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EFFECT OF FRACTION IV PORTION OF *Ximenia americana* STEM BARK ON *Trypanosoma congolense* - INDUCED SERUM ENZYMES CHANGES IN RATS

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ABSTRACT

The current study investigates the effect of fraction IV portion of *Ximenia americana* stem bark on *Trypanosoma congolense* induced serum enzymes changes in rats. Following infection with trypanosomes, the rats were monitored for levels of some serum enzymes. The results revealed that there was significant ($P<0.05$) elevation of serum enzymes Aspartate AminoTransferase (AST), Alanine AminoTransferase (ALT), Alkaline Phosphatase (AP), Gamma GlutamylTransferase (GGT) and Creatine Kinase (CK) in the infected animals. Treatment with 25 mg/Kg body weight fraction IV portion of *Ximenia americana* led to significant ($P<0.05$) reduction in levels of the enzymes. It is concluded that treatment with fraction IV portion of *Ximenia americana* was able to modulate the effect of trypanosomiasis induced serum enzyme levels of the infected rats.

Key words: Fraction IV, serum enzymes, *Trypanosoma congolense*, *Ximenia americana*

INTRODUCTION

African animal trypanosomiasis (AAT) is caused by *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei*. Trypanosomiasis is a debilitating disease of man, domestic and wild animals which is often characterized by anemia, reduced productivity and high mortality (Anosa, 1983; Allam *et al.*, 2011; Mbuthia *et al.*, 2011). Pathogenesis of trypanosomiasis is characterized by anaemia, leucopenia, thrombocytopenia, inflammatory changes observed during trypanosomiasis are caused by tissue damage stimulated by the presence of the parasites in the body, plasma biochemical changes, and various other lesions in some tissues and organs (Anosa and Kaneko, 1983; Igbokwe, 1995; Maudlin *et al.*, 2004; Abenga, 2014). The pathogenesis of trypanosomiasis disease has been extensively studied (Takeya *et al.*, 1987; Anosa, 1988; Maudlin *et al.*, 2004; Biryomumaisho and Katunguka-Rwakishaya, 20007; Serem *et al.*, 2013). Serum biochemical changes during trypanosomiasis have been extensively reviewed (Anosa, 1988; Farem and Ekanem, 2011; Abenga, 2014). The level of serum enzyme in the plasma frequently correlates with the extent of tissue damage by the parasites and the degree of elevation of these enzyme activities in plasma is determined by the strain of trypanosome and health status of the host (Anosa, 1988b).

Ximenia americana is a plant used in traditional medicine for the treatment of malaria, leproutic ulcers and infectious diseases (Ogunleye and Ibitoye., 2003; Arbonnier, 2004; James *et al.*, 2007; Maikai *et al.*, 2009). We have previously reported the trypanocidal activity of *Ximenia americana* aqueous extract against the bloodstream form of *Trypanosoma congolense* (Maikai *et al.*, 2008; 2009; 2014).

The current study was carried out to determine the effect of fraction IV portion of *Ximenia americana* stem bark on *Trypanosoma congolense* induced serum enzymes changes in rats.

MATERIALS AND METHODS

Experimental animals

Twenty five (25) adult male Wistar rats, weighing between 240-310 g were used for the experiment. They 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 of (CCAC, 1993) was strictly followed.

Source of Trypanosome

The trypanosome used for this study was *Trypanosoma congolense* (Federe strain) which was obtained from the Nigerian Institute of Trypanosomosis Research, Vom Plateau State Nigeria and passaged into rat. This was subsequently maintained by passages in rats.

Plant sample

The bark of *Ximenia americana* was collected from Afaka village 35 km to Kaduna (11° 10' N, 7° 38' E) and taken to 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 freeze dried. The solvent free extracts were then weighed and stored in brown bottles at 4°C until use.

Partial purification of aqueous crude extracts (Column chromatography)

The aqueous crude extract was partially purified using column chromatography. Briefly, slurry was prepared by shaking 120 g of silica gel (Qualikems, 60-120 mesh powder) with 200 ml of water and methanol in the ratio of (1:1) and then packed in a column (1.5X30). 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; benzene/methanol 19:1; acetic acid/methanol 1:1; water/methanol 1:1) in order of increasing polarity. The eluents were collected in separate beakers and dried at 50°C using a water bath. The dried fractions were kept at 4°C for use in *in vitro* experiments. The fractions were tested for antitrypanosomal activity and fraction IV which had the highest activity was used for the subsequent experiment.

