).

Preparation of plant extracts

The stem barks of the plants were rapidly washed under running tap water and air-dried in the laboratory at room temperature. The dried barks was ground into coarse particles and then milled to a powdery form. One hundred and fifty grams (150 g) of the resulting powder was soaked in 500 mL absolute methanol and stirred intermittently for 72 h at room temperature. The plant material was filtered using clean muslin cloth and the filtrates were further filtered using Whatmann filter paper 1. The extract was concentrated to dryness using water bath at 60°C. A standard drug Diminazene aceturate (Diminor®) used in the treatment of animal trypanosomes was used as the positive control in the study while the vehicle (Tween-80) was given as negative control. For oral administration of the extract, 30% ethanol was added to 70% Tween-80 and 1 part of this preparation was mixed with 9 part of distilled water (1:9). Tween-80 was used to enhance solubility of the extract. Tween- 80 is not toxic when less than 10% of it is administered to rats. (Food Safety Commission, 2007)

Parasite

Trypanosoma brucei brucei was obtained from Department of Veterinary Pathology, Faculty of Veterinary Medicine University of Ibadan, Ibadan, Nigeria. The parasites were maintained in the laboratory by continuous passage in rats, introducing 1×10^4 parasites intraperitoneally. Blood from the tail was used for the estimation of parasite density using the “rapid matching” method of Herbert and Lumsden (1976). This method involved microscopic counting of parasites in pure blood or blood appropriately diluted with buffered phosphate saline.

Experimental animals

Seventy adult albino male rats weighing 150-200 g (177.7±16.18) were obtained from animal facility of Faculty of Veterinary Medicine, University of Ibadan, Nigeria. They were kept and acclimatized in the animal facility of the Department of Zoology, University of Ibadan before commencement of the experiment. The animals were divided into two groups of 35 animals each for the suppressive test and repository test respectively. The experiments were conducted in compliance with the international guiding principles for research involving animals

Antitrypanosomal studies

The rats for each test were divided into five experimental groups (A-E) and two control groups (F-G).

Suppressive test

The Peters’4 day suppressive test was adopted (Peters, 1967). The animals were inoculated intraperitoneally with 1×10^4 parasites in 0.2 ml Phosphate buffered saline (PBS) solution and after 2 h the extract was administered orally and subsequently for four days.

Extracts were administered in the following combination ratios of *K. senegalensis* (KS) and *A. leiocarpus* (AL): 1:4, 2:3, 1:1, 3:2 and 4:1 at 250 mg/kg (this dose was adopted from the proposed thresholds for *in vivo* activity of antimalarial extracts Rasoanaivo et al., 2004) for the experimental groups A-E, respectively. The positive control group (F) was treated with a single dose 3.5 mg/kg of Diminazene aceturate (Diminor®) while the negative control (G) was given Tween-80. On days 5, 9, 14 and 17 post infection, rats were tailed and smears prepared for parasite count. Mean parasitaemia was calculated for each group and percentage chemosuppression was determined using formular described by Odeghe et al. (2012).

Chemosuppression (%) = (A-B / A) 100

Where A is the mean parasitaemia for negative control and B is the mean parasitaemia of the experimental groups / positive control.

Table 1. Repository (Prophylactic) activity of combined stem bark extract of *Khaya senegalensis* and *Anogeissus leiocarpus.*

Combination ratios (KS vs AL)	Parasitaemia Day 7	Prophylaxis (%)	Parasitaemia Day 10	Propylaxis (%)	Parasitaemia Day 13	Prophylaxis (%)	Parasitaemia Day 17	Prophylaxis (%)	Parasitaemia Day 21	Prophylaxis (%)
1: 4	1.44±0.15^{b}	68.0	3.28±0.11^{b}	59.56	12.06±2.13^{ab}	54.01	31.56±1.94^{c}	41.56	40.39±4.97^{d}	27.45
2:3	1.61±0.06^{b}	64.22	3.00±0.25^{b}	63.01	11.78±1.93^{ab}	55.07	29.17± 0.88^{c}	45.98	31.63±0.57^{c}	43.18
1:1	0.00±0.00^{a}	100	2.22±0.31^{b}	72.63	11.53±1.18 ^{ab}	55.84	20.89± 1.34^{bc}	61.32	26.17±3.26^{bc}	53.00
3:2	0.00±0.00^{a}	100	2.06±0.57^{b}	74.60	8.00±0.97^{a}	69.49	16.61±0.87^{b}	69.24	20.17±0.60^{b}	63.77
4:1	0.00±0.00^{a}	100	0.00±0.00^{a}	100	0.00±0.00^{a}	100	0.00± 0.00^{a}	100	0.72±0.72^{a}	98.71
Positive control	0.00±0.00^{a}	100	0.00±000^{a}	100	0.00±0.00^{a}	100	0.00 ±0.00^{a}	100	0.61±0.61^{a}	98.90
Negative control	4.50±0.19^{c}	-	8.11±0.72^{c}		26.22±1.24^{b}		54.00±8.54^{d}		55.67±4.09^{c}	

Columns with values bearing the same superscript are not significantly different ($p<0.05$).

