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Full Length Research Paper

Comparative *in vivo* evaluation of the trypanocidal activities of aqueous leaf, stem-bark and root extracts of *Khaya senegalensis* on *Trypanosoma evansi*

A. A. Adeiza[1]*, A. Mohammed[2] and M. Mamman[3]

[1]Animal Health and Husbandry Department, College of Agriculture and Animal Science, Ahmadu Bello University, P. M. B. 2134, Mando Road, Kaduna, Nigeria.
[2]Animal Production Technology Department, College of Agriculture and Animal Science, Ahmadu Bello University, P. M. B. 2134, Mando Road, Kaduna, Nigeria.
[3]National Institute for Trypanosomiasis Research, Surame Road, Kaduna, Nigeria.

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The effects of three different parts of *Khaya senegalensis*, commonly used in the traditional treatment of diseases in Northern Nigeria was examined in *Trypanosoma evansi* infected rats. At a dose of 120 mg/ml body weight for 3 consecutive days, the aqueous stem bark extract completely suppressed parasite establishment. The dose cured the experimentally infected rats in 9 days. The aqueous leaf extract showed a weak trypanocidal activity while the stem bark extract showed the most activity that is dose dependent. The results suggested that traditional use of *K. senegalensis* extracts has a pharmacological basis.

Key words: *Khaya senegalensis*, *Trypanosoma evansi*, trypanocidal activity, *in vivo*, parasitaemia.

INTRODUCTION

Trypanosomosis caused by *Trypanosoma evansi* called Surra is transmitted mechanically by biting flies of the genera *Tabanus, Lyperosia, Stomoxys* and *Atylotus* (Brun et al., 1998), displaying typical signs such as fever, anemia, weight loss, oedema, lymphadenopathy and sudden death (Touratier, 1992; Brun et al., 1998; Aquino et al., 1999). It is the most widely distributed of the pathogenic animal trypanosomosis (Lun et al., 1993) and is the most important single cause of economic losses in camel rearing areas, causing morbidity of up to 30.0% and mortality of around 3.0% (Pacholek et al., 2001; Njiru et al., 2002). Studies have shown that the parasite can infect all species of domesticated livestock in Africa beyond the northern most limits of tsetse fly belt and in parts of East Africa, camels are the most important host (Dia et al., 1997), in Asia, Bactrian camel and dromedaries, cattle, buffaloes, horses and pigs (Partoutomo et al., 1994; El-Sawalhy and Seed, 1999; Pacholek et al., 2001). It has also been found in goats and sheep (Luckins, 1998; Onah et al., 1998), cats (Tarello, 2005), dogs (De La Rue et al., 2000) and in a human in India where the parasite was found to have survived and proliferated for at least 5 months (Joshi et al., 2005). In wild animals it has been found in tigers (Manohar et al., 2003), deer, elephants, capybara and jaguars (Brun et al., 1998).

Camels are found in the Northern part of Nigeria, most commonly Borno, Kano, Katsina, Kebbi, Sokoto, Jigawa and Yobe states where they are extensively utilized as sources of meat and transport. Most of the camels found in these areas are traded from neighbouring Niger and Chad Republics (Ochappa, 1988). Surra has attracted international attention in recent years, with the hosting of an international symposium on strategies for research and control of the disease (Obihiro, 1998). Control of trypanosomosis rely principally on chemotherapy using the salts of three compounds, diminazene, an aromatic diamidine; homidium, a phenanthridine and isometha-midium, a phenanthridine-aromatic amidine (ILRAD, 1990; Anene et al., 2001). Unfortunately these existing trypanocides now show serious limitations in that they are either old, toxic and/or expensive (Atougouia and Costa,

*Corresponding author. E-mail: dearadeiza@yahoo.com.

1999), development of resistance by the parasite (Gutteridge, 1985), drug availability in the rural areas, distribution, differences in the epidemiology of the disease response to therapy, and relapses (Fairlamb, 1990; Onyelili and Egwu, 1995; Atougouia and Costa, 1999). There is therefore the need to search for cheaper more comprehensive, easily available and less toxic sources of trypanocides.