Infection

Three apparently healthy rats were infected with *T. congolense* at peak parasitemia (10⁸ parasite/ml of blood), (Herbert and Lumsden, 1976) the animals were sacrificed and blood immediately collected in heparinized tubes containing three (3) ml of phosphate buffer saline glucose. This was subsequently used to infect the experimental animals.

Experimental design

A total of twenty five rats were randomly divided into five groups (I, II, III, IV and V) of five animals each. Group II to IV were infected with 10³ *T. congolense* each as described by Adeyemi et al., (2012). When parasites were detected in the blood, group III and IV were given intraperitoneally, 25 mg / kg body weight of fraction IV extracts (for 3 days) and 3.5 mg / kg body weight Diminal[®] respectively. Group II were left untreated while group I were uninfected and untreated. Group V were uninfected but treated intraperitoneally, 25 mg / kg body weight of fraction IV extracts (for 3 days). Blood samples were taken from the tail vein on day 7, 14, 21, after treatment, and 28 days post infection. The blood samples were taken for serum enzyme studies.

Determination of Serum enzymes

Blood samples were collected from the rats into test tubes and allowed to coagulate at room temperature for 1 hour before centrifuging at 5000g for 5 minutes; serum was collected and stored at - 20°C. The serum was subsequently used for the evaluation of serum enzymes: Alanine AminoTransferase (ALT), Aspartate AminoTransferase (AST), Alkaline Phosphatase (AP), Gamma-Glutamyl Transferase (GGT) and Creatine Kinase (CK) using an auto-analyzer (Bayer[®] Clinical Chemistry Analyzer, Germany).

Statistical analysis

Values obtained are expressed as mean ± SEM. Data were subjected to one-way analysis of variance (ANOVA); followed by Tukey's multiple comparison *post-hoc* test, using GraphPad Prism version 4.0 for windows (GraphPad Software, San Diego, California, USA). Values of *P* < 0.05 were considered significant.

RESULTS

The results of experimental infection with *T. congolense* showed changes in levels of serum enzymes Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT), Alkaline Phosphatase (AP), Gamma Glutamyl Transferase (GGT) and Creatinine Kinase (CK) (Figure 1,2,3,4 and 5) respectively. The enzyme levels in the control animals (uninfected and untreated) group I were within the normal ranges. Group II infected and untreated showed significant (*P* < 0.05) elevation in levels of the serum enzymes AST, ALT, AP, GGT and CK with increasing parasite population, when compared to the other treated groups III and IV. The rats in group II however, died before the 28th day post infection as a result of massive parasitemia. There was however, no significant (*P* > 0.05) difference between the treated groups III and IV. Treatment with fraction IV, group V did not significantly (*P* > 0.05) increase the levels of the enzymes AST, ALT, AP, GGT and CK (Fig. 1,2,3,4 and 5) from the pre-treatment values.

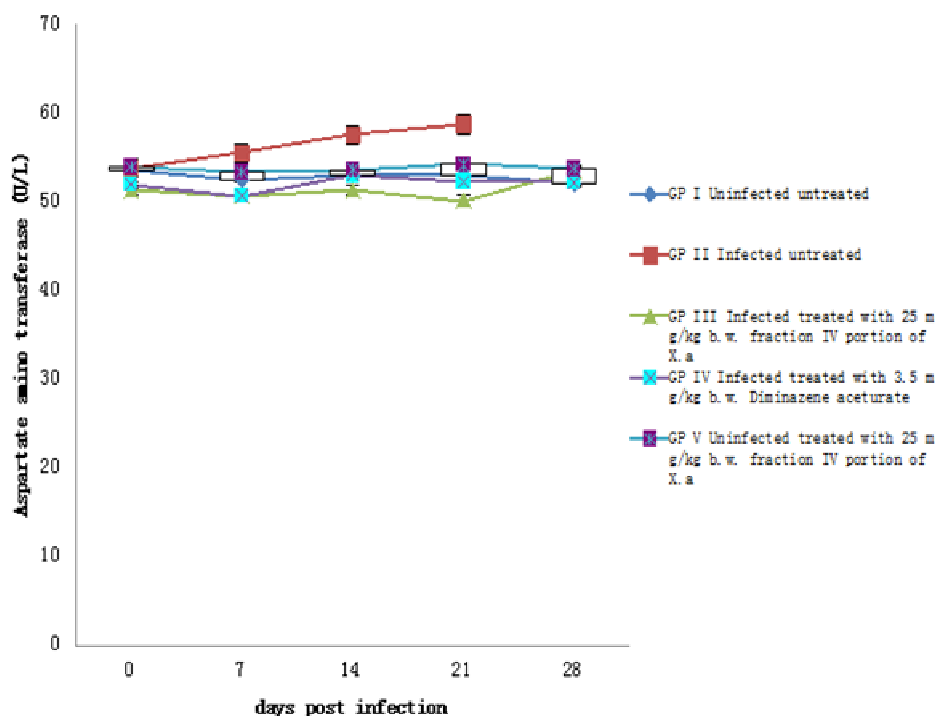


Figure 1. Effect of treatment with 25 mg/kg b.w. fraction IV portion of *Ximenia americana* on serum Aspartate Amino Transferase *T. congolense* infected rats

