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Anti Trypanosomal Activity of *Cantharellus cibarius* on *Trypanosoma brucei brucei*

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Abstract: *The present study investigates the therapeutic activity of Cantharellus cibarius on Trypanosoma brucei brucei In vitro and In vivo. Several extracts acquired from this plant, were tested for anti trypanosomal activity In vitro and at different concentrations. The ethanolic extract had the highest activity In vitro and was further analysed for In vivo activity on rats infected with Trpanosoma brucei brucei. The groups were treated intraperitoneally for 7 consecutive days with extract concentration ranging from 100-400mg/kg of body weight. The group treated with 400mg/kg of Cantharellus cibarius had significant reduction in parasiteamia and their life span was prolonged up to the 15 day post infection and all other groups treated with other doses, including the control, all died between days 6th-7th post infections. The group challenged with parasite 30-60 minutes after treating the animals with ethanolic extract of Cantharellus cibarius did not come down with parasiteamia. The Phytochemical screening showed appreciable amount of alkaloids and saponins.*

Keywords: *In vivo and In vitro, phytochemical screening, trypanosomal activity, Cantharellus cibarius on Trypanosoma brucei brucei.*

I. Introduction

African trypanosomosis is a parasitic haemoprotozoan wasting disease of animals and man. It is caused by trypanosome species. In Africa, the most important Trypanosoma species are transmitted by the tsetse fly of the genus Glossina. It occurs across more than a third of Africa, and almost all animal species, except poultry, are affected. The disease results in acute, sub acute or chronic disease characterized by intermittent fever, anemia, occasional diarrhea, and rapid loss of condition which often terminates in death [1]. The disease is endemic in 36 sub-Saharan Africa countries where there are tsetse flies that can transmit the disease. In 2000, WHO estimated that about 60 million people were at risk, with an estimated 300 000 new cases per year in Africa. And fewer than 30,000 cases are diagnosed and treated annually. Similarly about 3 billion pounds are lost annually in Africa from animal trypanosomosis in terms of different aspects of animal production and draught [2]. However after continued control efforts of the vector, the number of cases reported [3] has dropped below 10, 000 for first time in 50years and this trend has been maintained. Never the less the chemotherapy of Trypanosomiasis is beset by so many factors such as parasite resistance to trypanocides [4], drug toxicity [5-6], expensive nature of drug. Also, route of administration of drug is very combo some. Efforts to produce vaccine have been dwarf due to antigenic variation of the parasite [7-9].

African Trypanosomiasis is one of the tropical diseases in which new, safer and affordable drugs are needed thus, making the search for the development of trypanocidal agents from medicinal plants. In Africa, traditional medicine from plants, has a long history and still holds a strong position in medical and veterinary care [10]. Several reports on the evaluation of various chemical compounds isolated from plant on several diseases include the work of [11-15] just as interesting reports on the antitrypanosomal effects of plant extracts and plant derivatives [16-23]. Mushrooms have been used in humans' food since ancient times, which are low in calories. In recent years there has been a significant increase in the consumption of mushroom due to an increasing number of studies identifying the therapeutic properties of the substances against illness such as cancer, tumor, chronic hepatitis, diabetes, atherosclerosis, and hypercholesterolemia [24]. phylum Basidomycota include fungi with gills or pores and also the familiar mushrooms which are high in minerals, vitamins, fibres, and essential amino acids. This publication, present report on trypanocidal activity of extracts obtained from a b *Cantharellus cibarius* on *Trypanosoma brucei brucei*.

II. Materials And Methods

2.I Plant materials and extract preparation

The Mushroom (*Cantharellus cibarius*) were collected from Nigerian Institute for Trypanosomiasis research Kaduna and it's environ. They were identified and authenticated by a Mycologist at the Department of Biological Sciences, Ahmadu Bello University, Zaria.

2.2 Preparation of crude extracts

They were dried in a laboratory oven at a temperature of 50^0C. Dried materials were blended to powder with a laboratory blender. Two hundred grams (200g) of the pounded dried plants materials were weighed and extracted by maceration for 72h in 500ml of petroleum ether, the filtrate was concentrated under a rotary evaporator while the Mac was air dried and extracted exhaustively with acetone. The filtrate was also concentrated and the Mac was further extracted with 100% ethanol. All the filtrates were stored in capped bottles inside the refrigerator at 4°C until required.

2.3 Trypanosome stock

A Stabilate of *Trypanosoma brucei brucei* (Federe strain was obtained from Nigerian Institute for Trypanosomiasis Research Kaduna and they were maintained in Swiss albino rats by serial passage which served as donor animal.

