ABSTRACT

Context: Control of African trypanosomiasis relies on chemotherapy, but the development of resistance and the problem of drug residues require research for alternatives. Triterpenes and phenolics, the major constituents of *Pleurotus sajor-caju* (Fr.) Singer (Pleurotaceae), are reported to be effective against trypanosomiasis.

Objective: Trypanocidal effect of whole *Pleurotus sajor-caju* aqueous extract was investigated in vivo against *Trypanosoma congolense*.

Materials and methods: Mice (25–32 g) were divided into seven groups of six animals. Mice in groups A–F received 2.5 × 10^4 trypanosomes, while group G was uninfected. Extracts (100–250 mg/kg) were administered intraperitoneally for 5 days to groups A–D while diminazine aceturate (group E) and normal saline (group F) served as positive and negative controls, respectively. Parasitemia, survival time, body weight and haematological parameters were monitored for 60 days post-treatment.

Results: Parasitemia decreased significantly (*p* < 0.01) post-treatment with 200 and 250 mg/kg of the extract and became undetectable by day 16 and 12 post-infection, respectively; the ED50 was 221.5 mg/kg. The packed cell volume (PCV) and the weight of mice treated with 250 mg/kg extract were 46.20 ± 2.6% and 32.05 ± 3.63 g, respectively, which is higher than the group treated with diminazine aceturate. The mean survival time of animals in groups D and E was > 60 days, while that of group F was < 4 days. Differential leucocyte count on day 68 post-infection in groups C, D and E were not significantly different.

Conclusion: *Pleurotus sajor-caju* therefore could be a potential source of new trypanocidal drugs.

Introduction

African trypanosomes are protozoan pathogens, causing human sleeping sickness and nagana, a related disease in cattle. The major pathogenic tsese-transmitted trypanosome species are *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei* in cattle, sheep and goats, *Trypanosoma simiae* in pigs and *Trypanosoma brucei* in dogs. Within this region, ~46–62 million of cattle and other livestock species are at risk of the disease (Swallow 2000). Trypanosomiasis is an important constraint to livestock development in sub-Saharan Africa. The causative agents of the Human African trypanosomiasis are *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*. The disease is found in many regions of the world, but mainly in sub-Saharan Africa between latitude 14°N and 29°S (World Health Organization [WHO] 1998). Approximately 9 million km² of sub-Saharan Africa, representing about one-third of the total land, is invaded by tsese flies (Mattioli et al. 2004); these flies are found in 37 countries, mostly in Africa. The economic losses attributed to African animal trypanosomiasis are due to decreased meat and milk production as a result of infertility, morbidity and mortality.

