Journal of Microbiology and Antimicrobials Vol. 4(2), pp. 49-48, February 2012
Available online at http://www.academicjournals.org/JMA
DOI: 10.5897/JMA11.101
ISSN 2141-2308 ©2012 Academic Journals

Full Length Research Paper

Does treatment of African trypanosomosis, *Ganoderma applanatum* holds any hope? Findings from a preliminary analysis in Nigeria

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Accepted 1 November, 2011

Treatment of African trypanosomosis is becoming difficult due to the increasing wave of treatment failure occasioned by drug resistance of *Trypanosoma brucei species*. This study was therefore set up to assess the antitrypanosomal potential of extracts from a mushroom with acclaimed wide medicinal properties. Aqueous extracts of *Ganoderma applanatum* were obtained using hot water extraction method. These were injected into 24 laboratory reared rats comprising 12 rats infected with *Trypanosma brucei brucei* serving as test, and the other 12 rats uninfected which served as control. The rats were observed for up to two weeks for obvious clinical symptoms. Laboratory tests, mainly microscopy using thick and thin blood films, and haematocrit centrifugation technique (HCT) for parasites and haematological profiles were monitored on daily basis. Data obtained were analysed using simple descriptive methods. All the rats infected with *T. brucei brucei* died from overwhelming parasitaemia between day 6 and day 12 of observation. Uninfected counterparts who were injected with the *Ganoderma* extracts remained alive at day 12. Also rats that were uninfected and not injected with *G. applanatum* extracts remained healthy by day 12. *G. applanatum* hot water crude extract did not exhibit any antitrypanosomale action on the dose levels used. However, further work on anti-trypanosomal activity of the mushroom at higher doses using other methods of extraction is recommended.

Key words: African trypanosomiasis treatment, extracts, *Ganoderma applanatum.*

INTRODUCTION

Treatment of infections and infestations globally is increasingly becoming a daunting challenge due to wide-spread resistance against most of the front line drugs meant for treatment of such life threatening diseases (Lejon et al., 2010; Vodnala et al., 2009; Blum et al., 2009). This has in fact compounded the already existing problem in sub-Saharan Africa. Other developing countries of the world where poor planning and implementation of health policies is still a major factor also face this challenge. In addition, the lean budgetary provisions for health-related matters have made availability of quality drugs for proper management of infections a great challenge (Jombo et al., 2007, 2008; Bassey et

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al., 2009).

African trypanosomosis, a parasitic disease caused primarily by flagellated protozoa called *Trypanosoma brucei gambiense* or *T. brucei rhodesiense* is still prevalent in most parts of sub-Saharan Africa with at least 60 million people at risk of contracting the infection and about 40,000 new cases are reported each year (Arora and Arora, 2006; Steverding, 2008; Lutumba et al., 2007). The disease causes irreversibly fatal brain damage if left untreated and is generally grouped among the neglected tropical diseases (Robays et al., 2008; Matemba et al., 2010; Simarro et al., 2008).

In Nigeria, African trypanosomosis is still prevalent in several communities (Koffi et al., 2009; Simarro et al., 2008; Jamonneau et al., 2000; Jamonneau et al., 2010). The ongoing development of drug resistance by *T. brucei species* poses a serious global challenge towards effective control and eradication of the disease (Checchi et al., 2008; 2009; Priotto et al., 2008). In Uganda for instance, resistance to melarsoprol among those with advanced stages of the disease of up to 10% was recorded, with up to 70% deaths. In addition reports from Sudan, Congo DR and Angola also found that 30% of individuals with second stage trypanosomosis who were treated with melarsoprol failed to respond to treatment. These reports also indicated corresponding high fatalities in those cases where patients did not respond to drug therapy (Priotto et al., 2008; Lejon et al., 2007; Sanderson et al., 2008). Varying degrees of resistance of trypanosomes to eflornithine (diethyfluoromethylornithine), nifurtimox, pentamidine, benznidazole, suramin and rimantadine among others have been documented with increasing rates of treatment failures with serious accompanying health challenges (Sanderson et al., 2008; Balasegaram et al., 2006; Simarro et al., 2011).

In view of the aforementioned challenges confronting physicians in managing African trypanosomosis due to increasing drug resistance, there is an urgent need for a continuous search for newer anti-trypanosomal drugs with enhanced potency and efficacy compared to the present selection of drugs (Simarro et al., 2011). Such discoveries no doubt would be a boost to the on-going global trypanosomiasis control programme.

Ganoderma is a genus of polypore fungus grown on wood with over 250 species and is widely used in traditional Asian medicines (Guillamon et al., 2010; Tapseil et al., 2006; Wasser, 2011). Several studies have shown that extracts of *Ganoderma species* have been found useful in the treatment of diverse ailments in humans (Chan et al., 2005; 2009; Tang et al., 2006). These include: treatment of malignancies such as lung cancer; cardiac failure; bacterial and parasitic infections; and viral infections including HIV due to its immunopotentiation and immunomodulatory properties (Lindequist et al., 2005; Wasser, 2002; Rajewska and Balasinska, 2004). Based on its wide medicinal applications, the anti-trypanosomal properties of *Ganoderma applanatum* were assessed.

MATERIALS AND METHODS

Experimental design

Experimental albino rats were obtained from Nigerian Institute for Trypanosomias is Research (NITR), Vom. The rats were kept in laboratory cages, fed with commercially prepared feeds (Vital feed) and allowed to acclimatise for four weeks. Blood samples were then collected from the tail vein on a microscope slide and examined under the microscope to exclude the presence of trypanosomes and other haemoparasites. *T. brucei brucei* isolate were maintained in rat obtained from laboratory animal colony at NITR, Vom, which served as source of Trypanosomes for the study.