Repository (prophylactic) test

The method of Abatan and Makinde (1986) was adopted in the evaluation of the prophylactic potential of the combined extract of *K. senegalensis* and *A. leiocarpus*. Extracts containing KS and AL in ratios 1:4, 2:3, 1:1, 3:2 and 4:1 at 250 mg/kg were administered to experimental groups A-E, respectively, positive control (group F) was treated with a single dose of Diminazene aceturate (Diminor®) at 3.5 mg/kg while the negative control (G) was given the vehicle (Tween-80). All animals were infected with 1×10^4 *T. brucei brucei* on day 4 post treatment. Rats were tailed and smears prepared for parasite count daily and mean percentage parasitaemia was calculated for each combination ratio.

Packed cell volume determination (PCV)

The PCV was determined for all animals in both the repository and suppressive assays.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) software version 16.0 was used for data analysis. The results were expressed as Mean ± SEM. The statistical difference between groups was performed using one way analysis of variance (ANOVA) followed by Duncan multiple range test. Values of $p<0.05$ were considered significant.

RESULTS

Repository test

The prophylactic activity of the extracts on infected rats increased with increasing quantity of KS in the combination (Table 1). On day 7 post infection, ratios 1:1, 3:2 and 4:1 showed 100% chemoprophylactic activity. The activity of 4:1 combination ratio remained at 100% until after day 17 and decreased to 98.7% by day 21 post infection. This compared well with the standard drug Diminazene aceturate which had 98.9% prophylactic activity by day 21 post infection. The activity in the other combination ratios decreased progressively and by day 21 post infection, ratios 1:4, 2;3, 1:1 and 3:2 had reduced to 27.45, 43.18, 53.00 and 63.77%, respectively (Table 1).

Suppressive test

Table 2 shows the effect of the combined extract on early infection. Parasites were observed on day 9 post infection with highest parasitaemia recorded in the negative control animals. The highest chemo-suppressive activity (68.53%) was however observed in animals administered the 4:1 ratio. This is a similar trend to that observed in repository test were an increase in the quantity of KS gave an increased activity of the extract. The suppressive activity of the varying ratios of the combined extract decreased to between 4.35-57.26% by day 17 while the positive control remained at 100%.

Packed cell volume (PCV)

Packed cell volume of infected rats in both tests is presented in Table 3. The highest PCV values were recorded in animals receiving 4:1 dose ratio. In the two tests there was no significant difference ($p<0.05$) between PCV values of negative control animals and animals receiving 1:4 combination ratio. Only animals in the positive control group, treated with the commercial drug had PCV values within the normal range (35-57) in rats. In the repository test, PCV values increased as the ratio of *K. senegalensis* increased in the dose.

Table 2. Suppressive activity of combined stem bark extracts *Khaya senegalensis* of *Anogeissus leiocarpus.*

Combination ratios (KS vs AL)	Parasitaemia Day 5	Suppression (%)	Parasitaemia Day 9	Suppression (%)	Parasitaemia Day 14	Suppression (%)	Parasitaemia Day 17	Suppression (%)
1: 4	0.00±0.00^{a}	100	8.33±1.51^{c}	12.32	25.99±6.23^{c}	8.81	44.00±2.52^{c}	4.35
2:3	0.00±0.00^{a}	100	7.11±1.64^{bc}	25.16	22.88±4.03^{bc}	19.72	41.33±0.66^{c}	10.15
1:1	0.00±0.00^{a}	100	3.22±0.97^{ab}	66.11	22.67±1.76^{bc}	20.46	35.06±3.95^{c}	23.78
3:2	0.00±0.00^{a}	100	3.16±0.17^{ab}	66.74	18.13±1.49^{bc}	36.39	20.94±8.07^{b}	54.49
4:1	0.00±0.00^{a}	100	2.99±0.25^{ab}	68.53	12.57±3.56^{b}	55.90	19.66±4.79^{b}	57.26
Positive control	0.00±0.00^{a}	100	0.00±000^{a}	100	0.00±0.00^{a}	100	0.00±0.00^{a}	100
Negative Control	1.95±0.15^{b}	-	9.5±2.29^{c}		28.50±1.89^{c}		46.00±1.16^{c}	

Columns with values bearing the same superscript are not significantly different ($p<0.05$).