Although an increasing number of reports on drug resistance in animal trypanosomes in Africa have been published (Holmes et al. 2004), it is not clear whether this increase is due to a higher incidence of drug-resistant trypanosome strains or to a growing interest of the scientific community in this area of research. There is also the problem of the influx of fake drugs in Africa containing less active compound than required, leading to the mistaken impression that the strains are resistant, when in fact the drug is simply under-dosed. The estimation of its regional prevalence suggests 17 African countries (Delespaux et al. 2008), including Kenya (Murilla et al. 2002), Uganda, Tanzania (Eisler et al. 2000), Ethiopia (Tewelde et al. 2004), Zambia (Sinyangwe et al. 2004), Zimbabwe (Joshua et al. 1995), Cameroon (Mamoudou et al. 2008), Nigeria (Geerts et al. 2001), and Burkina Faso (McDermott et al. 2003), demonstrate area-wide resistance in at least one region of each country. The total value of the market for trypanocides for African farmers may exceed US$25 million, but this is considered insufficient by pharmaceutical companies to justify investment in the development of new drugs (FAO 2016). Therefore, the challenge remains to develop a cheap alternative chemotherapeutic control measure. Wild and cultivated mushrooms contain a diversity of biomolecules with nutritional (Kalac 2009) and/or medicinal properties (Poucheret et al. 2006). Due to these properties, mushrooms have been recognized as functional foods, and as a source for the development of medicines and nutraceuticals. Fruiting bodies, mycelia and spores accumulate a variety of bioactive metabolites with immunomodulatory, cardiovascular, liver anti-fibrotic, antiinflammatory, anti-diabetic, antiviral, antioxidant, antitumor and antimicrobial properties (Zaidman et al. 2005; Poucheret et al. 2006; Beattie et al. 2010). Ergosterol peroxide (5α, 8α-epidioxy-
22E-ergosta-6, 22-dien-3β-ol) isolated from *Pleurotus ostreatus* (Jacq.) P. Kumm. (Pleurotaceae) had an inhibitory concentration (IC$_{50}$) of 6.74 μg/mL on *Trypanosoma cruzi*, but showed no lytic action on erythrocytes and no cytotoxic effect on mammalian cells at concentrations higher than 1600 μg/mL (Ramos-Ligonio et al. 2012). The phytochemical screening of cold and hot aqueous and silver nanoparticles extracts of *Pleurotus sajor-caju* (Fr.) Singer revealed bioactive compounds such as cucurbitacin, triterpenes, sterols, alkaloids, vitamins and minerals, and has also proved to contain antioxidant and therapeutic properties (Devi & Krishnakumari 2015). Phytochemical screening of aqueous and methanol extracts of *Pleurotus sajor-caju* revealed the presence of similar bioactive compounds in comparable quantity. The bioactive compounds include phenolics, flavonoids, saponins, alkaloids, glycosides, sterols, tannins, β-carotene, ascorbic acid, carbohydrates, proteins, amino acids and fatty acids (Johnsy & Kaviyarasan 2014). The methanol and aqueous extracts of the *P. sajor-caju* have been reported to possess antimicrobial and antioxidant activities (Pandiaraajan et al. 2012; Johnsy & Kaviyarasan 2014). The triterpenes and phenolic content of *Pleurotus sajor-caju* is high and the information on the trypanocidal efficacy of these mushrooms is sparse. Thus, this study has provided evidence for the efficacy of *Pleurotus sajor-caju* and its effect on the hematological parameters in mice experimentally infected with *Trypanosoma congolense*.

**Materials and methods**

**Extraction of mushroom**

Fresh mushroom (*Pleurotus sajor-caju*) verified and cultivated in Forestry Research Institute of Nigeria, Ibadan, Nigeria, was collected April 2015 from the institute. The macro-fungus materials were air-dried at 45°C in a hot air drier and grounded to a powder using a grinder. The extract was prepared by maceration of mushroom dissolved in water (w/v). Appropriate aliquots of the mixture were filtered using a previously weighed filter paper. A dark brown filtrate was obtained. The residue was dried, weighed again and the difference in weight gave the amount of powdered mushroom dissolved in water (w/v). Appropriate aliquots of the aqueous extract were taken and diluted to prepare graded doses of the extract for the assay.

**Animals**

The study was conducted with the permission of the University of Ibadan Animal Ethics Committee (UIACUREC/App/2015/019) and in line with the guidelines of the committee. BALB/c white male mice (25–32 g) were bought from the National Veterinary Research Institute Vom, Nigeria. They were allowed to acclimate for 2 weeks at the experimental site. They were housed in cages under standard conditions of temperature (28–32°C) and relative humidity of 70–80% with a light period of 12 h/day and were allowed to access water and food *ad libitum*.

**Trypanosome stock**

A susceptible strain of *Trypanosoma congolense* (Karu strain) was collected from the Nigerian Institute of Trypanosome and Onchocerciasis Research (NITOR) Vom, Plateau State, Nigeria. It was kept in mice and transported to the animal house to be used as donor mice.

**Toxicity test**

Acute toxicity study was done according to OECD (2001) guideline for testing of chemicals using mice. Twelve BALB/c albino mice were randomly divided into three groups (4 mice). After being fasted for 2 h, mice in the first group were given water; the second and third groups were given (intraperitoneally) 200 and 400 mg/kg of the aqueous extract of *Pleurotus sajor-caju*, respectively, and observed for any signs of toxicity in the first 4 h and subsequently daily for 10 days to assess safety of the extract. Animals were observed for gross changes, such as loss of appetite, hair erection, lacrimation, tremors, convulsions, salivation, diarrhea and mortality. Blood samples were collected to determine white blood cell count, red blood cell count, haemoglobin concentration (Hb) and packed cell volume (PCV) on day 10 after the administration of extract.