Finding healing power in plants is an old idea. People, all over the world, long applied poultices and imbibed infusions of thousands of indigenous plants dating to prehistory. Disease management in Nigeria history also provides evidence of the relationship of plants and medicine (Bannerman et al., 1986; Raghavendra et al., 2006; Ayandele and Adebiyi, 2007). Fulani herdsman, in Nigeria, and others who keep animals as a means of livelihood have been involved in the treatment of animal diseases prior to the onset of modern medicine (Nwude, 1986). Collectively plants produce a remarkably diverse array of over 500,000 low molecular mass natural products also known as secondary metabolites (Fatope et al., 2001), such as alkaloids, glycosides, flavonoids, terpenes and coumarins (Rates, 2001). The exploitation of certain herbs and other plant materials said to be traditionally useful in the control of trypanosomiasis have increased (Asuzu and Chineme, 1990), providing better and cheaper alternatives (Nok et al., 1996; Freiburghans et al., 1996; Nok, 2005). The plant, *Khaya senegalensis* (Desr.) A. Juss (Arbonnier, 2004), is a dry zone mahogany belonging to the family Meliaceae, that is easily recognised by its round evergreen crown of dark shining foliage pinnate leaves and characteristic round capsules (Keay et al., 1989). The stems, barks, leaves and flowers of *K. senegalensis* have been found to contain limonoids. They include phragmalin limonoids named khayanolides D and E, khayanosides, 2, 6-dihydrofussinolide and two mexicanolides named khayanone and 2-hydroxyseneganolide (Olmo et al., 1997; Khalid et al., 2002). Three other mexicanolide limonoids named seneganolide A, 2-hydroxy-seneganolide A and 2-acetoxyseneganolide A have been reported (Abdelgaleil et al., 2004). They have a wide range of biological activities, which includes insect antifeeding and growth-regulating properties and medical activities in humans and animals. They also possess antiviral, antifungal and bactericidal properties (Abdelgaleil and Nakatani, 2003). *K. senegalensis* is highly reputed for its numerous medicinal uses (Arbonnier, 2004), it has been known to be used ethnomedicinally as a remedy for several human and animal ailments (Deeniad and Sadiq, 2002), active *in vitro* against *Trypanosoma brucei brucei* (Wurochekke and Nok, 2004; Atawodi, 2005), *Trypanosoma congolense* (Atawodi et al., 2003; Atawodi, 2005) and helminthiasis (Fajimi and Taiwo, 2005), showing moderate to high efficacy against *Haemonchus, Cooperia, Oesophagostomum* and *Trichostrongylus* spp. (Chiezey et al., 2000). The aqueous extract is taken against diarrhoea, gynaecological disturbances, digestive disorders and nervous confusions (Nacoulma-Ouedraogo, 1996). The stem-bark of the water fractions and the roots, stem-bark and leaves fraction of the ethanol fractions were active on *Staphylococcus aureus, Streptococcus* spp., *Escherichia coli, Pseudomonas aeruginosa, Salmonella* spp. and *Bacillus subtilis* (Kubmarawa et al., 2008). The stem-bark and leaves have been used in the forms of decoction and concoctions for the cure of vermifuge, abortifacient, antipyretic, malarial fever (Arbonnier, 2004). The dried stem-bark is used externally for the treatment of leprosy, dermatomes, sores and ulcers in adults (Le Grand. 1989), this part is also used in dressing ulcers on the back of sheep, camels and horses (Dalziel, 1956). *K. senegalensis* have also been claimed to be helpful in medico-magic (counter poison, insanity), amenorrhea, dysmenorrhea, blennorrhea, scabies, conjunctivitis, chicken pox, small pox, jaundice, lumbago in horses, urticaria, anaemia, colic, phagedenic ulcers, gastritis, sedative for cough, sickle-cell disease, magico-religious (sacred tree) and fodder for cattle (Arbonnier, 2004).