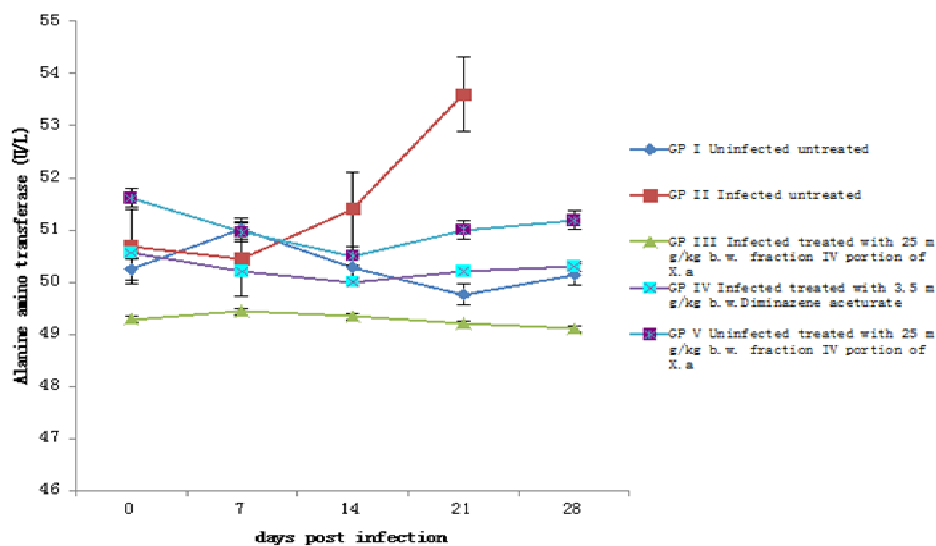


Figure 2. Effect of treatment with 25 mg/kg b.w. fraction IV portion of *Ximenia americana* on serum Alanine Amino Transferase in *T. congolense* infected rats

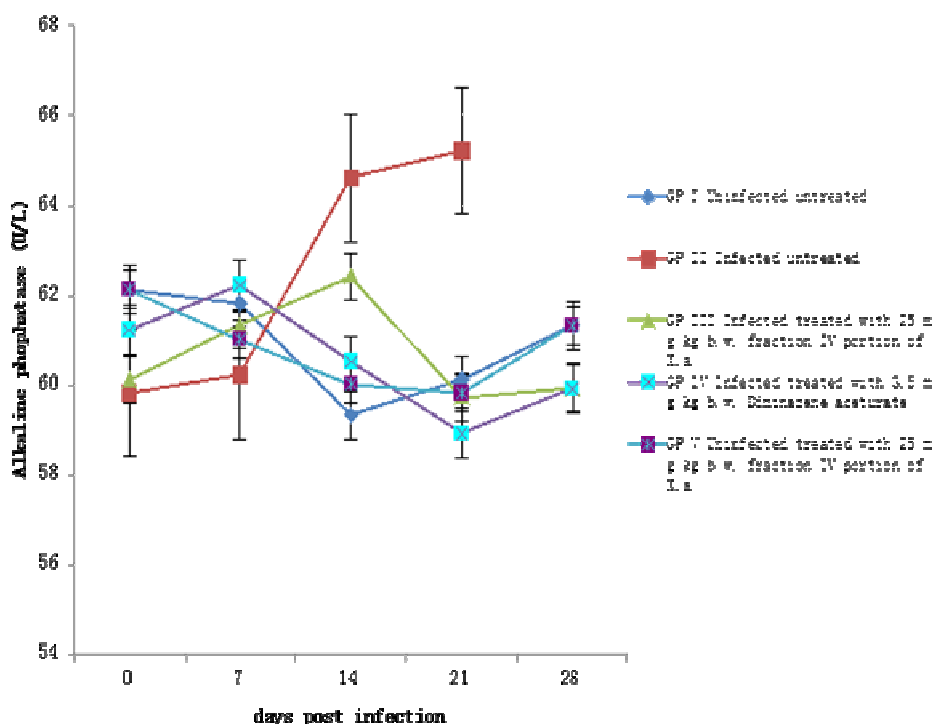


Figure 3. Effect of treatment with 25 mg/kg b.w. fraction IV portion of *Ximenia americana* on serum Alkaline Phosphatase in *T. congolense* infected rats

