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Effects of treatment with fraction IV extract of *Ximenia americana* on the survival rate, packed cell volume and total plasma proteins of *Trypanosoma congolense* infected mice

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The effect of treatment with fraction IV extract of *Ximenia americana* on the survival rate, packed cell volume and total plasma proteins of *Trypanosoma congolense* infected mice was investigated. Following infection with 10^4 *T. congolense*, the mice were treated with 25 mg/kg body weight fraction IV extract and 20 mg/kg body weight Diminazene aceturate, respectively, while a group was left untreated. Parasitaemia, packed cell volume, total plasma proteins and survival rate was determined. The results showed fraction IV portion of *X. americana* significantly ($P < 0.05$) enhanced the survival rate, packed cell volume of the treated animals, and also reduced the levels of parasite and total plasma proteins compared to the infected untreated groups. Fraction IV portion of *X. americana* could act as an adjunct therapeutic agent in the treatment of trypanosomosis.

Key words: *Ximenia americana*, *Trypanosoma congolense*, survival rate, total plasma proteins.

INTRODUCTION

African trypanosomes are protozoan parasites that cause sleeping sickness in humans and nagana in domesticated animals. It remains one of the most neglected human disease in Africa (Maudlin et al., 2004) and is of major animal health and economic impact in sub-saharan Africa (Mbuthia et al., 2011). The control of human and animal trypanosomosis is based on a limited number of compounds many of which are chemically related and have been used for more than 60 years (Leach and Roberts, 1981; Gutteridge, 1985; Kinabo, 1993; Anene et al., 2001; Maudlin et al., 2004). The repeated use of trypanocidal drugs in control of animal trypanosomosis has led to the development of drug resistant trypanosome population (Geerts and Holmes, 1998; Geerts et al., 2001; De Koning, 2001). Renewed interest in traditional pharmacopeias is increasing worldwide most especially among African people who are becoming reliant on herbal medicines for their health care needs. This is because medicinal plants are more accessible and affordable (Mander, 1998). Traditional knowledge of plants could help researchers target plants that are medicinally useful (Cox and Balick, 1994).

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Ximenia americana is a medicinal plant that has been reported to have shown several biological activities such as antimicrobial, antifungal, anticancer, antineoplastic, antitrypanosomal, antirheumatic, antioxidant, analgesic, moluscicide, pesticidal and also having hepatic and heamatological effects (Asres et al., 2001; Ogunleye et al., 2003; Elnima, 2003; Geyid et al., 2005; Voss et al., 2006; James et al., 2007; Maikai et al., 2009; Siddaiah et al., 2009; Soro et al., 2009; Maikai et al., 2010). The plant has been reported to contain biologically active compounds such as saponins, flavonoids, tannins, terpenoids, sterols, quinones, alkaloids, cyanogenetic glycosides, cardiac glycosides, saponosides, isoprenoids, fatty acids, triterpenes, sesquiternes and carbohydrates in the form of sugars and soluble starch (Ogunleye et al., 2003; Geyid et al., 2005; James et al., 2007; Araújo et al., 2008, 2009; Soro et al., 2009; Maikai et al., 2009; Maikai et al., 2010).

Therefore, the aim this study was to investigate if fraction IV extracts of *X. Americana* could extend the survival rate and reduce parasitaemia, total plasma proteins and modulate packed cell volume of *Trypanosoma congolense* infected mice.

MATERIALS AND METHODS

Plant sample

The bark of *X. americana* was collected from Afaka village 35 km to Kaduna (11°10' N, 7°38' E) and taken to the Department of Biological Sciences, Ahmadu Bello University Zaria for identification and confirmation with the voucher No. 1612. The voucher specimen (No. 1612) was deposited in the herbarium. The stem bark was dried at room temperature before crushing it into powder, then stored in air tight container and kept at 4°C until needed.