There is currently an absence of alternative drugs for treating trypanosomosis, the search for active substances of natural origin is of major importance since several available drugs including semi-synthetic and synthetic derivatives were originally isolated from natural compounds (Soerjarto, 1996; Cragg et al., 1997). This study was therefore designed to evaluate the anti-trypanosomal efficacy of the aqueous root, leaf and stem-bark extracts of *K. senegalensis* in rats experimentally infected with *T. evansi* and compare the plant part so as to see which is most active.

MATERIALS AND METHODS

Collecting plant materials

The stem-bark and leaves of *K. senegalensis* was collected from Nasarawa state in May, 2006. The plant was identified and authenticated as *K. senegalensis* at the Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria where the voucher specimen number 900181 was deposited.

Plant extraction

The roots, stem-bark and leaves were dried at room temperature for two weeks and they were ground into fine powder using pestle and mortar, sieved to remove excess coarse plant materials. One hundred (100) grams each of the powdered roots, stem-bark and leaves was soaked in1000 ml of distilled water for 24 h at room temperature. It was then filtered through a cheese cloth before further filtration using a Whatman No.1 filter paper. The filtrate was concentrated in hot air oven at 50°C for two days and subsequently air-dried. The brownish extract was pounded into powder using pestle and mortar and stored in air tight containers and kept at 4°C till needed.

Phytochemical screening of extracts

The aqueous root, stem-bark and leaf extracts were screened as described by Harbone (1973), Trease and Evans (1989) and Sofowora (1993).

***Trypanosoma evansi* stock**

T. evansi (Kano strain) was obtained from the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. The parasite were inoculated into 3 rats and then transported to the laboratory at the College of Agriculture and Animal Science, Division for Agricultural Colleges, Ahmadu Bello University, Mando Road, Kaduna, Nigeria. The organisms were maintained separately by serial passages in rats and monitored daily for parasitaemia using the Herbert and Lumsden (1976) method.

Experimental animals

A total of seventy (70) Wistar rats of both sexes used for the study were purchased from the National Institute for Trypanosomiasis Research, Kaduna, Nigeria. They were maintained in clean rat cages in a 12h light/dark cycle with litter changed every week. They were fed, pelleted commercial rat feed (ECWA, Nigeria PLc, Jos, Nigeria), and were watered *ad libitum*. A standard protocol was observed in accordance with the Good Laboratory Practice (GLP) Regulations of the WHO (1992). The animal laboratory care of CCAC (1993) was strictly followed.

***In vivo* trypanocidal activity of *Khaya senegalensis* extracts**

Seventy rats of both sexes were randomly selected and separated into fourteen groups (I - XIV) of 5 rats each and kept in clean rat cages. The fourteen groups of rats (I - XIV) were each inoculated intra-peritoneally with 10^4 *T. evansi* organisms. Day 6 post infection the rats in Group II was given a single dose of diminazene aceturate (Samorenil ® Animal Care) at 3.5 mg/kg body weight intra-peritoneally and were used as the infected/treated control for the three plant extracts under study. The rats in Groups III – VI were treated intra-peritoneally with aqueous stem-bark extracts of *K. senegalensis* at 30, 60, 90 and 120mg/kg body weight respectively for 3 consecutive days, the rats in Groups VII – X were treated intra-peritoneally with aqueous leaf extracts of the same plant at 30, 60, 90 and 120 mg/kg body weight respectively for 3 consecutive days, while the rats in Groups XI - XIV were treated intra-peritoneally with aqueous root extract of the same plant at 30, 60, 90 and 120 mg/kg body weight respectively for 3 consecutive days. The rats in Group I were used as infected/untreated control.

Monitoring of infected and control animals

The experimentally infected and control animals were observed for the development of clinical signs of trypanosomosis including morbidity and mortality. The initial detection of parasitaemia was by the wet film mount using the tail blood (Murray et al., 1983) while the degree of parasitaemia was estimated as described by Herbert and Lumsden (1976). The experiment was terminated after 23 days post-infection when the first mortality was observed in the infected/untreated population.