**Determination of in vivo antitypanosomal activity**

Mice were divided into seven groups (A–G) with six mice (26–32 g) in each group. Mice in groups A–F were inoculated intraperitoneally with trypanosomes (2.5 × 10^4) while group G was not infected. The mice were screened for trypanosomes three days post infection using rapid matching counting method (Herbert & Lumsden 1976). When the parasitemia was 5 × 10^6 trypanosomes/mL on day 9 post infection, the mice in groups A–D were treated daily with 100, 150, 200 and 250 mg/kg aqueous extract of *Pleurotus sajor-caju*, respectively. Animals were treated for 5 days intraperitoneally. Group E served as the positive control and received diminazine aceturate (Diminaveto®. Arendonk, Belgium) at 7 mg/kg. Group F (infected) and G (uninfected) served as untreated control groups, and received normal saline. Mice were monitored daily, during and after treatment for parasitemia using rapid matching counting method (Herbert & Lumsden 1976) for 60 days after treatment to observe for possible relapse. The trypanocidal activity was evaluated by the mean survival time of treated mice for each dose. Treatment was considered to be successful when the mean survival time exceeded 60 days and the mice remained parasitemic. Cure rates were calculated and expressed as percentages on day 60 post treatment. Blood samples for haematological analysis were obtained from the tail before treatment and day 60 post treatment. Haematological analysis was based on standard protocols (Jain 1986). White blood cell count (WBC) was done in a coulter counter (Jain 1986). Packed cell volume (PCV) was measured using micro haematocrit method (Schalm et al. 1975). Differential leucocyte counts were determined by microscopic examination of Giemsa stained blood smear (Jain 1986).

**Statistical analysis**

Determination of Effective Dose on 50% of the parasites (ED$_{50}$) of a sigmoidal concentration response (variable slope) curve and comparison of haematological parameters by one-way ANOVA and Tukey’s multiple comparison test were performed using GraphPad Prism version 5 for Windows (GraphPad, San Diego, CA). The results were expressed as means ± S.E.M. significant difference between control group F and the treated groups using the Student’s t-test. The ED$_{50}$ was determined by computing the concentration of extract that gave a response halfway between the minimum and maximum responses in a...
concentration–response sigmoid curve. The relation below gives the trypanocidal activity (TA):

\[
TA = \frac{\text{Number of trypanosomes on day 4}}{\text{Number of trypanosomes on day 1 in treated groups}} \div \frac{\text{Number of trypanosomes on day 4}}{\text{Number of trypanosomes on day 1 in control group F}}
\]

**Results**

**Yields of extract**

The aqueous extract of *Pleurotus sajor-caju* gave a yield of 2.58 g (25.8% w/w).

**Toxicity test**

The preliminary acute toxicity test conducted revealed that there was no sign of toxicity (eye blinking, panting, lethargy, salivation or incoordination) or mortality in any of the treated groups of mice including those that received 400 mg/kg body weight which is more than the highest dose of *Pleurotus sajor-caju* aqueous extract use for the study. The haematological parameters observed was within the normal range in all the treated groups including those that received 400 mg/kg body weight extract (Table 1).

**Assessment of parasitemia and death**

The statistical parameter of the curve fitting analysis and the best-fit ED_{50} values for the aqueous extract was 221.5 mg/kg (Figure 1). The *in vivo* study showed significant efficacy of the extract applied for 5 days (day 9–13 post-infection). Parasitemia decreased after initiation of treatment with 200 and 250 mg/kg of the extract and became undetectable by day 16 and 12 post infection, respectively (Figure 2). However, mice treated with 100 and 150 mg/kg died before day 60 post-treatment. The mice treated with 200 and 250 mg/kg of the extract remained aparasitemic until day 68 post infection. Animals in groups D and E exhibited the most potent trypanocidal activity with a mean survival of >60 days. The most effective dose treated with 250 mg/kg aqueous *Pleurotus sajor-caju* cured 100% of mice (Table 2).