Extraction of plant material

Two hundred (200 gm) grams of the stem bark powder was weighed into a thimble and then transferred into a Soxhlet extractor and extracted sequentially with petroleum ether, methanol and water. The extracts were individually collected after each extraction and concentrated using a rotary evaporator (Buchi, Switzerland) at 50°C under reduced pressure and then dried. The solvent free extracts were then weighed and stored in brown bottles at 4°C until use. The aqueous extract was used for column chromatography after determining activity against trypanosomes (Maikai et al., 2008).

Partial purification of aqueous crude extracts (Column chromatography)

The aqueous crude extract of stem bark of *X. americana* was partially purified using column chromatography. Briefly, slurry was prepared by shaking 120 g of silica gel (Qualikems, 60 to 120 mesh powder) with 200 ml of water and methanol in the ratio of (1:1) and then packed in a column (1.5 × 30) at a flow rate of 0.2 ml /min. The column was loaded with 20 ml of the aqueous extract that had been previously adsorbed from distilled water on 4 g of the silica gel, and then eluted with four solvent mixtures (ethyl acetate/methanol 19:1 (Fraction I); benzene/methanol 19:1 (Fraction II); acetic acid/ methanol 1:1 (Fraction III); water/methanol 1:1 (Fraction IV) in order of increasing polarity. The eluents (Fraction I, II, III and IV) were collected in separate beakers and dried at 50°C using a water bath. The fractions were tested for antitrypanosomal activity and fraction IV which had the highest *in vitro* activity (values not shown). The dried fractions were kept at 4°C for further experiments.

Experimental animals

A total of forty three healthy white Swiss albino mice of 6 to 8 weeks old and weighing between 28 to 32 g were obtained from the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University Zaria. The animals were housed in clean plastic cages in a 12 h light/dark cycle, and fed with diet made from chick grower's mash (Pfizer) and mixed with groundnut cake and flour. Water was given *ad libitum* and the cages were cleaned every week, throughout the duration of the work. A standard protocol was observed in accordance with the Good Laboratory Practice (GLP) Regulations of the WHO (1998). The animal Laboratory care was strictly followed (CCAC, 1993). The rodent protocols and procedures used in this study were approved by the animal welfare committee of the college.

Trypanosome (Parasite)

T. congolense NITR stabilate 212 (Federe strain) was obtained from the Nigerian Institute of Trypanosomosis Research, Vom Plateau State Nigeria and passaged into rat which was subsequently maintained by passages in mice.

Trypanocidal drug

Diminal® (Diminazene aceturate) (Eagle chemical company limited, Ikeja Lagos Nigeria) a commercial drug for the treatment of animal trypanosomosis was used as a standard reference and as positive control. A concentration of 20 mg/kg body weight dose was administered to control animals according to manufacturer's recommendation.

Experimental design

Forty (40) experimental mice were randomly grouped into four groups (I to IV) of ten animals each. Group I was uninfected and untreated served as control. Group II to IV were inoculated with 10^4 trypanosomes. When parasites were detected, group II and IV were treated with a single dose of 20 mg/kg body weight of Diminal® (Diminazene aceturate) and 25 mg/kg body weight of fraction IV portions of *X. americana* (for five days) intraperitoneally respectively, while group III were left untreated. Parasitaemia, Packed cell volume (PCV), survival rate and total plasma proteins were determined. For the determination of PCV, blood was collected in heparinized capillary tubes from their tail at the end of treatment in tubes which were sealed at one end with Crystaseal® and centrifuged at 2000 g for 5 min in micro-haematocrit centrifuge. The PCV was then read using a Hawskley haematocrit reader (Barbara, 1980). Total plasma proteins concentation was determined using the Goldberg refractometer method (Schalm et. al., 1978).