2.4 In vitro anti trypanosomal activity

Exactly 10mg of the extracts were weighed and dissolved in 1ml of phosphate buffered saline (PBS). The stock solution was further diluted serially to obtain an effective concentration ranging from 2.5mg - 10mg/ml using PBS. Parasites motility were monitored in a 96 well micro titre plate (Flow laboratories Inc., Mclean, Virginia, USA as follows : 30µl of each constituted extract was dispensed in duplicated mixed 60µl of blood obtained by cardiac puncture of a rat containing 10^8 parasite and incubated at 37^oC . For control wells, 30µl of extract was replaced with PBS and incubated with blood containing parasites. Parasite motility was then monitored and counted at 5min intervals under a microscope at ×40 magnification.

2.2 DIIT

After incubation for *In vitro* trypanosomal activity, a Drug Incubation Infectivity Test was performed as follows: the acetone extract suspensions with none motile trypanosomes was inoculated in duplicate into healthy rats and the level of parasiteamia was assessed as described earlier for two weeks.

2.3 Phytochemical Screening

Chemical test was carried out on the powdered specimen of acetone, ethanolic and aqueous extract of *Cantharellus cibarius* using standard procedure to identify the constituents as described by [25-26] this is to identify the presence of tannins, resin, glycosides, flavonoids, alkaloids, saponins among others.

2.4 Acute Toxicity Studies

Sixteen albino rats' of both sexes (weighing 200-250g) were obtained from the animal house of Nigerian Institute for Trypanosomiasis Research Kaduna (NITR). They were randomly divided into four groups (ABC& D), kept in clean cages fed with pellets and water *ad libitum*. Various doses of the ethanolic extract (50, 100, 500 1000mg/kg) were administered to group ABC&D respectively.

After administration of the extracts (0.5 ml in mice intraperitoneally) the animals were observed continuously for 72hrs and for any signs of behavioural changes, toxicity and mortality. The LD^{50} of the extracts were calculated by the method of Lorke *et al.*, 1983. The control group received vehicle alone.

2.5 Prophylactic properties

A total of 12 mice received a dose of extract at 200mg /kg each they were then divided into 4 groups of 4 animals each (A B Cand D) at the end of 30, 60,120 and 180 members of each respective group were challenged with 10^4 trypanosomes and were monitored for parasiteamia from tail blood smear.

2.6 In vivo trypanocidal activity of Cantharellus cibarius

Twenty five (25) rats were intraperitoneally infected with 10^4 and the level of parasitemia monitored daily by the Herbert and Lumsden method (1976). At the height of parasitemia such as 10^7 animals were divided into five Groups of 5 each and were treated with ethanolic extract of *Cantharellus cibarius* for seven (7) consecutive days as follows:

Group 1: received 200mg /kg b.w

Group 2: received 300mg
Group 3: received 400mg/kg b.w
Group 4: were infected but not treated (positive control)
Group 5: were not infected but given normal saline (negative control).

III. Results

The ethanolic extract of **Cantharellus cibarius** exhibithed strongest *in vitro* trypanocidal activity compared to other extracts and also showed different percentage of *in vitro* motility activity against *Trypanosoma brucei brucei* at various concentrations the extract was able to immobilise100% parasite motility within 10 minutes of incubation at a concentration 5mg/ml, this was followed by acetone extract which was able to immobilise 100% parasite motility at the end of 60 minute incubation (Table1). Parasites were still motile in aqueous extract of **Cantharellus cibarius**, by the end 120 minutes of incubation.

However Diminal^{R} eliminate parasite motility in all concentrations (20-50) minutes of incubation.

The toxicological studies showed that the extract produced dose related death in mice 0/5,100mg/kg; 1/5, 500mg/kg and 3/5,1000mg/kg. The LD^{50} was calculated to be 350mg/kg with 95% confidence limits of 115-335mg/kg.The prophylactic activity showed that challenging the animals with parasites 30- 60min. after the administration of the extract did not come down with disease. However those infected between two to three hours after been administered extract developed the parasite after 5days. Similarly the *in vivo* result presented in (Figure2), showed that there was no reduction in parasiteamia within the groups treated with 200 and 300mg/kg. However there was great reduction in parasiteamia with the group (3) treated with 400mg/kg intraperitoneally, which led to their life's been prolonged up to the 15th day post infection.

Also the infected positive control group E, parasiteamia increased progressively until the death of the animals between days sixth and seventh post infection.

TABLE 1. Trypanocidal activity of extracts recovered from **Cantharellus cibarius** *Trypanosoma brucei brucei*

Time in minutes	10	20	30	40	50	60	70	80	90	100	120
C. cibarius acetone 2.5mg/ml	100	90	75	50	40	30	25	15	15	-	-
C. cibarius acetone (5mg/ml	100	90	80	70	65	50	20	-	-	-	-
C. cibarius acetone (10mg/ml	100	95	70	50	35	25	-	-	-	-	-
C. cibarius ethanol (2.5mg/ml	100	50	-	-	-	-	-	-	-	-	-
C. cibarius ethanol (5mg/ml	-	-	-	-	-	-	-	-	-	-	-
C. cibarius ethanol (10mg/ml	-	-	-	-	-	-	-	-	-	-	-
C. cibarius aqueous (2. 5mg/ml	*	*	*	*	*	*	*	*	*	*	*
C. cibarius aqueous (5mg ml	*	*	*	*	*	*	*	*	*	*	*
C. cibarius aqueous (10mg ml	*	*	*	*	*	*	*	*	*	*	*

- = no motile parasite ; *= parasite highly motility.

