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EVALUATION OF THE ANTITRYPANOSOMAL ACTIVITIES OF NINE SELECTED NIGERIAN MEDICINAL PLANTS IN MICE

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CERTIFICATION

This is to certify that this study was carried out by ADELODUN Victoria of the Drug Research and Production Unit (DRPU), Faculty of Pharmacy, Obafemi Awolowo University, Ile – Ife, Osun State, Nigeria.

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DEDICATION

To God – My All in All (My Everything).

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ABSTRACT

The study investigated nine selected Nigerian medicinal plants for antitrypanosomal activity with a view to providing information on the potential role of any of the nine plants as an antitrypanosomal and/or anti-inflammatory agent.

The ethanolic crude extract and ethylacetate fraction of the stem bark of *Terminalia ivorensis* A. Chev, and with other plant extracts; *Harugana madagascariensis*(stem bark), *Landolphia dulcis* (leaves), *Curcuma longa* (rhizome), *Ocimum gratissimum* (stem), *Khaya senegalensis* (stem bark), *Alcornea cordifolia* (leaves), *Achyranthes aspera* (leaves), and *Aloe schweinfurthii* (leaves) were tested for their antitrypanosomal activity using *Trypanosoma brucei brucei* infected mice. A dose probing acute toxicity test was carried out on *L. dulcis* and *T. ivorensis*, while the LD_{50} data in literature were used for the other plants. The antitrypanosomal effects of the extracts were evaluated by monitoring changes in parasitemia level and mortality of infected mice. The anti-inflammatory property of the most promising plant extract was assessed using acute oedema of the mice paw model as a probable mechanism of antitrypanosomal action. The data were subjected to statistical analysis by employing the paired t-test.

Acute toxicity experiments established an oral LD_{50} for *T. ivorensis* and *L. dulcis* respectively as (> 5000 mg/kg and >1600 <5000 mg/kg) while the interperitoneal LD_{50} of *T. ivorensis* is 100 mg/kg. *T. ivorensis, H. madagascariensis, C. longa, O. gratissimum, K. senegalensis, A. cordifolia, A. aspera*, and *A. schweinfurthii* had weak antitrypanosomal effect against *T. brucei brucei* when compared with the positive control group (Diminal®), but shows some significance ($P < 0.05$) as compared with the negative control group. However, *L. dulcis* crude extract demonstrated a significant effect ($P < 0.01$) by parasite clearance on day 5 of treatment but there was a relapse thereafter from day 7. The activities of the 3 fractions of *L. dulcis* also showed a disappearance of the parasites from the bloodstream of the animals from day 3 of treatment and thereafter, a relapse with a lesser parasite count for the n-Hexane fraction with a value of (9.53 ± 0.56) when compared with the aqueous and ethyl acetate fractions with values of (25.53 ± 1.53) and (22.13 ± 1.93) respectively on day 9. Administration of n-hexane fraction of *L. dulcis* (1000 mg/kg) inhibited oedema induced by subplantar injection of egg albumin in systemic acute anti-inflammatory test by 107 %.

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The study concluded that *L. dulcis* has a potential as an anti-trypanosomal and anti-inflammatory agent because it increased the survival time of infected mice due to reduction in parasitemia caused by *T. brucei brucei*.

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CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Trypanosomosis remains a potentially fatal disease of human and domestic animals in tropical Africa and South America (Fairlamb, 1982). It has undergone a dramatic and devastating resurgence in recent years (Smith *et al*., 1998) especially in Sub-Saharan Africa (Welburn *et al*., 2001). Atawodi, 2005 noted that the significance of trypanosomosis to human health, nutrition and economy is enormous, thereby necessitating continuous research for better ways of eliminating the disease. Unfortunately, the scarcity of chemical drugs, with the high incidence of their side effects and the emergence of resistance strains have rendered existing chemotherapy, inadequate (Atouguia and Costa, 1999). Therefore, there is need to explore other agents, especially of plant origin for new generations of anti-trypanocidal agents that are more effective, less toxic, and readily available at affordable prices.

There have been reports that a vast majority of the population particularly those living in villages largely depend on herbal medicines (Gupta, 1994). According to Sülsen *et al.* 2007, natural products are important sources of new drugs because their derivatives are extremely useful as lead structures for synthetic modification and optimization of bioactivity.

In furtherance on efforts on sourcing for nouveau molecules from natural sources in combating African animal trypanosomosis, previous research on *in vivo* antitrypanosomal activity is very limited compared to the in *vitro* experiments and in the few *in vivo* studies found in literature, no complete cure without relapses were recorded for plants such as *Alstonia boonei* bark, *Annona senegalensis* root, *Morinda lucida* leaves and *Picralima nitida* (Table 1. 2).