RESULTS

Phytochemicals detected

The results revealed that the aqueous leaf extract of *K. senegalensis* had high contents of carbohydrates and tannins, while alkaloids, saponins, flavonoids and sugars were moderately present, terpenes and cardiac glycosides were faintly present, while phlobatannins was absent (Table 1). The aqueous stem-bark extract showed high phlobatannins, moderate amounts of carbohydrates, saponins, flavonoids and cardiac glycosides and faint amounts of alkaloids, tannins, terpenes and sugars (Table 1). The aqueous root extract showed high alkaloids, tannin, saponnin, moderate carbohydrates, flavonoids, a faint presence of terpenes, sugars and cardiac glycosides while phlobatannins was present (Table 1).

Anti-trypanosomal effects of the extracts

Following infection of all the respective groups with *T. evansi,* parasitaemia was first detected on day 6 post-infection, each group was given their respective doses intra-peritoneally for 3 days (Figures 1, 2 and 3). The infected untreated rats (Group I) developed clinical trypanosomosis characterised by pyrexia, weakness, rough hair coats and parlour of the mucus membranes and footpads. The clinical signs were progressive with mortalities in the group on day 23 post infection, and the experiment was terminated. Treatment with the extracts (aqueous root, leaf and stem-bark) of *K. senegalensis* suppressed parasite establishment and the manifestation of mild clinical diseases and it was dose dependent, with absolute clearance of the parasite from circulation on day 15 post treatment in those treated with 120 mg/kg (Group X), day 15 in those treated with 90 mg/kg (Group V) and day 9 in the group treated with 120 mg/kg (Group VI) with the different extracts (Figures 1, 2 and 3). Treatment of Group II with diminazene aceturate at 3.5 mg/kg (Samorenil® Animal Care) cleared the parasites from circulation within 24 h and suppressed the manifestation of clinical signs of the disease with no relapse or death within the experimental period.

DISCUSSION

The trypanocidal activities observed in these extracts confirm earlier *in vivo* studies that potential trypanocidal constituents could be present (Asuzu and Chineme, 1990; Youan et al., 1997). The extracts under study reduced the levels of parasitaemia and the severity of the manifested clinical signs and suggest its possible usefulness in the treatment of trypanosomosis. The results suggest that the stem bark extract was generally more efficacious against *T. evansi* than the other two

Table 1. Phytochemical screening *K. senegalensis.*

Plant	Portion of extract	Alkaloids	Carbohydrates	Tannin	Saponin	Flavonoids	Terpenes	Sugars	Cardiac glycosides	Phlobatannins
K. senegalensis	Leaf	++	+++	+++	++	++	+	++	+	-
	Stem-bark	+	++	+	++	++	+	+	++	+++
	Root	+++	++	+++	+++	++	+	+	+	-

+++ = highly present, ++ = moderately present, + = faintly present, - = absent.

