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EVALUATION OF ANTITRYPANOSOMAL ACTIVITY OF ETHYL ACETATE EXTRACT OF *ADANSONIA DIGITATA* SEED EXTRACT IN *T.B. BRUCEI* INFECTED ALBINO MICE

Hassan M. Ibrahim[1]*, Emmanuel O. Ogbadoyi[2], Kabiru Y. Adamu[2], Maimuna U. Bello[2] and Ilumi J. Yemisi[2]

[1]Faculty of Veterinary Medicine, Usman Danfodiyo University Sokoto, State, Nigeria

[2]Biochemistry Department, Federal University of Technology, Minna, Niger State, Nigeria

ABSTRACT

Adansonia digitata, a shrub used in traditional medicine for the treatment of cancer was investigated for the treatment of experimental trypanosomiasis. Ethyl acetate extract of the seed of *Adansonia digitata* was investigated for in-vivo antitrypanosomal activity in albino mice infected with *Trypanosoma brucei brucei* and treated intraperitoneally with the extracts 24 hours post infection, at doses ranging from 50-500mg/kg for 14 days, while the positive control was administered a single dose of 3.5 Mg/kg body weight standard berenil. The parasitaemia in all the mice was monitored for the period of study. Seed extracts at dose of 400mg/kg showed significant antitrypanosomal activity ($P<0.05$) compared with the untreated control. Although parasites were not completely cleared from circulation, 67% of the experimental animals survived for over 18 days with 300mg/kg up to 38 days. The treated control (berenil at dose of 3.5mg/kg) showed 100% survival of the mice and parasites were completely cleared. Acute toxicity studies of the crude ethyl acetate extract of Adansonia digitata seed showed that the L.D50 value was well above 2900mg/kg. Phytochemical screening of the crude extract indicated the presence of carbohydrate, glycosides, cardiac glycosides, saponins, steroids and triterpene, flavonoids and alkaloids. The result of this study shows that *Adansonia digitata* has great potential as anti-trypanosomal agent, which could be developed into an alternative drug to complement treatment options for African trypanosomiasis.

Keywords: Trypanosomiasis, Anti-trypanosomal, Intraperitoneally, Parasitaemia, Phytochemical screening.

INTRODUCTION

Human African trypanosomiasis (HAT) or sleeping sickness is a severe fly-borne disease caused by protozoan of the species *Trypanosoma brucei* (*T.b.*). This disease was first described by European explorers by the late 1800s and early 1900s even if this disease has probably existed in Africa for many centuries (Hide, 1999). The disease occurs in foci in the tsetse fly (Glossina spp) "belt", a vast geographical region ranging from the Sahara to the Kalahari Desert equivalent to "the combined size of the United States, India and Western Europe" where these flies have their habitat (Gooding and Krafsur, 2009). Three major epidemics of HAT occurred in Africa during the last century, of which the most devastating (which killed millions of persons) occurred from the 1930s to the 1960s (WHO, 2010). The colonial administrations established mobile teams which systematically screened people in the endemic areas, curing those found with the disease. This initiative resulted in a significant roll back of the disease. In the early 1960s, HAT ceased to be a public health problem, and was no more considered (Simarro, 2008). From the 1970s to

