Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Antitrypanosomal potentials of the extract and fractions of *Abrus precatorius* seeds

Nwodo NJ[1*], Nwodo OFC[2]

[1]*Department of Pharmaceutical Chemistry, University of Nigeria, Nsukka Nigeria*
[2]*Department of Biochemistry, University of Nigeria, Nsukka Nigeria*

ARTICLE INFO

Article history:
Received 10 March 2012
Received in revised form 15 May 2012
Accepted 15 July 2012
Available online 20 November 2012

Keywords:
Abrus precatorius
Seed extract
Trypanocidal effect
Parasiteamia
Fractions
Trypanosome brucei brucei
Berenil

ABSTRACT

Objective: To evaluate the *in vivo* trypanocidal activity of the methanol extract and fractions of *Abrus precatorius* seeds in mice. **Methods:** Parasiteamia was induced unto mice by intraperitoneal injection of 1.25×10^5 *Trypanosoma* in normal saline. Five days when a high level of parasiteamia was established treatment commenced until ten days. The mice were treated with 10, 20 and 40 mg/kg bt. of the extract and 5 and 10 mg/kg bt. of the fraction (F_2), respectively for 5 days. Diminazene aceturate at the dose of 3.5 mg/kg bt. for two days was used as the reference drug. The level of parasitaemia and packed cell volume (PCV) of the animals estimated. **Results:** At doses of 10, 20 and 40 mg/kg the crude extract showed a sharp reduction in the level of parasitaemia in mice compared with the untreated group. The mice treated with F_2 at doses of 5 and 10 mg/kg showed a sharp reduction in the level of parasitamia to zero in day 9, and a gradual recovery from the 12th day of treatment. This effect is comparable to that of the mice treated with 7 mg/kg of standard drug diminazene aceturate. The PCV of the treated showed a gradual decrease with time, but not as much as the untreated group. Phytochemical screening revealed the presence of glycosides, alkaloids, carbohydrates, tannins and proteins in the *Abrus precatorius* powder while F_2 was rich in alkaloids. **Conclusions:** This study shows that both the extract and the fractions of *Abrus precatorius* seeds exhibited a promising trypanocidal property. Alkaloids may be responsible for the observed activity.

1. Introduction

Trypanosomiasis is one of the major public health problems in sub-Sahara Africa. It is a serious handicap to populations and countries striving for social and economic development. Hence, it is an important priority for biomedical, public agencies, agricultural sector and scientific community[1]. The disease caused by the *Trypanosome brucei* sub group is associated with anaemia, hepatocellular degeneration and glomerulonephritis[2]. There is little or no hope for the production of antitrypanosomal vaccine in the near future because of antigenic variation exhibited by the parasites[1]. This coupled with the limitations of the present trypanocidal drugs such as toxicity, drug resistance, and being inconvenient to administer to local populations increase the need for urgent search for more effective plant derived therapeutic agents against the disease. African trypanosomiasis (sleeping sickness) occurs in 36 African countries, south of Sahara, where about 50 million people are at risk of acquiring infection[3-5].

The plant kingdom is known to posses enormous resources for novel lead bioactive compounds. There are numerous plant species in the tropics of which some have been verified for their trypanocidal actions[6-8]. It is estimated that about 5%-15% of the plant species have been investigated for the presence of bioactive compounds. These plant-derived products have been shown to have certain advantage over synthetic medicines which include minimal toxicity effects on the organic functioning of the body, consistent potency, inability of microbes to develop resistance to them and the fact that they are well absorbed and distributed in the body[9]. *Abrus precatorius* (Fabaceace) (*A. precatorius*) is a perennial shrub and the seed commonly

*Corresponding author: Dr. Nwodo Ngozi Justina, Department of Pharmaceutical and Medicinal Chemistry, University of Nigeria, Nsukka 410001, Nigeria.
Tel: +2348062957580
E-mail: ngozi.nwodo@unn.edu.ng

known as rosary beads is used traditionally for several medicinal purposes.

The seeds of *A. precatorius* Linn (Fabaceae) are used widely in Africa, US and Asia for a wide range of pharmacological possibilities[10-11]. In many parts of world and in Nigeria, they are used for the treatment of inflammatory conditions such as arthritis which has been scientifically proved[12]. The substance also contracts ethystiboesterol primed and grand uterus[13]. It is used in Hindu medicine from early times, as well as in China and other ancient cultures, in the treatment of Ulcer and skin diseases externally[14,15].