Parasitaemia

Parasitaemia was monitored in the blood obtained from the tail pre-sterilized with alcohol. Detection of parasites was by wet film and

buffy coat methods. The number of parasites was determined microscopically at ×400 magnification using an Olympus Microscope (Japan). The method of Herbert and Lumsden (1976) was used to determine parasitaemia. Briefly, the method involves microscopic counting of parasites per field of the blood. Logarithm values of these counts were obtained and matched with the table of Herbert and Lumsden (1976), which was then converted to antilog to provide absolute number of trypanosomes per ml of blood. Negative samples were further examined by the more sensitive buffy coat technique as described by (Murray et al., 1977).

Toxicity studies

A total of three (3) mice were administered with 25 mg/kg body weight each of fraction IV extract of *X. americana* to check for acute toxicity. General toxicity signs were evaluated by observing the clinical signs. If none of the mice died then the extract was considered not acutely toxic.

Statistical analysis

Data obtained were expressed as mean ± Standard deviation and subjected to one-way analysis of variance (ANOVA). The log rank test was used to examine the null hypothesis that the survival curves were identical. A p-value of < 0.05 was considered to be statistically significant.

RESULTS

Effect of fraction IV on parasite population

Parasites were detected after three days post infection in the infected groups (Figure 1). There was a progressive rise in parasite population in group III (infected, untreated) peaking at day 14 with subsequent death (Figure 1). A significant ($P < 0.05$) reduction in parasite population was recorded in group II and group IV treated with 20 mg/kg body weight Diminazene aceturate and 25 mg/kg body weight fraction IV respectively.

Effect of treatment on packed cell volume

There was a decrease in packed cell volume for all the infected animals which coincided with the appearance of the parasites day 7 to 10 post infection (Figure 2) compared to group I (uninfected, untreated). Treatment with 25 mg/kg body weight fraction IV (group IV) and 20 mg /kg body weight Diminazene aceturate (group II) improved the value of the packed cell volume significantly compared to group III (infected untreated) (Figure 2). However, the PCV values did not achieve the pre-infection values.

Effect of fraction IV on survival rate

The effect of treatment with fraction IV portion of *X. americana* on the survival rate of mice experimentally infected with *T. congolense* is shown in Figure 3. The infected group treated with fraction IV (Group IV) and Diminazene aceturate (Group II) showed significant ($P < 0.05$) survival rates respectively when compared to the infected untreated group (Group III). However, treatment with Diminazene aceturate had a longer survival rate than fraction IV treated group.

Effect of fraction IV on total plasma proteins

The effect of treatment on total plasma proteins is shown in Figure 4. There was a steady rise in total plasma proteins for (group III) infected group (Figure 4), which peaked with the population of parasites day 14 post infection (dpi). Treatment with 25 mg/kg b.w. fraction IV extract of *X. americana* and 20 mg/kg b.w. Diminazene aceturate was significantly ($P < 0.05$) reduce than the value of total plasma proteins by day 14 post treatment compared to the group infected and untreated (group III) which steadily increased till the animals died.

Toxicity effect

The animals did not show any clinical signs of toxicity in the form of anorexia, dehydration, starry hair coat, depression, difficulty in respiration, coma or death as a result of administering 25 mg/kg b.w. of the extract. Thus, suggesting the marginal safety of the extract.

DISCUSSION

X. americana has been previously reported to have *in vitro* anti trypanosomal activity (Maikai et al., 2008, 2010), experimental infection of mice with blood stream trypanosomes results in pathological conditions which could result in the death of the animals if not treated. This study earlier reported that *X. americana* had no toxic effect in mice (Maikai et al., 2008). The study toxicity result of fraction IV extract did not show any clinical signs of toxicity, which was considered marginally safe as the extract was tested at a therapeutic dosage of 25 mg/kg b.w. The mice infected with *T. congolense* showed a decrease in PCV which was associated with the first wave of parasitaemia in the blood. The result corroborates with the report of Duncan et al. (1994) who reported that infection with *T. congolense* results in a significant reduction in the PCV. PCV reduction and anemia are the common cardinal features in the pathogenesis of trypanosome infection contributing to the morbidity and mortality thus curtailing longevity (Kagira et al., 2006; Karori et al., 2008). Treatment of the infected mice with fraction IV and Diminazene aceturate however, modulated the values of the PCV of the animals which corroborates earlier report of Mbaya et al. (2010) that