the 1990s, favored by dramatic events such as wars and population movements, HAT re-emerged and became an ongoing epidemic. WHO, private partners, and local governments took action, resulting in a significant decrease of the number of new cases reported which, in 2009, was lower than 10,000 for the first time in 50 years (WHO, 2010). Despite this encouraging development, HAT is still a considerable burden for life quality and economy in many sub-Saharan Africa countries, where there may be 200 foci and 15–20 million persons at risk (Cecchi *et al.,* 2008), as a large number of new infections may remain unreported or undiagnosed because of remote accessibility of many areas of the endemic region and ongoing wars (Fevre *et a*l., 2008). Besides, it is generally assumed that new epidemics of HAT could occur, originating from these uncontrolled areas where there still are very active foci (Berrangford *et al.,* 2011. HAT affects poor and remote rural populations dependent on agriculture, fishing, or hunting. Until very recently, this disease was receiving very few attention, and health interventions and research and development were inadequate to the need (WHO 2010). In the last 50 years, only one drug, eflornithine, has been developed even though a huge amount of knowledge of African trypanosome biology has been accumulated in the meantime (Barrett 2010) . Overall, the current drugs used to cure HAT are expensive, highly toxic, need parenteral administration, and parasites increasing resistance has been observed (Barrett *et al.,*2007) . Therefore, less toxic, more efficient, easy-to-administer and non expensive drugs are urgently needed in the field. WHO and some private partners have been recently multiplying initiatives, offering funding for research activities for this purpose. Some encouraging results have already been reported. The research activities have been aiming at developing new field suitable, easy to-use, and cheap tools to solve the HAT diagnosis, staging, and follow-up issues observed in the field.

Baobab or *Adamsonia digitata L.* belong to the Malvaceae family (Bremer *et al.,* 2003). And is deciduous tree native to arid central Africa (Yezzie *et al.,* 1994). Its distribution area is larger and this species can be found in most of sub-Sahara Africa's semi-arid and sub-humid regions as well as in Western Madagascar (Diop *et al.,* 2005). It has been introduced to areas outside Africa and grown successfully (Sidibe & Williams, 2002). Baobab is very long-lived tree with multipurpose uses. The different plant parts are widely used as foods, medicines and the bark fibres are also used (Sidibe and Williams, 2002). The tree provides foods, shelter, clothing and medicine as well as material for hunting and fishing (Venter and Venter 1966; Gebauer *et al.,* 2002). Every part of the baobab tree is reported to be useful (Owen, 1970; Igboeli *et al.,* 1997 and Gebauer *et al.,* 2002).

MATERIALS AND METHODS

Plant Collection

Fresh seeds of *Adansonia digitata* were collected between the months of September and October in Maiduguri, Borno State, Nigeria. The seeds were washed and dried at room temperature. The dried seed sample was then grinded to powdered form and stored in an envelope until required for use.

Animal and Parasite

Albino Mice were purchased from the Department of Pharmacology, Faculty of Pharmacy, Ahmadu Bello University (A.B.U) Zaria, Nigeria. The mice were transported to Federal University of Technology Minna (F.U.T Minna) via road transport and were allowed to acclimatize in of Department of Biochemistry Federal University of Technology Minna (F.U.T Minna) for two weeks. The mice were fed with poultry feed (chick mash) purchased from vita feed and water *ad libitum*. The experiments were conducted in compliance with the internationally accepted principles for laboratory Animals use and care as contained in the Canadian Council on Animal Care (CCAC,1997) , guidelines for Animal use. A stabilate of pleomorphic *Trypanasoma brucei brucei* was obtained from the Nigeria Veterinary Research Institute (NVRI) VOM, Plateau State, Nigeria. The parasites were inoculated into albino mice and transported to

Federal University of Technology Minna (F.U.T Minna) Niger State, Nigeria.

Preparation of the Crude Extracts

The extract was prepared by slight modification of the method described by kiuchi *et al*. (1978) as carried out by Ogbadoyi, *et al.* (2007). Seventy grams (70g) of the dried seed powder was sequentially extracted using reflux method in 400 ml hexane, ethylacetate, and methanol respectively. The extraction lasted for two (2) hours in each case. Extracts were filtered hot using muslin cloth, and the solvents were evaporated using steam bath. The dried extract obtained after evaporating the solvent was transferred into sterile universal bottles and kept in the refrigerator until required for use.