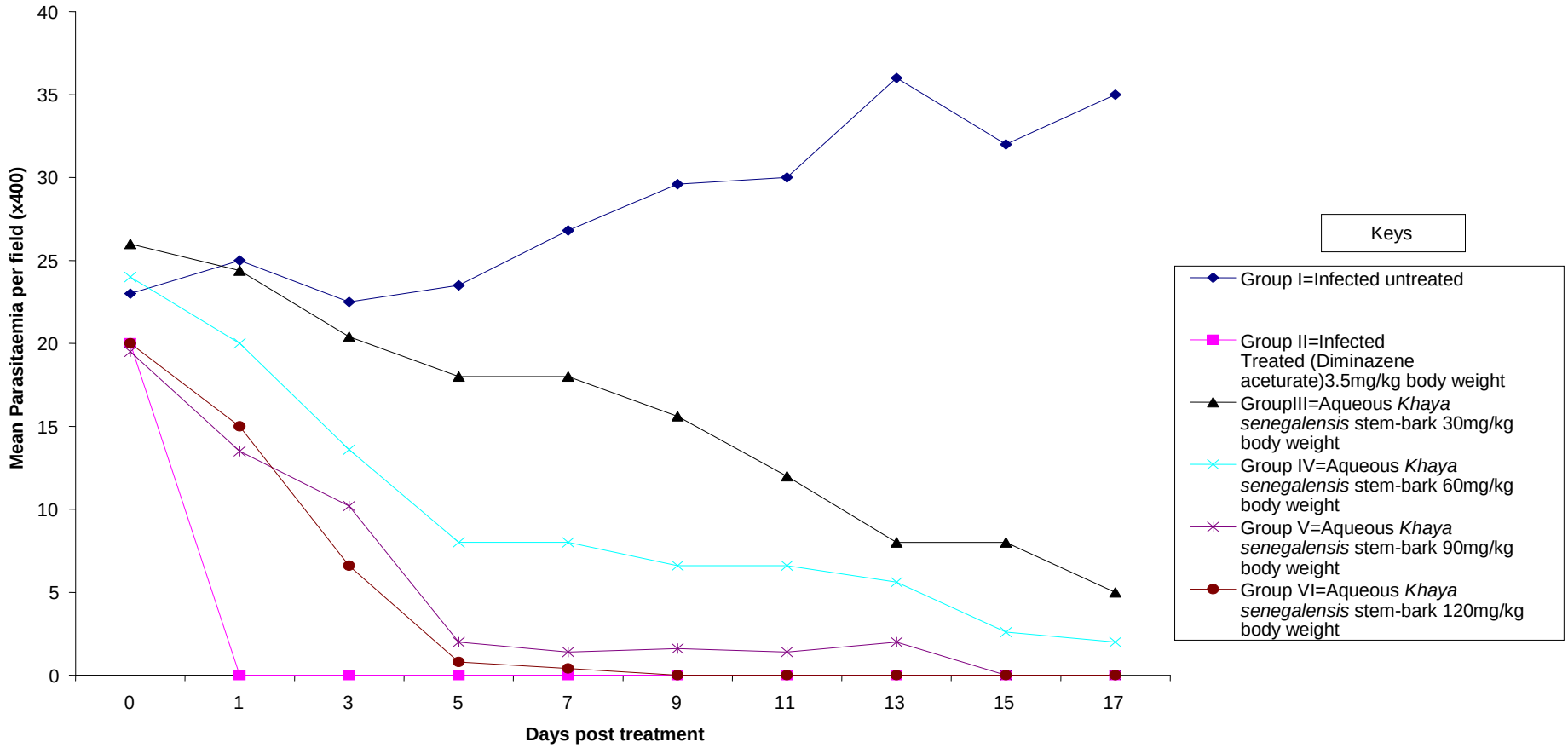

Figure 1. The effect of different doses of aqueous stem bark extractor of *K. senegalensis* on parasite population.