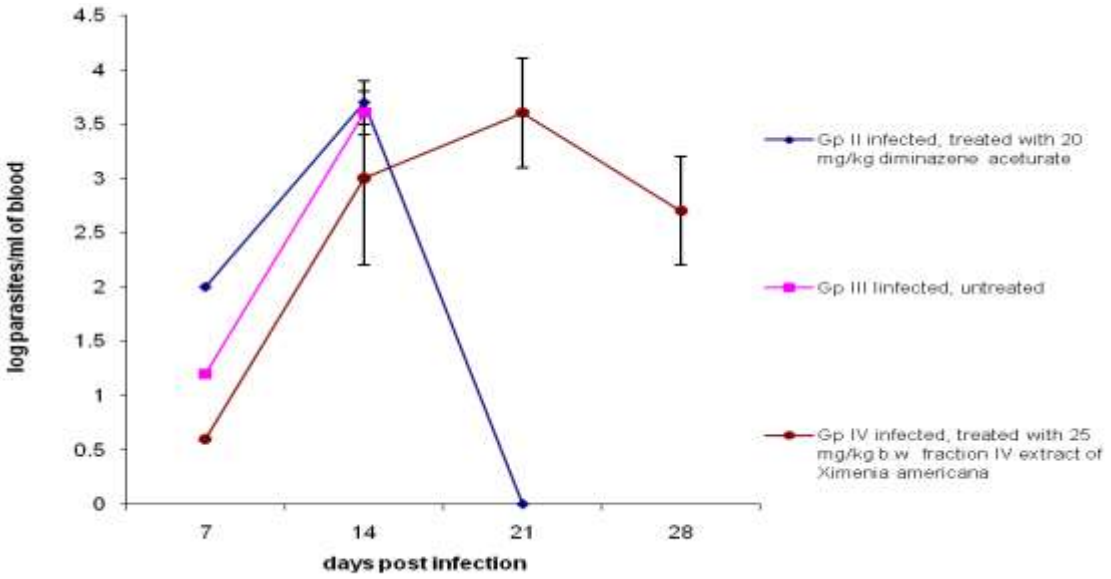

Figure 1. Effect of treatment on parasite population of mice experimentally infected with *T. congolense* (geometric mean and standard error of parasitaemia).

