

**Research Paper**

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ANTI-TRYPANOSOMAL POTENTIAL OF MOMORDICA BALSAMINA LINN FRUIT PULP EXTRACT AGAINST *TRYPANOSOMA BRUCEI BRUCEI* INFECTION

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Abstract

The search for new trypanocides has not been keenly pursued due to high cost of design and development with no promise of financial returns. *Momordica balsamina* fruit pulp extract was screened for antitrypanosomal activity in experimental *T. brucei brucei* infection in rabbits. The extract was administered prior to parasite inoculation, 24 hours post parasite inoculation and on establishment of infection. The treatment was by oral administration of the extract at 500mg/kg body weight for 14 consecutive days. Parasitaemia was monitored daily while body weight and packed cell volume (PCV) were determined before commencement of studies and subsequently at weekly intervals for 28 days. The result showed a significant ($P<0.05$) delay in the establishment of *T. b. brucei* infection in rabbits treated at 24 hours post parasite inoculation. Packed cell volume also increased significantly ($P<0.05$) in all treated groups when compared to the untreated group (control). This was less in the group treated on establishment of infection. Administration of the extract to the curative group resulted in body weight gain. The other groups suffered weight loss. The infected but not treated group died at day 39 post infection while those treated before parasite inoculation, 24hours post parasite inoculation, and on the establishment of infection survived for 45 days, 53 days, and 61 days respectively. We conclude that *M. balsamina* pulp extract reduces anaemia in experimentally infected rabbits.

Key words : *Momordica balsamina*, Chemotherapy, Antitrypanosomal, Trypanosomiasis, *Trypanosoma brucei brucei*

Introduction

Trypanosomiasis remains a major public health problem to man and his livestock over much of tropical Africa. It is caused by *Trypanosoma* species. The disease is difficult to treat and deadly, if untreated. It is difficult to estimate the overall burden of African trypanosomiasis because of under diagnosis in the most heavily infected countries. However, it is believed to be in the vicinity of 100,000 new cases per year, with between $\frac{1}{3}$ and $\frac{1}{2}$ of cases remaining undetected and untreated (Pepin and Meda, 2001).

Chemotherapy is the most widely used means of controlling the disease. However, effectiveness of the drugs available is limited by a number of factors, which include increasing parasite resistance (Afewerk et al., 2000) and treatment failures, unacceptable toxicity (Akanji and Ngaha, 1989; Triolo, 1990;

Onyekwelu, 1999) unavailability, logistics of administration, long period of treatment, and high cost. Furthermore, the high cost of developing new drugs, with little hope of a reasonable financial return for their investment in research and development, are serious disincentives for most pharmaceutical companies. In the search for new trypanocides, a wide range of medicinal plants have been screened for antitrypanosomal activity and quite a number of them have been reported to have significant antitrypanosomal activity (Adewumi *et al.*, 2001; Ogbadoyi *et al.*, 2007). *Momordica balsamina* linn (Cucurbitaceae) is an annual or perennial vine belonging to the family cucurbitaceous, indigenous to tropical Africa. It has been used for various ailments and it is believed to possess significant immune boosting effect (Okeke, 2004) and also used as anti-tumor (Bourinbaier, 1995) and anti-HIV agent (Bot, 2004). The phytoconstituents of *M. balsamina* include glycoside, flavonoid, saponins and resins with glycosides highly present (Abubakar *et al.*, 2005).

There is a significant link between cancer and trypanosomiasis chemotherapy (Barret and Barret, 2000). It is also known that the immune system of the host is usually overwhelmed due to antigenic variation. Since available information on the medicinal use of *M. balsamina* indicate that it possesses anti-tumor and immune boosting effects, this study was aimed at screening the fruit pulp extract for possible antitrypanosomal property using *T. b. brucei* infected rabbit as a model. We report here that *M. balsamina* pulp extract has trypanostatic activity, reduces anaemia and promotes weight gain in experimental trypanosomiasis.

Experimental

Animals

Adult male rabbits (1.69-2.94kg) were purchased from Miango, Plateau state, Nigeria. Animals were handled in compliance with internationally accepted standards for the humane treatment of animals.