Monitoring the Course of Parasitemia and Administration of Extract

Blood was collected by cardiac puncture with clean sterilized syringe from an infected Mouse and immediately diluted with physiological isotonic solution to serve as diluents for the inoculation of the parasite. Apparently healthy mice were infected intraperitoneally with 0.02 ml of the inoculum containing trypanosome cells. The course of parasitemia was monitored at two days interval throughout the study period by microscopic examination of blood stains taken from the tails of infected animals. In order to determine the activity of the extract, six groups of mice, each consisting of three mice were intraperitoneally treated with the crude methanol extract of A. digitata seed at doses of 50mg/kg, 100mg/kg, 200mg/kg, 300mg/kg, 400mg/kg and 500mg/kg body weight. Another group that was not infected was treated with 500mg/kg seed extract. The seventh group consisting another three mice was infected but not treated to serve as negative control, while the eight group was infected and treated with a single dose of the standard drug Berenil at a dose of 3.5mg/kg bodyweight to serve as positive.

Acute Toxicity Studies and LD_{50} Determination of Crude Methanol Seed Extract

The acute toxicity study was carried out as described by Lork, D *et al.,* (1983). Three groups (A,B and C), each consisting of three mice were used. The extract was administered intra-peritoneally at different doses of 1000mg/kg, 100kg/mg and 10 kg/mg body weight respectively. The second phase of the acute toxicity study involved the use of four groups of mice with each group consisting three mice. The extract was administered intra peritoneally at different doses of 2900mg/kg, 1600mg/kg, 1000mg/kg, and 600mg/kg, respectively.

Phytochemical Analysis of the Partially Purified Extract

The ethylacetate seed extract was screened for the presence of saponins, carbohydrates, tannins, terpenes, flavonoids, anthraquinones, alkaloids, steroids, glycosides and resins using simple chemical tests as described by Odebiyi and Sofowara (1982-1986) and Trease and Evans (1989).

RESULTS AND DISCUSSION

The result as shown on Figure 4.1 indicates that ethyl acetate extract of *A. digitata* seed at varying test dosages of 100 - 500mg/kg bodyweight produced significant antitrypanosomal effects ($P<0.05$). Mean number of parasites ranged from 0.00 for the positive control (Berenil) to 14.02 in the infected but untreated group. The highest activity of the extract was recorded at 400 mg/kg bodyweight (3.59 parasites) while 300mg/kg concentration produced the least trypanosomal activity (11.42), which was not significantly different ($P>0.05$) from that of the infected but untreated mice (14.02). The trypanocidal effects of the ethyl acetate hot seed extract of A. digitata at 400 and 500mg/kg concentrations were statistically comparable ($P<0.05$) to that of the control (Berenil). There were fluctuations in the level of parasitaemia in all the treated groups, which were however, kept at relatively very low level. The ethyl acetate hot seed extract of *A. digitata* exhibited mild to moderate antitrypanosomal activity at the different concentrations studied (100-500mg/kg) but did not completely clear the parasite. On the other

hand, Berenil had total clearance of parasitaemia from the 2nd day post infection without relapse of infection throughout the study.

DISCUSSION

The results obtained in this study show that the ethylacetate hot seed extract of *A. digitata* exhibited appreciable antitrypanosomal activity compared to the standard drug, berenil, since it was able to prolong the lifespan of the test animals beyond that of the untreated control by 18 days. This observation is similar to that of Abdel-Sattar *et al.,* (2009) who reported strong activity of methanol extracts of *Solanum schimperianum* and *C. tuberculata.* Maikai (2011) also made similar observations for *Ximenia Americana.*