Several constituents which include abrin, anthocyanins, N, N-dimethyltryptophan metho cation, precatorine, hypaphorine, choline, trigoneline, steroids, flavonoids toxins and agglutinins have been previously isolated from the seeds of *A. precatorius*[10,16].

Our current report on the trypanocidal property of the crude extracts and fractions of *A. precatorius* seeds is an effort to further explore the chemical and pharmacological diversity of this plant species. Besides, it has been observed that some chemical agents that possess abortificent effect also exhibit trypanocidal potentials.

2. Materials and methods

2.1. Plant material

A. precatorius seeds were collected from Nsukka, Enugu state of Nigeria, between the months of September and November. The plant material was authenticated by Mr. Alfred Ozioko, a taxonomist, in the Department of Botany, University of Nigeria, Nsukka. A voucher specimen 89 was deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.

2.2. Extraction and fractionation

The pulverized seeds (400 g) were macerated in 1 200 mL chloroform/methanol (2:1) for 18 h, with two changes of the solvent and then filtered. The filtrate was mixed with 400 mL of water in a separating funnel and allowed to stand. The upper aqueous methanol layer was drained out and dried in a water bath.

About 8 g of the dried upper aqueous methanol layer was separated in Sephadex LH-20 column eluting with 100% methanol. Aliquots of 3 mL were collected and monitored with TLC and similar fractions were bulked to obtain fractions F_1-F_5. All the fractions were tested for phytochemical constituents and fraction F_2, which contained mainly alkaloids, was screened for pharmacological activity.

2.3. Phytochemical analysis

The Phytochemical analysis of both the *A. precatorius* powder, the extract and fractions were carried out using standard methods[17].

2.4. Pharmacological tests

Albino mice (20-25 g) were obtained from the Department of Veterinary Parasitology, Faculty of veterinary Medicine, University of Nigeria, Nsukka. They were housed in steel cages under standard conditions of temperature (28-32 ℃) and relative humidity of 70%-80% with a light for a period of 16 days and were allowed access to water and food *ad libitum*. The animal experiments in this study followed the principles of laboratory animal care (U.S. National Institutes of Health, 1985). Ethical permission for the use of laboratory animals was obtained from the local ethical committee of our Institution.

A resistant strain of *Trypanosome brucei brucei (T. brucei brucei)* was collected from the Nigerian Institute of Trypanosome Research Vom, Jos, Plateau state, Nigeria.

2.5. Estimation of parasitaemia and determination of in vivo activity

Thirty-six albino mice (20-25 g) of either sex were separated into six groups of six mice per group to investigate the effect of the extracts on *T. brucei. brucei* infection by a standard method[18]. The mice were infected by intra-peritoneal injection 0.1 mL of 1.25×10^5/mL trypanosomes in normal saline, fed *ad libitium* and allowed free access to clean water. Three days post-inoculation, the mice were screened individually for the presence of *T. brucei. brucei* infection using rapid matching counting method as previously reported[18,19].

The screening continued until a high level of parasiteamia was established and treatment with the extract commenced. Animals in groups A-C were treated respectively with 10, 20 and 40 mg/kg doses of the extract for 5 days. Groups D, which served as the positive control received Berenil (Diminazene aceturate) (Hoechst AG Frankfurt, Germany) at the dose of 3.5 mg/kg for two days. Group E which served as an infected control was treated with normal saline placebo. Group F served as the uninfected, untreated control. Mice were monitored daily during and after treatment for the level of parasitaemia and packed cell volume.

The experiment was also conducted with F_2 at doses of 5 and 10 mg/kg.

2.6. Acute toxicity studies

The acute toxicity, LD_{50} of both the crude extract and the fractions were determined using a standard method[17].

2.7. Statistical analysis

The results are expressed as means ± SEM. Significant difference between control and treated groups were determined using the student's *t*-test. Furthermore, the

difference within means was analysed using the one-way analysis of variance (ANOVA).