Hence this work was undertaken to screen nine selected Nigerian medicinal plantsused locally for *in vivo* antitrypanosomal activity. This will be followed by solvent partitioning of the most active plant using solvents of increasing order of polarity. Infection models in mice have indicated that African trypanosomosis trigger anti-inflammatory responses (Namangala *et al*., 2001). Therefore, the most active partitioned fraction will be evaluated to determine its anti-inflammatory response in mice.

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1.2 Literature Review

1.2.1 Trypanosomosis

1.2.1.1 History of Trypanosomosis

Trypanosomosis is a disease - complex associated with the tsetse fly *Glossina sop*, which is found throughout sub-Saharan Africa affecting wildlife, humans and livestock. It causes serious health problems, economic losses in livestock in form of anaemia, loss of condition and emaciation thus repressing the economical and cultural developments of the affected areas (Bourn *et al*., 2005). The World Health Organization in listing major problems facing mankind places trypanosomosis high on the list with malaria, filariasis and leishmaniasis (WHO/TDR, 2002).

The causative agents of the disease are flagellated protozoa that belongs to the family Trypanosomatidae, Order Kinetoplastida, so called because of the large DNA containing structure (the kinetoplast) found at the base of the flagellum and they live and multiply extracellularly in blood tissue fluids of their mammalian hosts (Barret *et al.,* 2003).

Phylogenetic reconstruction based on the genes coding for the small subunit ribosomal RNA suggested that all Salivarian trypanosomes (to which African trypanosomes belong) separated from other trypanosomes approximately 300 million years ago (Haag *et al.,* 1998). Probably soon after their emergence, Salivarian trypanosomes became gut parasites or commensals of early insects, which evolved around 380 million years ago. With the appearance of tsetse flies some 35 million years ago, trypanosomes have been transmitted to mammals by these bloodsucking insects. The long co-existence of both tsetse flies and game animals may explain why most African wildlife species are tolerant of trypanosomosis: they become infected by the parasite but show no ill effects (Lambrecht, 1985).

In contrast, domestic animals have yet been unable to develop tolerance or resistance to trypanosome infections within the 13000 years of their breeding. Scottish missionary and explorer David Livingston (1813–1875) was the first to suggest that "Nagana" is caused by the bite of tsetse flies (African animal trypanosomosis is also called "Nagana"which is derived from a Zulu term meaning "to be in low or depressed spirits") (Maré, 2004). In 1852, he reported the occurrence of a disease in the valleys of the Limpopo and Zambezi rivers as well as at the banks

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of the lakes Nyasa and Tanganyika from which all the cattle he carried died after they have been bitten by tsetse flies. However, it took another 40–50 years until trypanosomes were identified as the causative agents of nagana and sleeping sickness. In 1895, the Scottish pathologist and microbiologist David Bruce (1855–1931) discovered *T. brucei* as the cause of cattle trypanosomosis (Bruce, 1895). The first unequivocal observation of trypanosomes in human blood was made by the British Colonial surgeon Robert Michael Forde (1861–1948) in 1901 when he examined a steamboat captain in the Gambia (Forde, 1902). He first thought that the organisms he found were worms but the English physician Joseph Everett Dutton (1874–1905) identified them as trypanosomes a few months later and proposed in 1902 the species name *Trypanosoma gambiense* (now *T. b. gambiense*) (Dutton, 1902). In the same year, the Italian physician and pathologist Aldo Castellani (1878–1971) found trypanosomes in the cerebrospinal fluid of sleeping sickness patients and suggested that they cause sleeping sickness. One year later, Bruce provided conclusive evidence that sleeping sickness is transmitted by tsetse flies (WHO, 2005). It was the German military surgeon Friedrich Karl Kleine (1869–1951) who showed in 1909 the cyclical transmission of *T. brucei* in tsetse flies (Kleine, 1909)

There are three different sub-species of *T. brucei*, which cause different variants of trypanosomosis.

T. brucei gambiense - Causes slow onset of chronic trypanosomosis in humans. Most common in central and western Africa, where humans are thought to be the primary reservoir it is transmitted by the tse tse fly) (Barrett *et al.*, 2003).

T. brucei rhodesiense - Causes fast onset of acute trypanosomosis in humans. Most common in Southern and Eastern Africa, where game animals and livestock are thought to be the primary reservoir and it is transmitted by the tse tse fly *Glossina morsitans* (Ormerod, 1967).

T. brucei brucei - Causes animal African trypanosomosis or nagana disease along with several other species of trypanosome (Plate 1). It is transmitted by the bite of *Glossina pallidipes, T. b. brucei* is not human infective due to its susceptibility to lysis by human apolipoprotein L1 (Vanhamme *et al.*, 2003). However, as it shares many features with *T. b. gambiense* and *T. b. rhodesiense* (such as antigenic variation) it is used as a model for human infections in laboratory and animal studies.

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