The mechanism by which the crude extract of this plant exert their trypanocidal activities is unknown since the active ingredient(s) were not isolated. However, previous reports indicate that a number of tropical plants contain constituents that have been demonstrated to be clinically efficacious against many protozoal diseases (Le Grand, 1989; Oliver-Bever, 1986; Etkin, 1981; Bodley *et al.,* 1995; Gbile and Adesina, 1987). Similarly, it is known that existing trypanocidal drugs exert their therapeutic action through a variety of mechanisms (Atawodi, 2005). Sepulveda-Boza and Cassels (1996) reported that many natural products exhibit their trypanocidal activity by virtue of their interference with the redox balance of the parasites acting either on the respiratory chain or on the cellular defenses against oxidative stress. This is because natural products possess structures capable of generating radicals that may cause peroxidative damage to enzymes that are very sensitive to alterations in redox balance. Some agents also act by binding with the kinetoplast DNA of trypanosomes (Atawodi *et al.,* 2003). Different phytochemical constituents might also be responsible for the antitrypanosomal activities (Asres *et al.,* 2001; Antia *et al.,* 2009). Several studies have reported the presence of phytochemicals such alkaloids in *A. digitata* (Proll *et al.*, 1998; Osman, 2004). According to Mann *et al.,* (2003), antitrypanosomal activity could also be attributed to the presence of alkaloids. The DNA intercalation in combination with protein biosynthesis inhibition is reported to be the mechanism of action responsible for the observed anti-trypanosomal effect of the active alkaloids (Merschjohann *et al.,* 2001). The trypanocidal activity of several flavonoids such as quercetagetin (Hoet *et al.,* 2004); hispidulin and santin (Sülsen *et al*., 2007) has been previously reported. Furthermore, the trypanocidal activity of the methanolic seed extract at 100mg/kg-500mg/kg concentration was statistically comparable to that of the positive control. Generally, there were fluctuations in the level of parasitaemia in all the treated groups, which were however, kept relatively at very low level. This could be as a result of resistance put forward by the parasites or some limitations on the part of the extract itself such as the inability of the extract to permeate other tissues where the flagellates are known to hide as a way of evading trypanolytic action of drugs (Anosa, 1988; Awodi *et al.,* 2011). It has been shown in previous studies that different parts of the same plant could show varying levels of antitrypanosomal activity just as extracts of different parts of the same plant (Atawodi *et al.,* 2003; Igwe and Onabanjo, 1989). In this present study the extracts had varying degrees of antitrypanosomal activity based on the solvent used for extraction. Therefore, the statement that a plant extracts is efficacious or not should be taken in the context of the solvent used. In some instances, where extracts obtained by reflux (hot) extraction were compared to those acquired through cold extraction, it was observed that more activity was observed with cold extract, indicating that trypanocidal components of many plants are heat-labile (Atawodi, 2005). This may explain why some plants reported to be traditionally useful for treating trypanosomiasis are not active when scientifically evaluated in the past. It may therefore be advisable to use cold rather than hot extraction, where possible, when evaluating the trypanocidal activity, or indeed, other biological activities of medicinal plants.

The seed extracts of *A. digitata*, at different concentrations used in this study showed considerable trypanocidal activity. This finding is in line with earlier reports (Freiburghaus *et al.,* 1996, 1997, 1998; Nok *et al.,* 1993; Asuzu and Chineme, 1990; Atawodi *et al.,* 2003; Mikail and Ajagbonna, 2007) that clearly indicated that plants of different families could possess potent trypanocidal activity. In fact, natural products with trypanocidal activity and belonging to a variety of phytochemical classes have been identified (Hopp *et al.,* 1976; Sepulveda-Boza and Cassels, 1996; Mikail, 2009).

The various extracts of *A. digitata* exhibited mild to moderate antitrypanosomal activity at the different concentrations studied (100-500mg/kg) but did not completely clear the parasite. On the other hand, Berenil had total clearance of parasitaemia from the 2nd day post commencement of treatment without relapse of infection throughout the study. The result of this study further showed that *A. digitata* hot seed extract has potential in the management of African Animal trypanosomiasis. The hot seed extract of this plant has demonstrated some level of antitrypanosomal activity by reducing the parasite levels compared to the untreated control. There was also the extension of lifespan of the treated groups beyond that of the untreated control. However, the fluctuations observed points to limitations in the use of these extracts alone. But, since most traditional practices consist of herbal concoctions from different plants (Atawodi *et al.,* 2002), it is possible that in such combined herbal therapy involving A. digitata, the contribution of its extract is either to suppress the parasitemia and allow active principle from other component of the herbal mixture to easily wipe out the residual A. digitata - resistant trypanosomes or that the extract is able to interact and weaken the parasites' membrane thereby enhancing the accessibility of organelle-specific active component from other plants in the herbal recipe.