Table 3. Packed cell volume (PCV) of *Trypanosoma brucei brucei* infected rats in repository and suppressive test.

Combination ratios (KS vs AL)	PCV	
	Repository test	Suppressive Test
1:4	26.50±0.65^{a}	31.33±0.88^{b}
2:3	29.67±1.45^{ab}	24.67±5.04^{ab}
1:1	30.00±2.35^{abc}	16.33±2.33^{a}
3:2	30.25±2.02^{abc}	28.00±2.31^{b}
4:1	33.75±2.78^{bc}	30.50±1.50^{b}
Positive Control	36.20±0.97^{c}	42.80±0.86^{c}
Negative Control	26.50±1.50^{a}	31.25±3.77^{b}

Columns with values bearing the same superscript are not significantly different ($p<0.05$).

DISCUSSION

K. senegalensis and *A. leiocarpus* are among the plants used by local farmers in northern Nigeria in the treatment of trypanosomiasis. Reports have shown that *K. senegalensis* and *A. leiocarpus* when used singly in treatment of trypanosomes infected rats possess antitrypanosomal activity (Umar et al., 2010; Ibrahim et al., 2008; Wurochekke and Anyanwu, 2012). The antitrypanosomal activity of the combined extracts compared well with the standard drug in both repository and suppressive tests. This suggests a high efficacy of the combined extract in parasite clearance. *K. senegalensis* has been reported to contain saponins, tannins, alkaloids, glycosides, steroids, terpenoids and flavonoids (Makut et al., 2008) while *A. leiocarpus* contains alkaloids, glycolsides, phenols, steroids, tannins, anthraquinones, saponins and flavonoids (Mann et al., 2010; Kaboré et al., 2010). Nok (2001) attributed the trypanocidal activity of a number of tropical plants to flavonoids (azaanthraquinone), highly aromatic planar quaternary alkaloids, barbarine and harmaine.

In both repository and suppressive tests, the highest antitrypanosomal activities were observed when KS and AL were combined in ratio 4:1. This suggests that the antitrypanosomal activity of the combined extract was dependent on the quantity of KS in the combination. An increased amount of KS, may have resulted in a higher concentration of active phytochemical components needed for parasite clearance.

Detection of parasites 21 days post infection both in animals treated with 4:1 combination of KS: AL and positive control group in the repository test indicates a similar efficacy in both treatments. The presence of parasites on day 21 however, indicates the inability of both the extract and Diminazene aceturate to completely clear the parasites when administered at 250 mg/kg and 3.5 mg/kg respectively. This is in consonance with the findings of Jatau et al. (2010) which reported relapse of *T. evansi* infection in rat treated with 3.5 mg/kg of Diminazene aceturate on day 17 post treatment. They however reported no relapse of the infection at a higher dose of 7mg/kg. Mamman et al., (1994) suggested that the relapse of *T. brucei* infection could result from the inaccessibility of the drugs to privilege sites where trypanosomes were shielded from exposure to curative concentration of the drug.

In suppressive test, lower parasitaemia observed in the experimental groups in comparison to untreated animals (negative control) suggests a modulation of infection by the extracts. The daily decrease in the suppressive ability of the extracts compared to Diminazene aceturate suggests a quicker break down of the plant metabolites resulting in a lowering of the antitrypanosomal activity of the extracts.

Anaemia is a constant feature of trypanosome infections and its severity is linked to the level of parasitemia (Umar et al., 2000). Karori et al. (2008) reported that trypanosomes generated reactive oxygen species that attack red blood cells' membranes, inducing oxidation and subsequently haemolysis. The increased PCV values recorded with an increase in the quantity of KS in the extract suggest the prophylactic ability of KS which protects the animals from anaemia, This improved PCV in the experimental groups, suggest that the extract could reduce the severity of *T. b. brucei* infection if used as prophylaxis. The low PCV observed in suppressive test could be as a result of slow absorption of the extract.

The findings from this study show that a combination of KS and AL in ratio 4:1 has a high antitrypanosomal activity and a good prophylactic potential. This justifies the use in combination of *K. senegalensis* and *A. leiocarpus* in the treatment of animal trypanosomiasis. A combination with a higher ratio of *K. senegalensis* is suggested because of its better potential for the management of animal trpanosomiasis.

Conflict of interests

The authors did not declare any conflict of interest.

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