The packed cell volume (PCV) and the weight showed a similar course in mice treated with aqueous extract and diminazine aceturate (Figure 3). After a drop to between 40% and 45% by day 9 post infection, the PCV recovered and reached normal values of 50–55% by day 68 post infection. The weight ranged between 25–32 g before treatment and 27–38 g 60 days after treatment. However, mice treated with 100 and 150 mg/kg exhibited signs of anorexia and weight loss, while the groups treated with 200 and 250 mg/kg had an appreciable weight gain as parasitemia decreases, suggesting relative effectiveness of *Pleurotus sajor-caju*. There was no significant difference (p > 0.05) in the PCV and weight of the groups treated with aqueous extract and the group treated with diminazine aceturate. However, the PCV and the

**Table 1. Blood parameters of mice tested for *Pleurotus sajor-caju* toxicity.**

| Group | Pre-Rx WBC | Post-Rx WBC | Pre-Rx RBC (×10^5) | Post-Rx RBC (×10^5) | Pre-Rx PCV (%) | Post-Rx PCV (%) | Pre-Rx Hb (g/dl) | Post-Rx Hb (g/dl) |
|-------|------------|-------------|---------------------|---------------------|----------------|----------------|----------------|----------------|
| A1    | 8800       | 11,000      | 9.83                | 9.17                | 48             | 49             | 13.7           | 11.5           |
| A2    | 10,500     | 8900        | 10.7                | 10.34               | 49             | 47             | 10.9           | 14.1           |
| A3    | 8100       | 7900        | 8.79                | 9.11                | 49             | 51             | 15.1           | 16.1           |
| A4    | 9700       | 10,000      | 9.96                | 11.1                | 47             | 48             | 15.7           | 16.3           |
| B1    | 9800       | 9000        | 10.74               | 9.98                | 51             | 51             | 14.4           | 14.3           |
| B2    | 7800       | 9100        | 11.13               | 10.64               | 49             | 48             | 10.7           | 14.2           |
| B3    | 9500       | 9500        | 9.33                | 9.92                | 49             | 51             | 14.1           | 12.7           |
| B3    | 9500       | 9500        | 9.33                | 9.92                | 49             | 51             | 14.1           | 12.7           |
| B4    | 9100       | 8700        | 10.1                | 10.2                | 46             | 47             | 15.8           | 16             |
| C1    | 11,000     | 10,000      | 8.76                | 8.12                | 53             | 52             | 13.2           | 13.9           |
| C2    | 8900       | 8600        | 9.93                | 9.96                | 46             | 50             | 12.4           | 13.1           |
| C3    | 8700       | 9800        | 8.88                | 8.97                | 48             | 48             | 12.8           | 13.7           |
| C4    | 9300       | 9000        | 9.23                | 9.56                | 45             | 45             | 14.9           | 15.2           |

Group A: control group, Group B: given 200 mg/kg of *Pleurotus sajor-caju* and Group C: given 400 mg/kg of *Pleurotus sajor-caju*. Rx = Treatment.
weight of the group treated with 250 mg/kg extract was higher than the group treated with diminazine aceturate (Figure 3). The differential white blood cell counts on day 68 post infection are shown in Figure 4.