3. Results

Phytochemical screening revealed the presence of glycosides, alkaloids, carbohydrates, tannins and proteins in the *A. precatorius* powder. The LD_{50} of the extract and that of the fraction were 1 265 and 570 mg/kg (*i.p.*) body weight respectively.

The results of the pharmacological studies are shown in Figures 1 and 2 for the crude extract and in Figures 3 and 4 for the fractionated component (F_2). At doses of 10, 20 and 40 mg/kg the crude extract showed a sharp reduction in the level of parasitaemia in mice compared with the untreated group (Figure 1). The packed cell volume (PCV) of the treated mice showed a reduction up to day 4 and gradual increase subsequently as compared to a steady reduction showed by the untreated group (Figure 2). As shown in Figure 3, the mice treated with F_2 at doses of 5 and 10 mg/kg showed a sharp reduction in the level of parasitamia to zero in day 9, and a gradual recovery from the 12th day of treatment. This effect is comparable to that of the mice treated with 7 mg/kg of standard drug diminazene aceturate. The PCV of mice treated with F_2 at 5 and 10 mg/kg showed a gradual degrease with time, but not as much as that of untreated group (Figure 4).

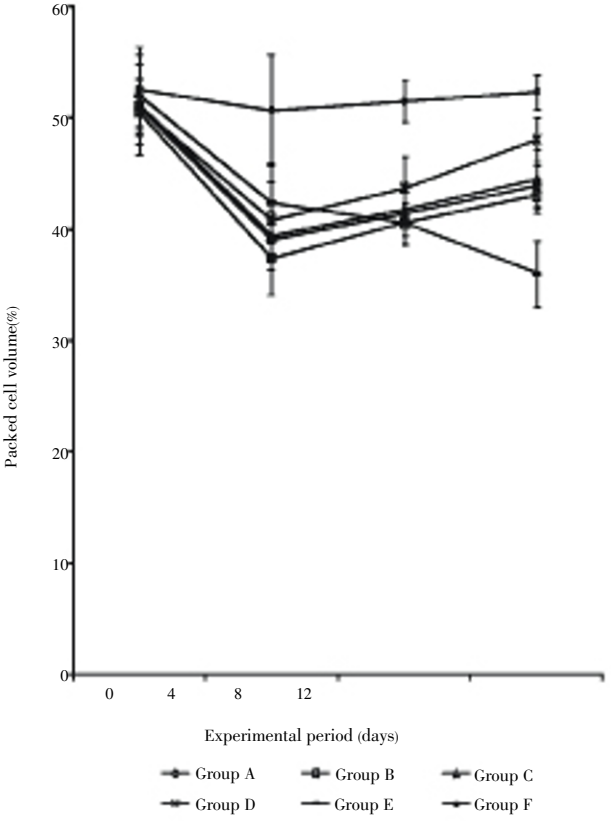

Figure 2. PCV of mice groups infected with *T. brucei* and treated with doses of *A. precatorius* seed extract (with standard deviation bars).

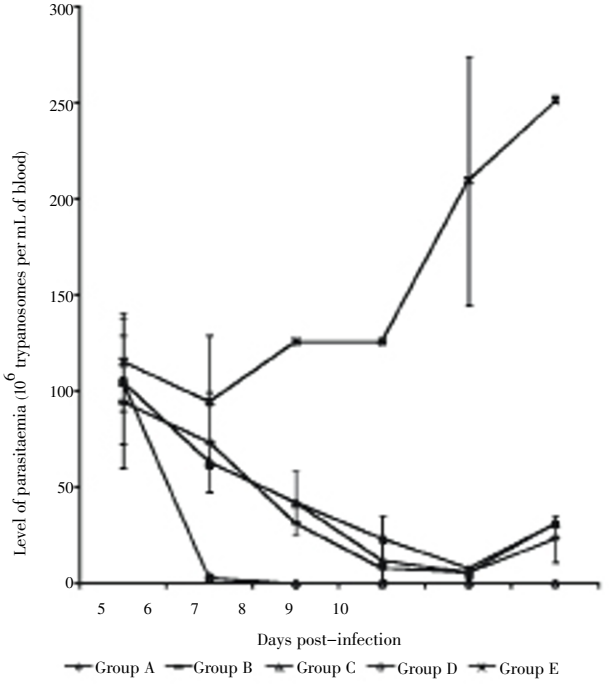

Figure 1. Level of parasitaemia of *A. precatorius* seed extract (with standard deviation bars) and *T. brucei* infected mice groups .