Trypanosomes

Trypanosoma brucei brucei, Federe strain was isolated from cattle at Federe, in Plateau State, Nigeria, and preserved in liquid nitrogen in Parasitology Unit of the Nigerian Institute for Trypanosomiasis Research, Vom, Nigeria.

Plant material

Ripped *M. balsamina* fruits were collected within and around Kaduna Vom, Plateau State, Nigeria in November 2006. The fruit was identified by Mallam Abdulkareem of the Federal College of Forestry, Jos, Plateau State, Nigeria, and a specimen was deposited in the college herbarium.

Preparation of the fruit pulp extract.

Momordica balsamina fruit was cut into two and the pulp containing the seed squeezed out. The seed was separated from the pulp using a wire mesh to obtain the pulp extract. The extract was dried in an oven at 50°C for 36 hrs. The yield was 32.11%. The dried extract was kept in the refrigerator until required.

Parasite inoculation.

Fifteen (15) adult male rabbits were weighed and divided into 5 groups (A – E) of 3 rabbits each. A stablate of *T. b. brucei* taken from liquid nitrogen was inoculated into donor rats. Heavily infected blood sample from donor rats was collected through ocular vein puncture and immediately diluted with physiological saline to give 1×10^7 parasites per ml to obtain inoculums. Healthy rabbits (groups A - D) were then inoculated with 0.1 ml of the diluted blood sample containing 1×10^6 trypanosome cells. Group A animals were pretreated with the extract for 14 consecutive days prior to parasite inoculation. Group E was not infected. Infection was monitored daily by wet film examination of blood sample taken from marginal ear veins of infected animals.

Administration of extract

Administration of extract to group B commenced at 24 hrs post parasite inoculation while group C was at establishment of infection. Group D animals were only inoculated with the parasite but not treated with the extract while the group E animals were neither inoculated nor treated. Groups D and E served as positive and negative controls respectively. All administrations were through oral route at 500mg/kg body weight for 14 consecutive days.

Therapeutic Effects of extract on infected animals

This was done by monitoring the weight of all the animals, determining the packed cell volume (PCV), and parasitaemia of the animals. PCV was determined using the haematocrit centrifuge technique while parasitemia was determined daily as described by Herbert and Lumsden (1976).

Results

Parasitemia

The results are presented in Figures 1-3. There were fluctuations in the level of parasitaemia of all the treated groups, which were however, kept at relatively very low level. The prepatent period for the group treated at 24 hours post parasite inoculation was 5-11 days while for other groups it was 5-7 days. When compared to the infected but untreated control, the treatment resulted into the prolongation of life by 6, 14 and 22 days for groups A, B and C respectively. Treatment of the infected group also resulted in the improvement in PCV when compared with the infected but untreated. The treated animals gained weight while the untreated animals suffered weight loss (Figures 4-6)