Table 2: Phytochemical screening of extracts obtained from Cantharellus cibarius

Compound tested	**Acetone**	**Ethanol**	**Aqueous**
Alkaloids	+	++	-
Saponins	+	++	+
Free Antraquinone	-	-	-
Bound Antraquinone	-	-	-
Tannins	-	-	-
Terpenoids	+	+	+
Flavournoids	-	-	-
Cardiac glycosides	-	+	-
Phlobabtanins	_	-	-

Sterols	_	-	-

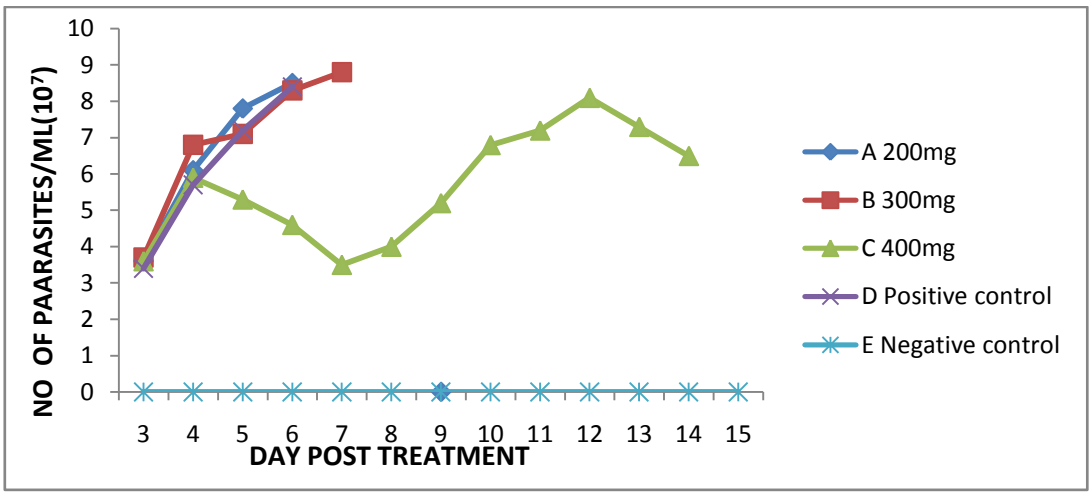

Figure I Fig. 2: Trypanocidal activity of various doses of ethanolic extract of ***Cantharellus cibarius*** on infected rats

IV. Discussions

Mushrooms species have exhibit tremendous activity on pathogens such as parasites, virus, cancers etc. The efficacy of the extract was assessed on the basis of percentage of motile parasite in parasite population; As such the cessation or drop in motility in the *invitro* result of trypanosomes in the ethanolic extract incubated blood compared to the parasite loaded control blood with no extract was taken as a measure of anti-trypanosomal activity. This investigation supports earlier reports which indicate that different extract from the same plant may exhibit Different activity, [16and 22] due to composition and concentration in different chemical components. *In vitro* experiment remains a useful method for pre selection of plant for anti-trypanosomal activity [16]. Therefore, extract found to be active in this report must be tasted *in vivo* before a definite statement can be made on their trypanocidal potentialities.

The result tends to be in line with similar work carried out with plant extract [28, 16,29 -31).

The anti-trypanosomal activity of the extracts could be as a result of the presence of appreciable amount of alkaloid, saponins among others found in the phytochemical examination (Table1) Also in the *in vivo* experiment, it should be noted that infected animals developed parasite between 3-4 days post infection which is usual with *Trypanosoma brucei brucei* (Federe strain), and causes sudden death in animals if not treated. The group (C) treated with ethanolic extract at 400mg/kg body weight was able to reduce the number of parasite, while those treated with 200-300mg cumulated in death 4-5 days post treatment which suggests the dose dependent nature of the extracts. The extension of the life of the animals treated in group C. Even though the parasite was not cleared completely, maybe connected with the release of extracellular factors by the trypanosomes which has been implicated to have pathological effects on the animal [32-35]. That the anti-trypanosomal effect is attributable to the ethanolic extract is confirmed by the death of the control group left untreated. The toxins release into the mammalian system renders the antibodies produced by the host against the parasite ineffective because the parasite has the ability to produce a large repertoire of antigens. The host defense mechanism is only partially specific and often lagging behind the progress of the disease in terms of antigen antibody interaction [36]. Eventually there is a breakdown of the host immune system coupled with parasite invasion of the central nervous system leading to coma and death. Furthermore the prophylactic experiment showed a promising result, which suggest that the active principle is been metabolize after five hours such a property can be investigated for the prevention of Trypanosomiasis during blood transfusion

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