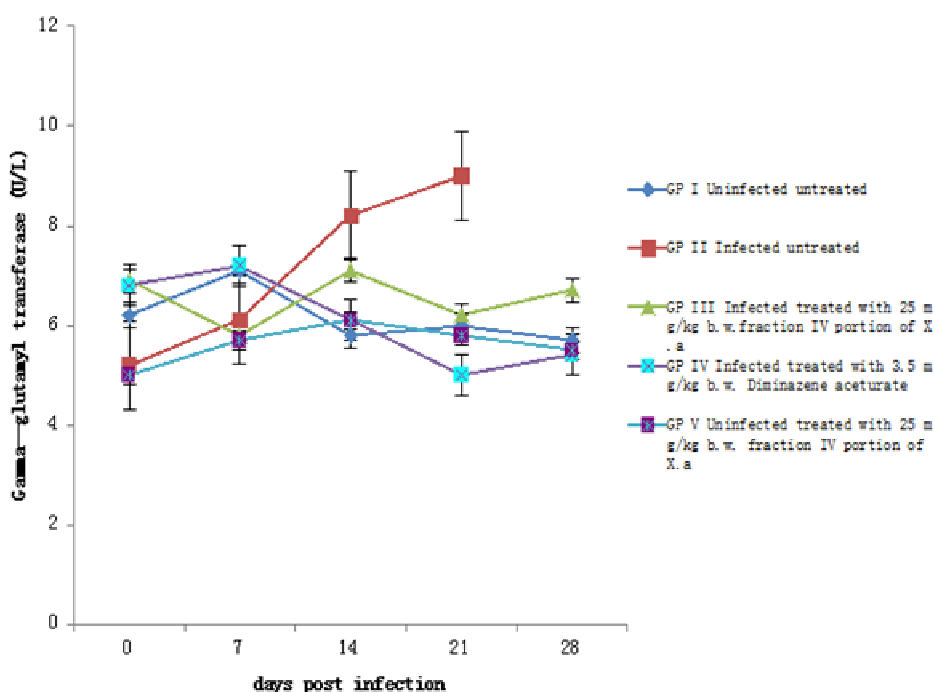


Figure 4. Effect of treatment with 25 mg/kg b.w. fraction IV portion of *Ximenia americana* on serum Gamma-Glutamyl Transferase in *T. congolense* infected rats

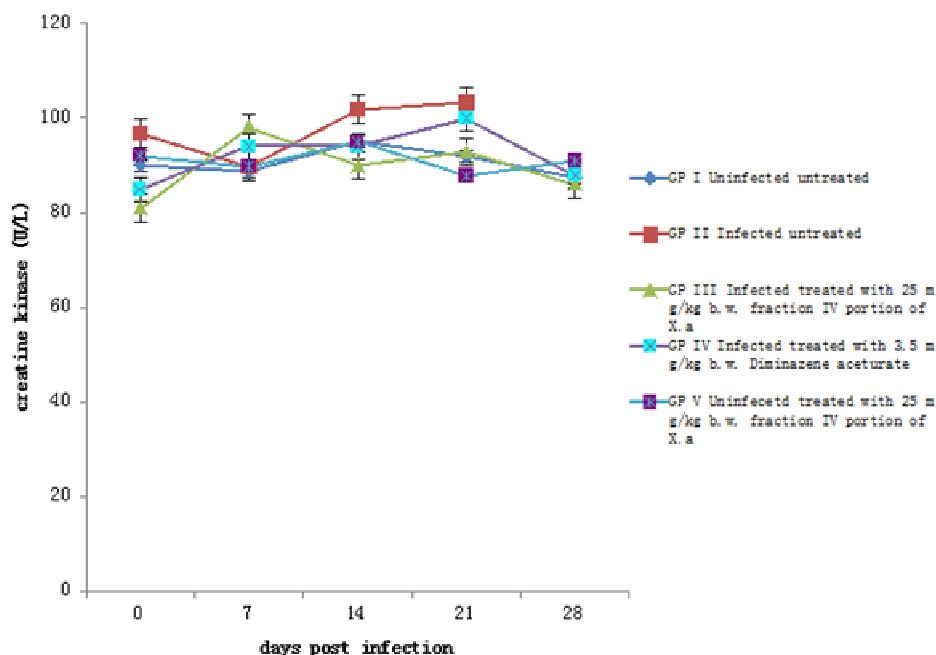


Figure 5: Effect of treatment with 25 mg/kg b.w. fraction IV portion of *Ximenia americana* on serum Creatine Kinase in *T. congolense* infected rats

DISCUSSION

The emergence and spread of resistance to antitrypanocidal drugs has highlighted the need for the discovery and development of novel antitrypanocidal leads. The evaluation of serum enzymes have been reported to be of diagnostic value, and can serve as early warning signs for certain diseased conditions (Tietz, 1999; Awobode, 2006; Ekanem *et al.*, 2006; Shittu *et al.*, 2013).