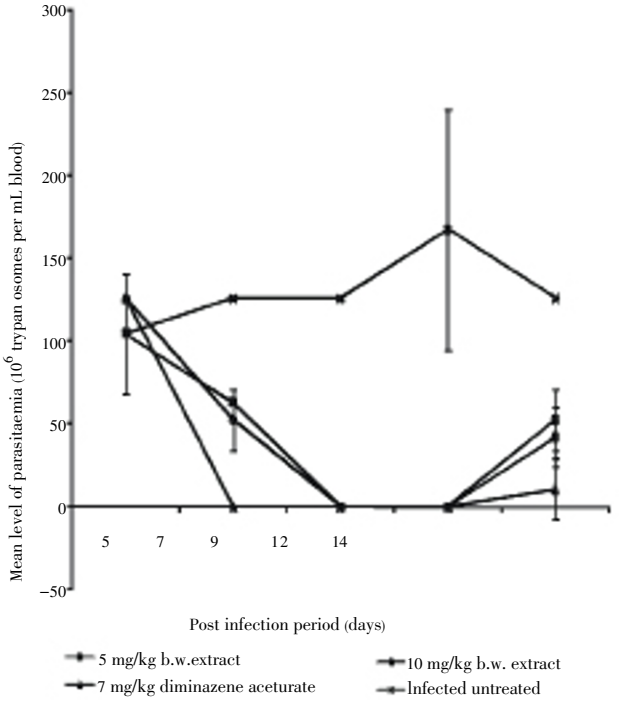

Figure 3. Level of parasitaemia in mice groups infected with trypanosomes and treated with varied doses of *A. precatorius* fractions and diminazene aceturate.

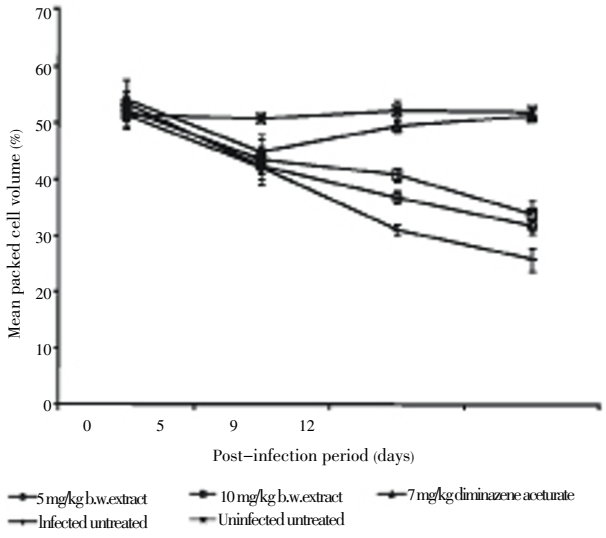

Figure 4. PCV of mice groups infected with trypanosomes and treated with varied doses of *A. precatorius* fraction and diminazene aceturate.

4. Discussion

The leaves, roots and seeds of *Abrus precatorius* Linn (Fabaceae) have been used widely in Africa, US and Asia for various medicinal purposes. Recent studies on the aqueous seed extract showed some protective effect against alcohol-induced renal damage, which could be attributed to a reduction in alcohol-induced lipid peroxidation[20,21]. The estimated global burden have been as high as 300 000[22]. Our current results showed that the crude extract possess significant trypanocidal effect in mice treated with *T. brucei brucei*. The reduction in parasitaemia was found to be comparable with that of standard drug, diminazene aceturate. The phytochemical analysis exhibited the presence of alkaloids, proteins, carbohydrates and traces of glycosides were found in the extract. The fractions exhibited the presence of alkaloids mainly. This is in line with other works, which stated that most compounds isolated from plants with activity against protozoan and other parasitic diseases contain mainly alkaloids and flavonoids[6].