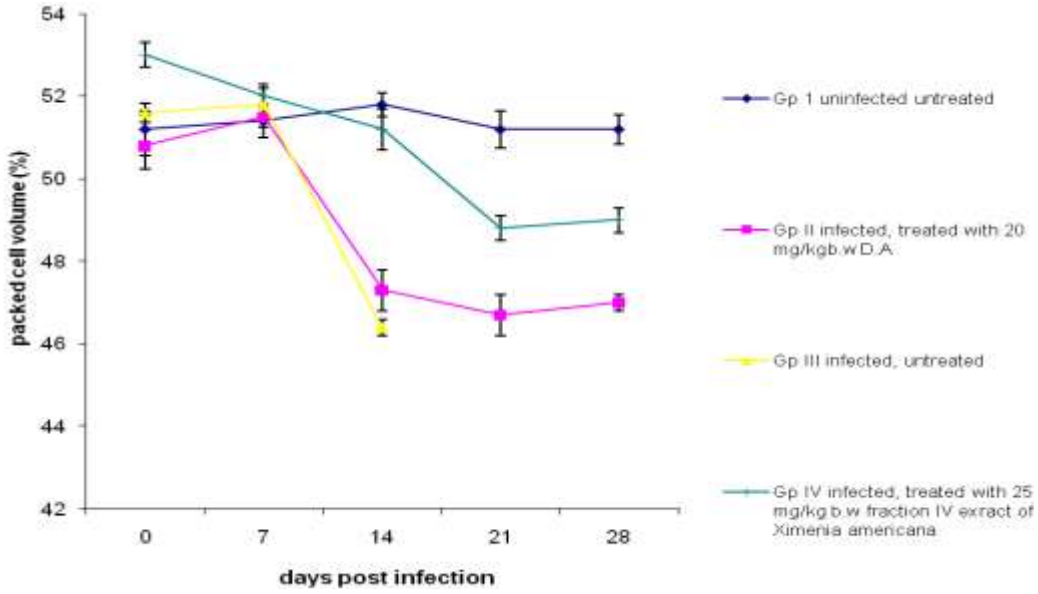

Figure 2. Effect of treatment on packed cell volume of mice experimentally infected with *T. congolense* (geometric mean and standard error of packed cell volume).

that mice infected with *T. brucei* when treated with *Azadirachta indica* had modulated values of PVC. Treatment with fraction IV portion of *X. americana* was not able to eliminate the parasites however, reduced the level of parasitaemia and thus extended the survival rate of the infected animals compared to the untreated group. The result of this study coincides with the study of Chen et al. (2004) and Mbuthia et al. (2011) who reported prolongation of survival rate in mice treated with tea extracts. They attributed it to the ability of tea flavonoids to counter the trypanosomosis induced inflammatory reaction and aiding antioxidant defense system. It has been reported that severity of trypanosomosis influences the total proteins levels of infected animals (Anosa, 1988; Ogunsanmi and Taiwo, 2001). The total plasma proteins were significantly ($P < 0.05$) higher in the infected

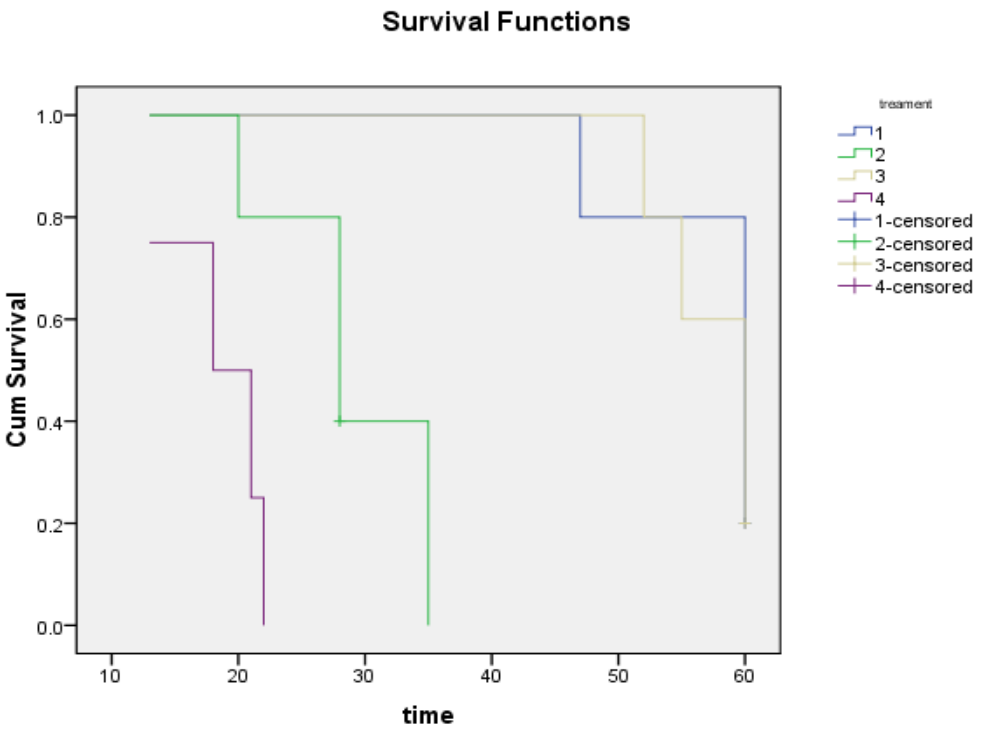

Figure 3. Kaplan meier survival curves comparing survival rates in *T. congolense* infected mice treated with fraction IV extract of *X. americana*. Key: 1 = uninfected untreated control: 2 = treated with 25 mg/kg b.wt fraction IV: 3 = treated with 20 mg/kg b.wt diminazene aceturate: 4 = infected untreated.