**Discussion**

The problem of trypanocidal resistance and an increasing concern over the presence of drug residues in animal products, when pure compounds are administered, has led to a resurgence of interest in the use of phytomedicines, in the form of extracts containing a mixture of compounds. This study was stimulated by a report of antimicrobial potential of edible mushrooms (Mendel 2015). The objective of the present study, therefore, was to evaluate the trypanocidal activity of the aqueous extract in mice models. *Pleurotus sajor-caju* extract was shown *in vivo* to exhibit trypanocidal activity in a concentration-dependent manner. The aqueous extract demonstrated an ED$_{50}$ value of 221.5 mg/kg on day 13 post infection, which is the fourth day of treatment.
The cure rate of animals treated with 250 mg/kg of the extract was 100% and the animals remained aparasitemic for more than 60 days post infection. This is remarkable because previous studies (Ekanem et al. 2008; Abu & Uchendu 2011; Tsegabirhan et al. 2014; Feyera et al. 2014) reported relapse when mice infected with trypanosomes are treated with either plant extracts or the commercial trypanocide (diminazine aceturate). For instance, Feyera et al. (2014) reported that 400 mg/kg dichloromethane and 80% methanol extracts of Artemisia abyssinica Sch. Bip. ex A. Rich. (Asteraceae) did not completely eliminate T. congolense from the blood stream of infected mice, but considerably reduced the level of parasitemia. In a similar study with Basidiomycota, ergosterol peroxide extracted from Pleurotus ostreatus had an inhibitory concentration (IC₅₀) of 6.74 µg/mL on T. cruzi, but showed no lytic action on erythrocytes and no cytotoxic effect on mammalian cells at concentrations higher than 1600 µg/mL (Ramos-Ligonio et al. 2012). The results of these studies reinforce the potential of Basidiomycota fungi as sources of bioactive natural products that may be developed into new therapeutic agents for neglected diseases, such as trypanosomiasis and leishmaniasis. Basidiomycota could also be a potential source of nematocide as shown by Studler et al. (1994) after screening for nematicidal activities in cultures of Basidiomycetes, and reported that cultures of Pleurotus pulmonarius and Hericium coralloides exhibited toxic effects towards the saprophytic nematode Caenorhabditis elegans.

The positive effect of trypanocides can further be assessed from weight measurements of the animals. Animals treated with the extract, on average, maintained their body weight at comparable levels to pretreatment values while those in the untreated infected group showed progressive reduction in body weights until death. Mice in groups A and B treated with 100 and 150 mg/kg, respectively, exhibited signs of weight loss, anorexia and death, while groups C and D treated with 200 and 250 mg/kg, respectively, had an increased weight gain as parasitemia decreases although the weight gain is not significantly different compared with the weight before treatment, suggesting relative effectiveness of Pleurotus sajor-caju. Peter et al. (2009) also reported progressive weight increase with decrease parasitemia in mice infected with trypanosome. Tukey’s multiple comparison tests (post-ANOVA) revealed the relative parasitemia level at different dose and level of significance within the groups. It has also been shown that the level of anaemia gives a reliable indication of the disease status and productive performance of trypanosome-infected animals (Ekanem et al. 2006). The level of parasitemia was significantly (p < 0.05) higher in group A (100 mg/kg) compared to groups D and E treated with 200 and 250 mg/kg of the extract and diminazine aceturate, respectively. However, there was no significant difference (p > 0.05) in the level of parasitemia between the animals treated with 250 mg/kg of the extract and diminazine aceturate indicating that if the active compound in the extract is isolated and the dose reduces, their efficacy could be comparable to that of diminazine aceturate. There was no significant difference (p > 0.05) in the PCV and weight of the groups treated with 250 mg/kg extract and diminazine aceturate. However, the PCV and the weight of the groups treated with the extract were higher than the group treated with diminazine aceturate. The result of this study showed that P. sajor-caju extract could possess hematinic potential since they are able to control anaemia, especially at the later stages of the infection, by minimizing drops in PCV values. The differential white blood cell count on day 68 post infection in groups C, D and E were not significantly different. The leucocytosis observed could be a result of increase in the number or proportion of lymphocytes in the blood have been reported in trypanosomiasis and these conditions might be the result of wax and wear syndrome on the immune system of the animal, which is due to the changing variable surface glycoprotein of the trypanosomes (Abubakar et al. 2005) that demands the immune system to continuously produce antibodies and hence keep the lymphocytes level high. However, the groups treated with higher doses of the extract that significantly lowered parasitemia seemed to prevent lymphocytosis, which suggests that this phenomenon is a result of parasite burden. Hence, the relatively reduced percentage of lymphocytes post treatment suggests that the extracts contributed to the attenuation of the immunological reaction by reducing parasitemia.