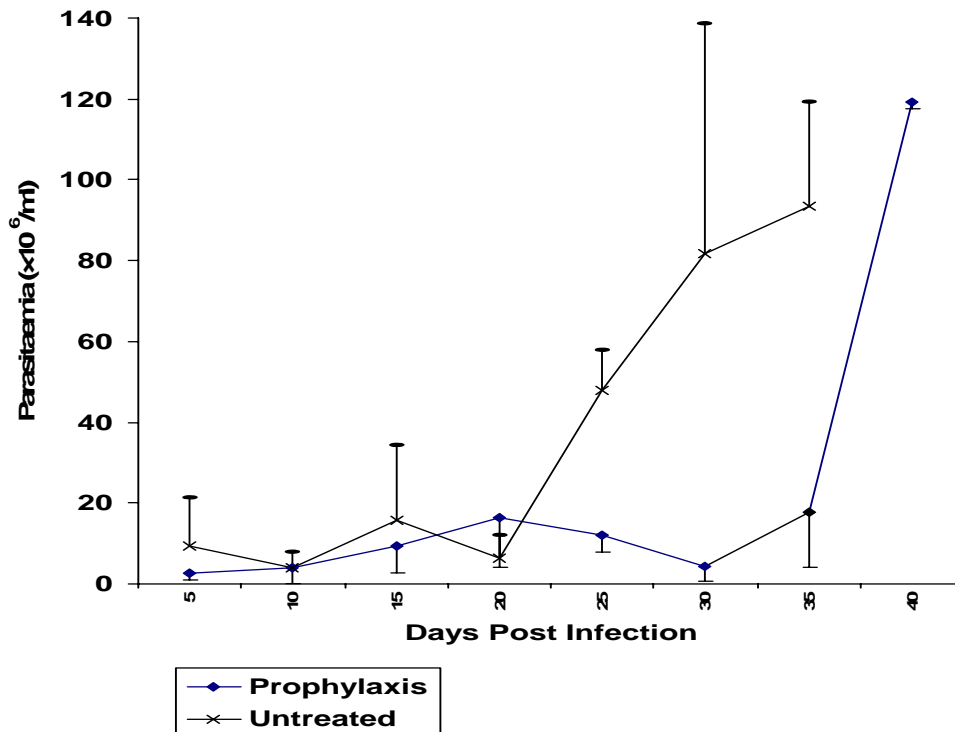


Figure 1: Changes in parasitaemia ($\times 10^6/\text{ml}$) of *T. b. brucei* infected rabbits and prophylactically treated with *M. balsamina* pulp extract

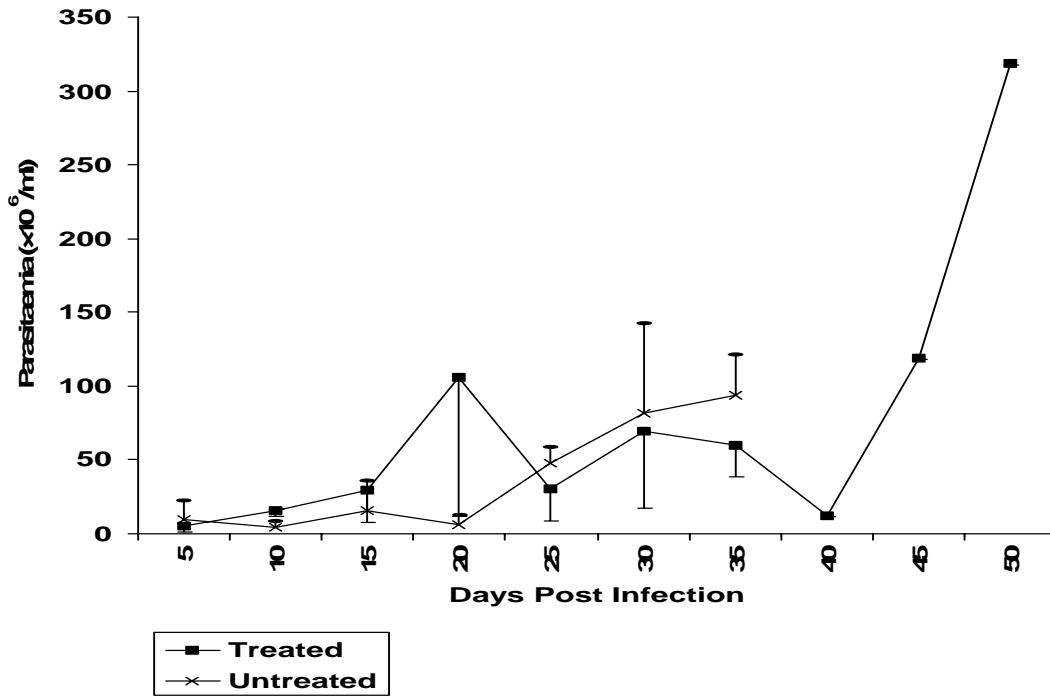


Figure 2: Changes in parasitaemia ($\times 10^6$ /ml) of *T. b. brucei* infected rabbits and treated at 24 hours post inoculation with pulp extract of *M. balsamina*.