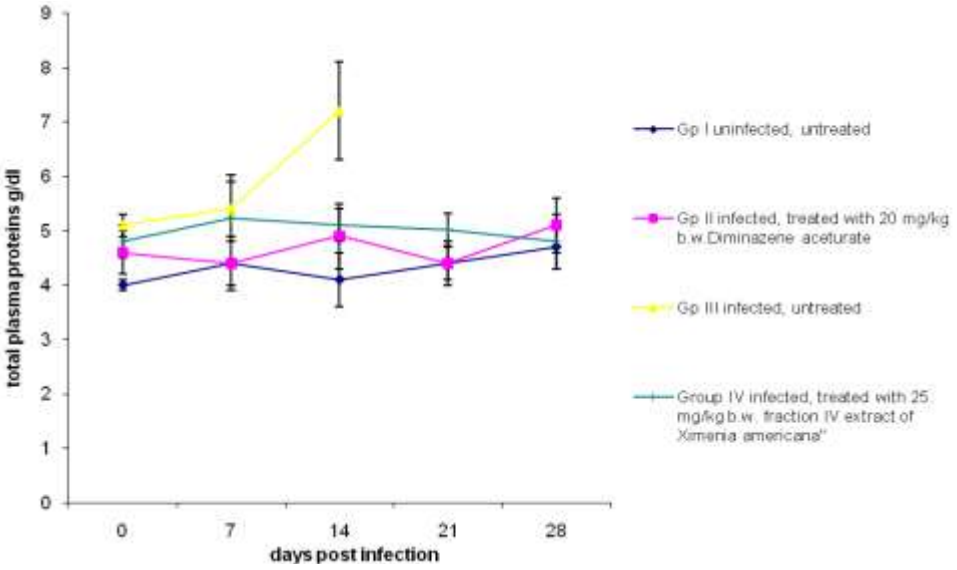

Figure 4. Effect of treatment on total plasma proteins of mice experimentally infected with *T. congolense* (geometric mean and standard error of Total plasma protein).

untreated groups when compared to the uninfected controls. The results of this study are similar with the results of Ogunsanmi and Taiwo (2001), who reported significant increases in total plasma proteins in *T.*

congolense infected sheep and goats.

Orhue et al. (2005), also reported significant increases in total plasma proteins and globulin in *T. brucei* infected rabbits. The increases in serum total plasma proteins could be due to increase release of tissue specific enzymes, and other intracellular proteins secondary to parasite induced cell membrane disruption, or increase in proteins might be due to increase in circulating antibodies as a result of the infection. It is also likely that the increase in total plasma proteins may be due to increase in mass of parasite proteins as a result of growing infection or possibly increases in parasite derived intracellular enzymes and proteins as the parasites are lysed by the host immune system. Treatment with fraction IV portion of *X. americana* led to reduction in the levels of total plasma proteins of infected animals when compared to the infected untreated group. The mechanism of action is unclear at this stage of investigation. Fraction IV extracts could be composed of a mixture of complex substances; it is not possible at this stage to identify the compounds responsible for the observed activity, but one is tempted to speculate that the antioxidants might play a protective role in countering the radicals generated as a result of the infection.

Conclusion

The study revealed that fraction IV extract of *X. americana* modulated the PCV value, prolonged the survival rate and reduced the total plasma levels of the treated animals. The results are promising, while research is ongoing to identify the type of compound responsible.

Conflicts of interest

Authors have none to declare.

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