The results were very significant with a pronounced reduction of parasiteamia in mice as compared to the positive and negative controls. There was significant decrease of parasiteamia with the respective doses of the crude extract especially on day 9, which corresponds to that of berenil before relapse occurred. The fractions exhibited very significant reduction of parasiteamia consistently from day 9 and became undetectable. The mice remained aparasitaemic until day 12 and relapsed immediately afterwards. The PCV showed a similar course in *Abrus* extract and Berenil treated mice. The PCV also improved with the fractions. The LD_{50} values indicated some safety margin in the use of the extract and the fractions[17]. Though the seeds of *Abrus precatorius* are poisonous, several efforts are being made to overcome its toxic effects, by method of preparation of the extracts, routes of administrations and recently an attempt has been made to scientifically study the process of detoxifying this seeds[23,24]. The statistical analysis of the results of this study showed a significant difference between the untreated control group and the treated group with both the extract and the fractions ($P<0.01$). The difference between the group treated the fractions and the group treated with Berenil was also significant with ($P<0.05$). Recent studies have also shown that *Abrus precatorius* seeds have immunomodulatory properties and that treatment of *T. brucei brucei* infected animal with vitamins with antioxidant ability ameliorated anemia and organ damage[25,26].

The results of this study are evident and show the potential of *Abrus precatorius* seeds as a source of trypanocide. Further purification and characterization of the pure compound responsible for this activity is ongoing, which may lead to molecules that can be relied on for the treatment of African trypanosomiasis. The study also proved the fact that some abortificents are possible trypanocides. To the best of our knowledge, no other work has been reported in this regard.

Conflict of interest statement

The authors declare no conflict of interests.

Acknowledgements

The authors are grateful to Dr L.U. Ngongeh, Vet. Parasitology of our university for his assistance on the parasitology assay. ICTP Trieste, Italy, is well acknowledged for providing the facilities for writing up this work.

References

[1] Ibrahim MA, Aliyu AB, Bashir M, Yunusa I, Umar TS. *Senna occidentalis* leaf extract possesses antitrypanosomal activity and ameliorates the trypanosome-induced anemia and organ damage. *Pharmacog Res* 2010; **2**: 175-180.

[2] Umar IA, Rumah BL, Bulus SL, Kamla AA, Jobin A, Asueliman BI, et al. Effects of intraperitoneal administration of vitamins C and E or A and E combinations on the severity of *Trypanosoma brucei brucei* infection in rats. *Afr J Biochem Res* 2008; **2**: 88-91.

[3] Corbel V, Henry MC. Prevention and control of malaria and sleeping sickness in African: Where we are and where are we going. *Parasite & Vectors* 2011; **4**: 37.

[4] Simarro PP, Diarra A, Ruiz Postigo JA, Franco JR, Jannin JG. The human African trypanosomiasis control and surveillance programme of the World Health Organization 2000-2009: The way forward. *PLoS Negl Trop Dis* 2011; **5**(2): e1007. doi:10.1371/journal.pntd.0001007

[5] Shuaibu MN, Wuyep PTA, Yanagi T, Hirayama K, Ichinose A, Tanaka T, et al. Trypanocidal activity of extracts and compounds from the stem bark of *Anogeissus leiocarpus* and *Terminalia avicennoides*. *Parasitol Res* 2008; **102**: 697-703.

[6] Nwodo NJ, Brun R, Osadebe PO. *In vitro* and *in vivo* evaluation of the antitrypanosomal activity of the fractions of *Holarrhena africana*. *J Ethnopharmacol* 2007; **113**: 556-559.

[7] Nwodo NJ, Agbo MO. Antitrypanosomal effects of methanolic Extract of *Nauclea diderrichii* (Merr) and *Spathodea campaunlata* stem bark. *J Pharma. & Allied Sci* 2010; **7**(5): 1219-1227.

[8] Antia RE, Olayemi JO, Aina OO, Ajaiyeoba EO. *In vitro* and *in vivo* animal model antitrypanosomal evaluation of ten medicinal plant extracts from southwest Nigeria. *Afric J Biotech* 2009; **8**(7): 1437-1440.

[9] Amin A, Muhtasib HG, Ocker M, Stock RS. Overview of major classes of plant derived anticancer drugs. *Int J Biomed Sci* 2009; **5**(1): 1-11.

[10]Xiao ZH, Wang FZ, Sun AJ, Li CR, Huang CG. A new triterpeniod saponin from *Abrus precatorius lin. Molecules* 2012; **17**: 295-302.