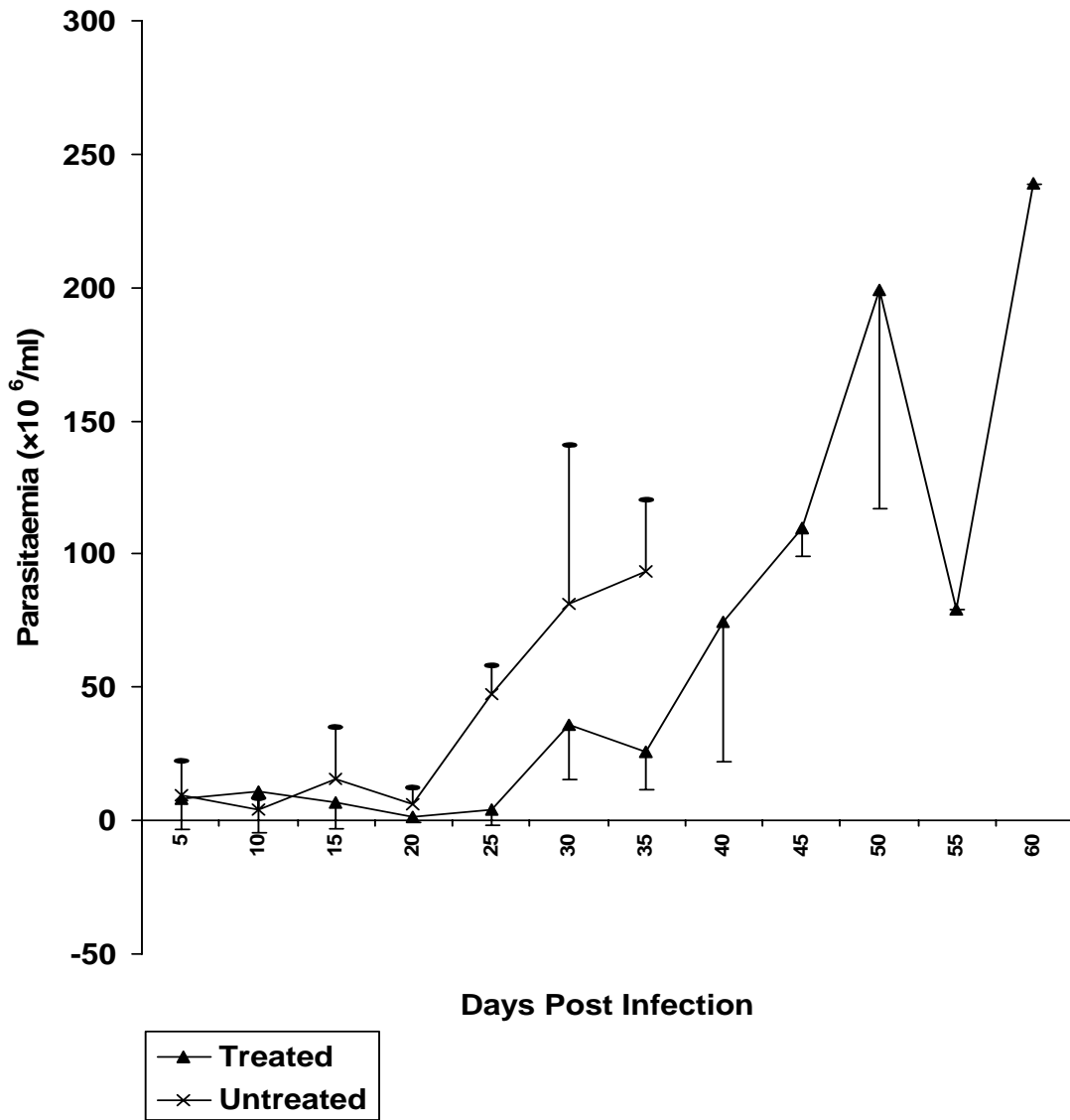


Figure 3: Changes in parasitaemia ($\times 10^6/\text{ml}$) of *T. b. brucei* infected rabbits and treated with establishment of infection with *M. balsamina* pulp extract