Hot water extraction of *Ganoderma applanatum*

One kilogram of the powder of *G. applanatum* was dissolved in three litres of distilled water. The sample was boiled for three hours with stirring every 30 min. It was allowed to stand for 24 h and thereafter filtered using whatmann no. 1 paper. The filtrate was evaporated to dryness in hot air oven set at 45°C, the extract obtained was reconstituted using sterile distilled water to obtain concentrations 500 mg/ml and further diluted to obtain 250 mg/ml (Crewe, 1997).

Rat groupings

The 24 rats were shared into six groups with 4 rats in each and were weighed:

Group A- rats were infected and treated with 250 mg of aqueous *G. applanatum* extract/kg body weight.
Group B- rats were infected and treated with 500 mg of aqueous *G. applanatum* extract/kg body weight.
Group C-rats were infected and not treated with the extract.
Group D- rats were uninfected but treated with 250 mg of aqueous *G. applanatum* extract/kg body weight.
Group E- rats were uninfected but treated with 500 mg of aqueous extract/kg body weight.
The group F- rats were uninfected and untreated.

Procedure

0.5ml blood collected from the parasite donor rat was diluted (50:50) with normal saline. The parasite donor rat was only used as a source of the parasites but was not part of the experiment. A drop of the diluted blood was examined under the microscope with an average of 5 trypanosomes per field of view. 0.1 ml of the diluted blood was used for injecting the infected group of albino rats intraperitoneally (Erah et al., 2003). Rats were bled through the ocular vein into Ethylene diamine tetraacetic acid (EDTA) bottles. Fresh blood samples collected each day were analysed for 12 consecutive days for the presence of trypanosomes using thick and thin blood films, and haematocrit centrifugation technique (HCT) (Luckins, 2003).

Data analysis

Data obtained was analysed using simple descriptive methods of

Table 1. Impact of extracts of *Ganoderma applanatum* on rats infected with *Trypanosoma brucei brucei* in Vom, Nigeria. Parasite per field of infected rats.

Day	Group A				Group B				Group C			
	1	2	3	4	5	6	7	8	9	10	11	12
1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4	6/1	10/1	ND	ND	3/1	ND	ND	1/30	ND	ND	5/1	ND
5	30/1	35/1	ND	20/1	25/1	10/1	10/1	2/1	ND	1/30	L	1/15
6	H	M	M	H	H	H	H	18/1	3/1	M	D	L
7	D	H	H	D	LM	D	D	M	L	H	-	L
8	-	D	H	-	D	-	-	H	H	H	-	H
9	-	H	-	-	-	-	-	H	H	D	-	H
10	-	-	H	-	-	-	-	H	H	-	-	D
11	-	-	D	-	-	-	-	D	H	-	-	-
12	-	-	-	-	-	-	-	-	D	-	-	-

ND = Not detected; L = Light (36 to 39/field); M = Medium (40 to 45/field); H = High (46 to 50/field); LM = Light Massive (51 to 60/field); M = Massive (Above 60/field); D = Death.

arithmetic sum and mean.

RESULTS

Infected rats with *T. brucei brucei* in groups A, B and C treated with *G. applanatum* crude extracts on day zero had no parasites detected in their blood on days 1 to 3. On day 4 and 5, parasites were detected in the range of 2 per field. Parasitaemia continued to increase progressively on daily basis from light (36 to 39/field), medium (40 to 45/field), high (46 to 50/field), light massive (51 to 60/field) and massive (> 60/field). From day 6 to 8 when the first mortality was recorded both the treated and untreated infected rats began to die progressively and simultaneously until all died by day 12. On the contrary, uninfected rats in groups D, E and F treated with or without the extracts were all alive by day 14 of the experiment (Table 1).

DISCUSSION

Antitrypanosome activity of *G. applanatum* was investigated in rats infected with *T. brucei brucei*. The extracts were applied in three graded concentrations on both infected and uninfected control. Our findings showed that all the infected rats (groups A,B and C) died by day 12 from overwhelming parasitaemia is an indication that the drug lacks any anti-trypanosome activity *in vivo*, however showed no obvious toxic effects on uninfected rats.

The findings from this study showed that crude extracts of *G.a applanatum* probably had no anti-trypanosomal properties. The possibility of the presence of potent anti-trypanosomal substances inherent in the mushroom in their inactive form may not be completely ruled out. *In vitro* assay of the extract may however be needed to establish this assertion (Bhaskar et al., 2010; Cui et al., 2007; Ma et al., 2003). The timing of the treatment of the infected rats on day zero may probably have contributed to this outcome as it might have been too early to attain therapeutic bioavailability for the rats.

The available documented anticancer, antibacterial, antiviral and antiparasitic effects among others lays credence to the fact that the medicinal potential of Ganoderma may probably not have been exhausted; hence, more advanced search into the molecular biology of its active ingredients should be carried out to ascertain its suitability or otherwise in the management of African trypanosomiasis (Lindequist et al., 2005; Mothana et al., 2000; Mothana et al., 2003). Such studies should as well involve increase in dosages so as to ascertain the impact of high dosages (Gao et al., 2003; Chairul et al., 1991). This study, being a preliminary study could not accommodate such procedures.

In conclusion, this study showed that crude extracts of *G. applanatum* are unsuitable for treatment of African trypanosomiasis. Exploring the efficacy of other methods of extraction of the fungus is open for further investigation. Also multiple doses of the extract at intervals may need to be tried to rule out the possibility of under-dosage.

ACKNOWLEDGEMENT

We wish to express our deep appreciation to the staff of the Haematology laboratory, Federal College of Veterinary and Medical Laboratory Technology, Vom, for their assistance towards sample collection and processing.

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