CONCLUSION

This work has demonstrated that *A. digitata* possesses some potential for a chemical lead for a new trypanocidal drug. Based on the above, it can be concluded that the use of *A. digitata* for management of trypanosomiasis in traditional medicine is scientifically justified.

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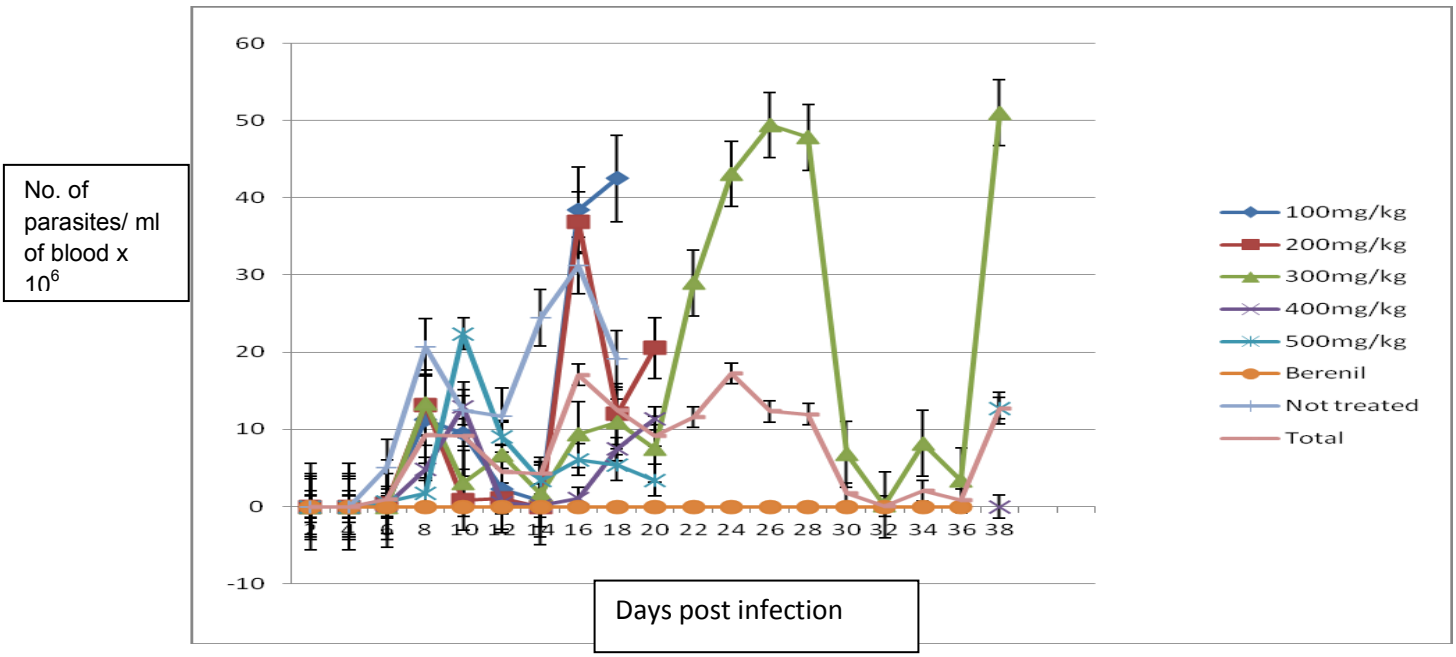

Figure1: Antitrypanosomal activity of Ethyl acetate extract expressed as mean number of parasites in of blood

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