The elevation of the activities of AST, ALT, AP, GGT, and CK in trypanosome- infected rats was simultaneous with establishment of infection by the trypanosomes proliferating rapidly in population in the host (Poltera, 1985; Kennedy, 2004; Allams *et al.*, 2011). This result corroborates earlier reports of (Chaudhary and Iqbal, 2000; Ogunsanmi and Taiwo., 2001; Orhue and Nwanze, 2004; Akpa *et al.*, 2008; Allams *et al.*, 2011; Nwoha *et al.*, 2013; Shittu *et al.*, 2013). Our study also agrees with similar observations in goats infected with *T. congolense* (Adah *et al.*, 1992). Several factors have been reported to influence the nature and severity of trypanosomiasis in the infected host (Anosa, 1988a) such include strain of parasite, age of animal and nutritional status. This suggests that infection by trypanosomes had led to tissue break down and inflammation in the host particularly of the liver, heart, muscle and kidney which resulted in the leakage of these enzymes from their intracellular stores into the plasma thus elevating their levels. AST is found mostly in cell organelles and its activity raises when there is a considerable damage to the heart, kidneys, skeletal muscle, liver and hemolytic anemia (Tietz, 1999; Nwoha *et al.*, 2013; Shittu *et al.*, 2013; Ajakaiye *et al.*, 2014). ALT is a specific liver enzyme found in the cell cytoplasm and its elevation is associated with cell membrane damage (Tietz, 1999; Yakubu *et al.*, 2005;

Nwoha *et al.*, 2013). Increase AP activity occurs in inflammatory conditions of the gastro-intestinal tract and liver (Tietz, 1999; Yakubu *et al.*, 2001, 2005; Wurochekke *et al.*, 2008; Oyewale and Malomo, 2009). The results also showed elevation of GGT levels which is a prompt response by the infected animal to infection by trypanosomes. The result agrees with earlier report of Ogunsanmi and Taiwo, (2001) who reported similar increases in the levels of GGT in grey duiker. There was elevation of CK levels which agrees with Allam *et al.*, (2011) who reported similar elevated levels of CK in *T. brucei* infection of gilts. Our result on CK levels however, differs from Hilali *et al.*, (2006) who reported normal levels of CK in *T. evansi* infection of water buffalo calves. CK activity is significantly elevated in myocardial infarction (Tietz, 1999). Since anaemia is a cardinal feature of trypanosomiasis in animals (Igbokwe and Mohammed, 1992; Anosa, 1988a; Omotainse *et al.*, 1994) the lysis of the red blood cells might result in the elevation of these enzymes in the plasma. It is also suggested that the elevation of enzyme levels seen, may also result from effect of trypanosome lyses resulting from the host defense mechanism (Kennedy, 2004).

One of the approaches used in chemotherapy of parasites relies on testing for biological activity of plant extracts. Since, they offer novel possibilities of obtaining new compounds that could be active against parasites. We have earlier reported the effect of fraction IV on DNA of mice experimentally infected by *T. congolense* (Maikai *et al.*, 2014). The administration of fraction IV portion of *Ximenia americana* on *T. congolense* infected rats appears to reduce the extent of organ damage, caused by the trypanosomes as evidenced by a decrease in levels of the liver enzymes (AST, ALT, AP, GGT and CK) activities.

Though the parasites were not completely eliminated by the treatment with fraction IV portion of *Ximenia americana*. We suggest that this decrease could be attributed to the antioxidant property of fraction IV (Maikai *et al.*, 2009) manifested in its protection of the vital organs including the liver, from the deleterious effects of *T. congolense*. The mechanism of action is however, unclear and the specific flavonoid is not known for now, investigations are still on going to identify it.

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CONCLUSION

This study has demonstrated that treatment with fraction IV portion of *Ximenia americana* showed a reduction in the parasite induced elevation of serum enzyme levels as well as modulate the effect of trypanosomiasis.

Conflict of interests

All authors confirm that there is no conflict of interests.

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