[11]Taur DJ, Patil RY. Effect of *Abrus precatorius* leaves on milk induced leukocytosis and eosinophilia in the management of asthma. *Asian Pac J Trop Biomed* 2011; **1**(1): 40-42.

[12]Georgewill OA, Georgewill UO. Evaluation of the anti-inflammatory activity of extract of *Abrus precatorious*. *East J Med* 2009; **14**: 23-25.

[13]Okoko II, Osinubi AA, Olabiyi OO, Kusemijiu TO, Noronha CC, Okanlawon AO. Anti-ovulatory and anti-implanatation potential of the methanolic extract of seeds of *Abrus precatorius* in the rat. *Endocr Pract* 2010; **16**: 554-560.

[14]Adelowotan O, Aibinu I, Adenipekun E, Odugbemi T. The *in vitro* antimicrobial activity of *Abrus precatorius* fabaceae extract in some clinical pathogens. *Niger Postgrad Med J* 2008; **15**: 32-37.

[15]Bobbarala V, Vadlapudi V. *Abrus Precatorius* L. seed extracts antimicrobial properties against clinically important bacteria. *Int J Pharm Tech Res* 2009; **(4)**: 1115-1118.

[16]Bagaria A, Surendranath K, Ramagopal UA, Rmakumar S, Karade AA. Structure-function analysis and Insight into the reduced toxicity of *Abrus precatorius* Agglutinin I in relation to Abrin. *J Biol Chem* 2006; **281**(45): 34465-34474.

[17]Danmalam UH, Abdullahi LM, Agunu A, Musa KY. Acute toxicity studies and hypoglycemic activity of the methanol extract of the leaves of *Hyptis suaveolens* poit. (Lamiaceae) *Nig J Pharm Sci* 2009; **8**(2): 87-92.

[18]Ngure RM, Ongeri B, Karori MS, Wachira W, Maathai RG, Kibugi JK, et al. Anti-trypanosoma effects of *Azadirachta indica* (neem) extract on trypanosome brucei rhodesiense- infected mice. *East J Med* 2009; **14**: 2-9.

[19]Olukunle JO, Abatan MO, Soniran OT, Takeet MI, Idowu OA, Akande FA, et al. *In vivo* antitrypanosomal evaluation of some medicinal plant from Ogun State Nigeria. *Sci World J* 2010; **5**(1): 17-19.

[20]Ligha AE, Jaja B1, Numbere NF. Protective effect of *Abrus precatorius* seed extract following alcohol-induced renal damage. *Euro J Sci Res* 2009; **25**(3): 428-436.

[21]Arrais-Silva WW, Rodrigues RSV, Nazario de Moraes L, Venere PC, Lunardi RR, Souza IL, et al. First report of occurrence of *Triatoma williami* Galvão, Souza e Lima, 1965 naturally infected with *Trypanosoma cruzi* Chagas, 1909 in the State of Mato Grosso, Brazil. *Asian Pac J Trop Dis* 2011; **1**(3): 245-246.

[22]Fevre EM, Wissmann BV, Welburn SC, Lutumba P. The burden of human African trypanosomiasis. *PLoS Negl Trop Dis* 2008; **2**: e333.

[23]Upadhay S, Ghosh AK, Singh V. Anti-lice activity of *Abrus precatorius* LINN (FAM. Fabaceae) seeds oil. *Egyptian Dermatol Online J* 2011; **7**(2): 4: 1-6.

[24]Bapat SP, Sane RT. Bio-analytical studies on the process of detoxification and safety evaluation of *Aconitum laciniatum* and *Abrus precatorius* for use in ayurvedic preparations. *Inter J Pharm Sci Res* 2012; **3**(3): 914-921.

[25]Anita T, Narmada PS, Umar DP. Immunomodulatory activity of the aqueous extract of seeds of *Abrus precatorius* Linn Jequirity in mice. *Iran J Immunol* 2011; **8**(2): 96-103.

[26]Umar A, Ogenyi E, Okodaso D, Kimeng E, Stancheva GI, Omage JJ, et al. Amelioration of anaemia and organ damage by combined intraperitoneal administration of vitamin A and C to *Trypanosoma brucei brucei* infected rat. *Afric J Biotech* 2007; **6**: 2083-2086.