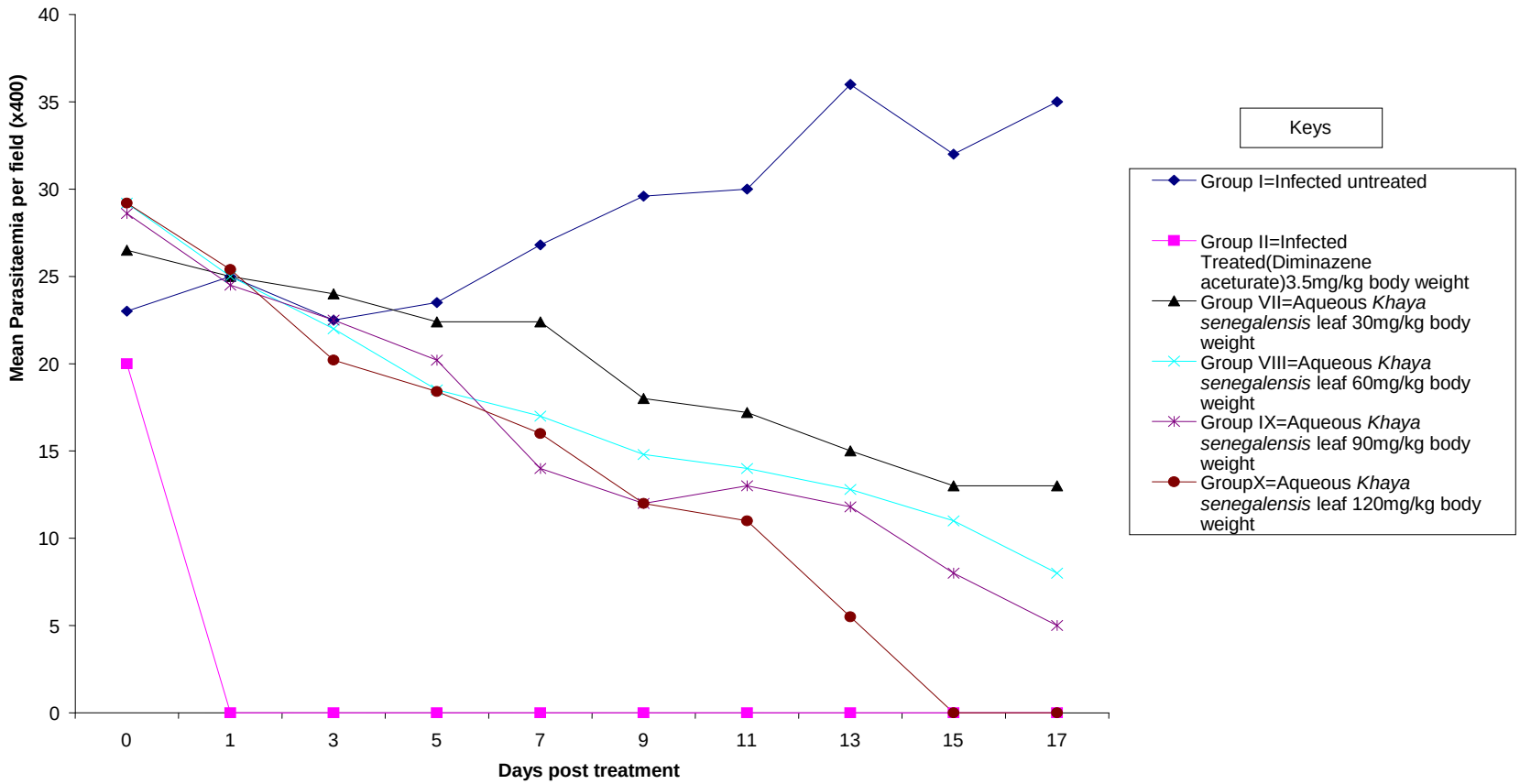

Figure 2. Show the effect of different doses of aqueous leaf extractor of *K. senegalensis* on parasite population.

plant parts studied. The plant parts showed differences in the degree of trypanocidal activity confirming earlier works by Atawodi et al. (2003) that a plant's trypanocidal activity should be taken in context of the plant part and the solvent extract tested. The stem bark extract of *K. senegalensis* reduced the parasite number to zero by day 9 post treatment at the dosage rate of 120 mg/kg body weight. This showed that the action on *T. evansi* was dose-dependent (Figure 1). Clearance of parasites from the blood of some Wistar stock rats showed the efficacy of the extracts. This result is similar to the findings of Nok et al. (1994) and Awotunde (2002), who recorded parasite clearance when animals infected with *T. brucei* were treated with leaf extracts of *Cannabis sativa* and *Ricinus communis*, respectively.

The anti - trypanosomal action of this plant extracts is unknown since the active ingredient(s) were not isolated. Reports in the past have shown many tropical plants contain constituents that are clinically efficacious against many protozoal diseases (Gbile and Adesina, 1987; Le Grand, 1989) and that the trypanocidal activity of certain plant extracts are due to the alkaloids and other constituents present (Oliver-Bever, 1986) or the lavonoids (Taurus et al., 2002).