The mice infected with T. brucei showed leucopenia, lymphocytosis, neutropenia and monocytosis in all the groups before treatment, except the uninfected control, before treatment commenced. The leucocyte count increased in the groups of mice treated with 200 and 250 mg/kg body weight. However, the leucocyte number was low in the group of mice treated with diminazine aceturate. Similarly, an increased neutrophil count was observed in the groups of mice treated with 200 and 250 mg/kg body weight, but the neutrophil number was lower in the group of mice treated with diminazine aceturate compared with the uninfected control. Pathogenic microorganisms are principally phagocytized by neutrophils, while lymphocytes are responsible for humoral and cellular immunity (Schalm et al. 1975; Baker & Silverton 1985). The decrease in differential leucocyte count following trypanosomes infection is consistent with reports that leucopenia characterized by neutropenia was seen in cats experimentally infected with T. brucei (Nfon et al. 2000). There was an increase in the number of lymphocytes (lymphocytosis) in the infected group before treatment compared to the uninfected control, until after treatment when the number of lymphocyte decreased (lymphopenia) except in the group of mice treated with diminazine aceturate with a higher number of lymphocyte. The decrease in lymphocyte numbers of leucocytes and neutrophils suggest that T. brucei caused marked antigenic stimulation leading to accelerated transformation of lymphocytes to plasma cells and transferred lymphocytes resulting to lymphopenia (Marrison et al. 1982; Anosa 1988b). Similarly, the severe fall in neutrophil number in the infected mice might have been caused by marked depression of precursor cells and marked phagocytosis of neutrophil precursor cell in the bone marrow (Anosa 1988b; Anosa et al. 1997) and spleen (Anosa & Kaneku 1984; Anosa 1988a). Eosinophilia was observed in the group of mice treated with 100 and 150 mg/kg body weight before treatment, however, mice treated with 200 and 250 mg/kg and diminazine aceturate showed eosinopenia. Anosa and Kaneku (1984) also reported eosinophilia in T. brucei-infected deer mice. The decrease in the number of eosinophil is due to the therapeutic effect of the extract and diminazine aceturate, which reduced the number of T. brucei. The number of monocytes (monocytosis) in the infected group before treatment increased compared to the uninfected control, until after treatment when the number of monocyte decreased. The monocytosis observed in mice infected with T. brucei before treatment may be due to increased demand for removal of particulate matter arising from severe pathology.

Treatment of parasitic diseases still represents a major challenge. Drug efficacy is mostly limited by the inability of the pharmaceutical to reach its target in a sufficient concentration and for a sufficient duration. In a study by Rosa et al. (2009),
Basidiomycota fungi representing 84 species and 17 genera were screened in a bioassay of enzyme trypanothonine reductase (TryR) from *Trypanosoma cruzi*, and amastigote forms of *Leishmania amazonensis* and reported that 34 of the extracts inhibited the activity of TryR and extracts from *Gymnopilus aracelatus* Murrill (Cortinariaceae), *Irpex lacteus* (Fr.) Fr. (Meruliciaceae), *Lentinus strigosus* Fr. (Polyporaceae), *Nothopanus hygrophanus* (Mont.) Singer ex Pegler (Marasmiaceae), *Pleurotus flabellatus* Sacc. (Pleurotaceae), and unidentified Basidiomycetes were toxic to *Leishmania amazonensis* Ross (Trypanosomatidae). Since *Pleurotus* spp. extract used in their study demonstrated trypanocidal activity, this suggests that investigations into the trypanocidal efficacy of the extract of *Pleurotus sajor-caju* in *vivo* may also be of benefit.

**Conclusions**

Based on this *in vivo* study, *Pleurotus sajor-caju* was effective in reducing and eliminating *Trypanosoma congolense*. Therefore, the use of the extract may be a useful and effective product for the control of African human and animal trypanosomiasis. However, further spectroscopic studies on the development of quality assurance protocols involving the use of reference substance of mushroom origin for this extract is warranted. Unambiguous structure elucidation of the active principle could provide leads for trypanocidal drug discovery and suitable bioactive marker compounds for standardization of the extract as a phytomedicine.

**Disclosure statement**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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