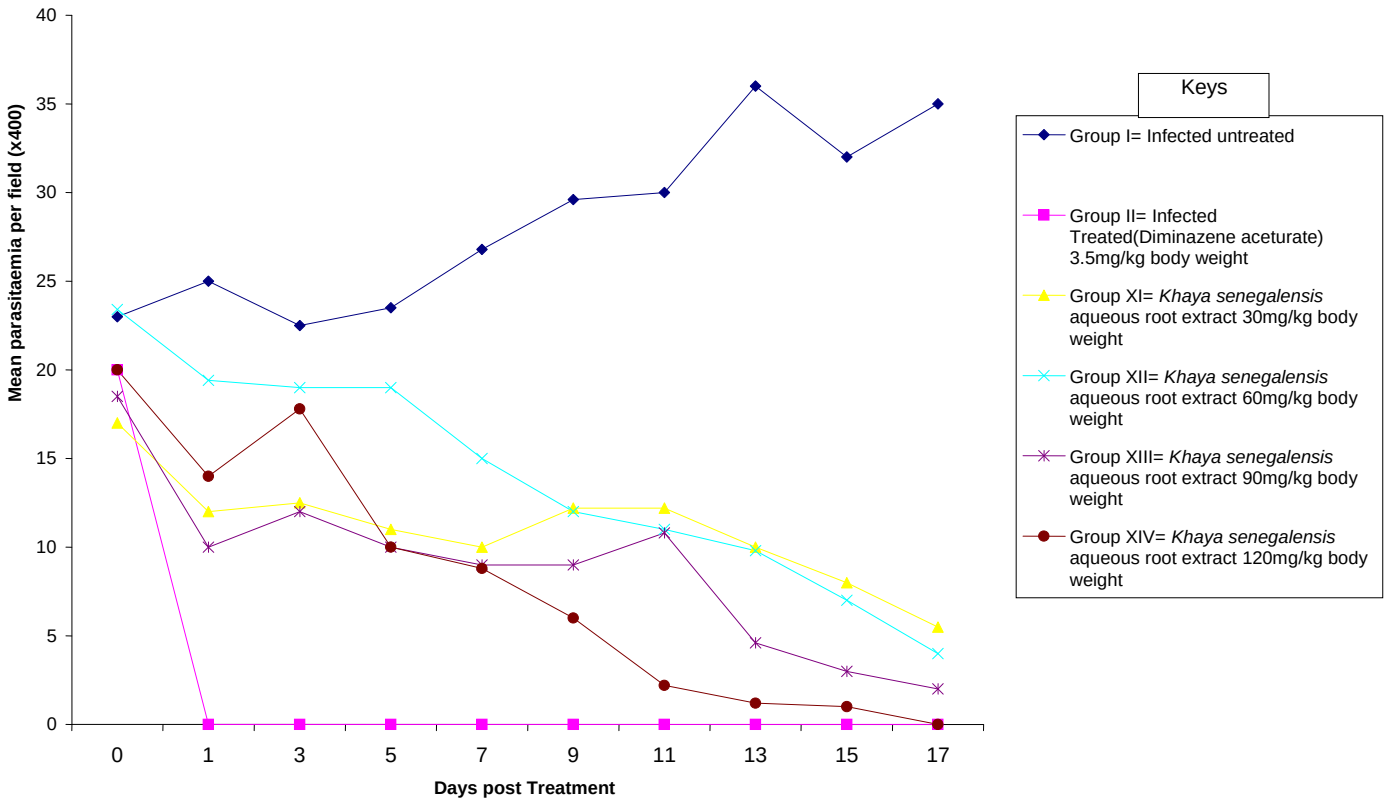

Figure 3. Show the effect of different doses of aqueous root extractor of *K. senegalensis* on parasite population.

Conclusion

Commercially produced trypanocidal drugs like Suramin and Pentamidine all have undesirable side effects (Fairlamb et al., 1977). The use and misuse of drugs has contributed to the development of drug resistance in trypanosomes. An urgent need arises therefore to develop newer drugs to counter resistance. The demonstration of activity in this study suggests optimism and it makes this work significant, because up till now this is the first report of activity against *T. evansi* in extracts involving *K. senegalensis.* The tradomedicinal use of *K. senegalensis* has a pharmacological basis, as it is a promising source for active compounds against trypanosomosis.

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