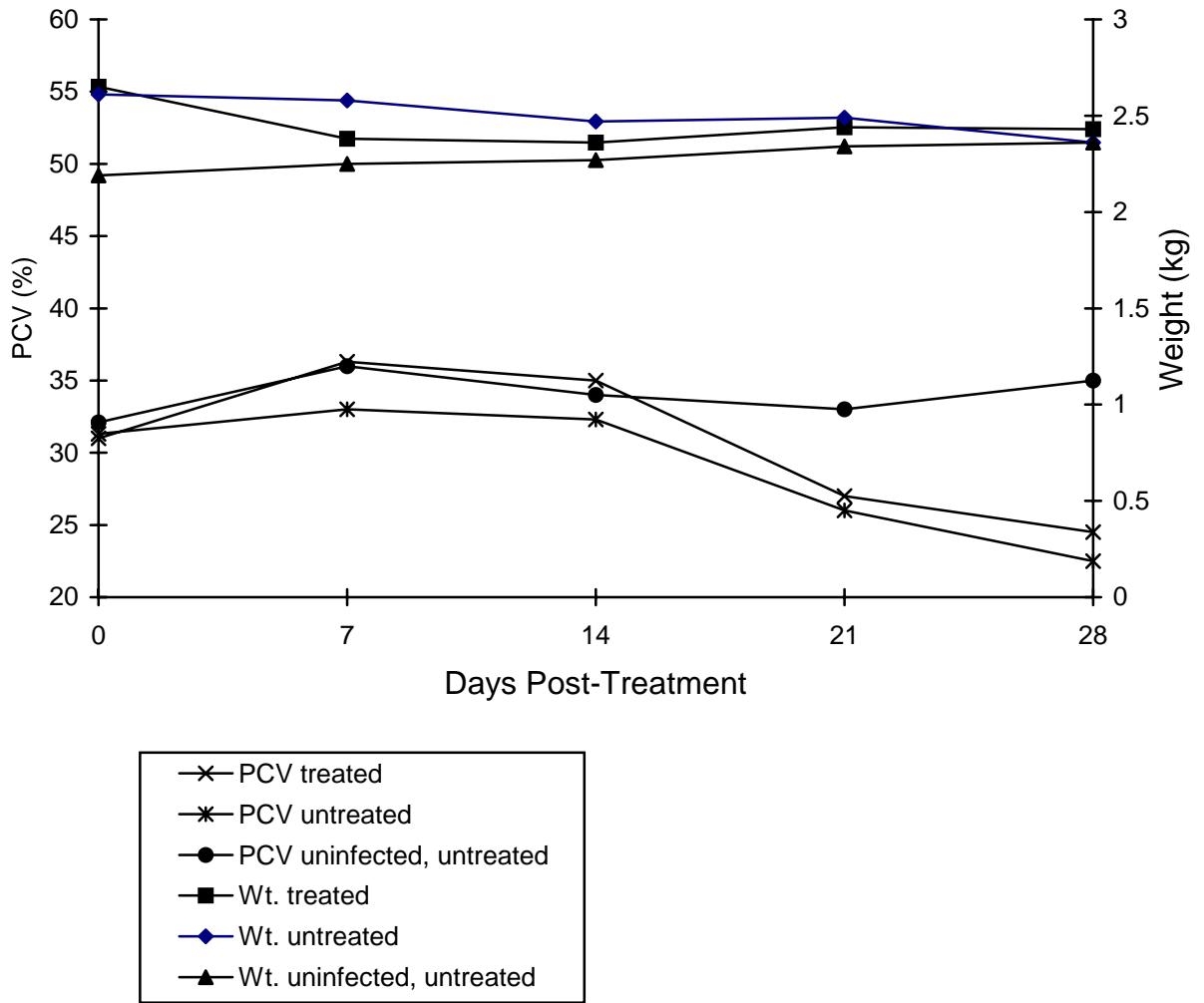


Figure 4: PCV and weight of infected rabbit and prophylactically treated with *M. balsamina* pulp extract.

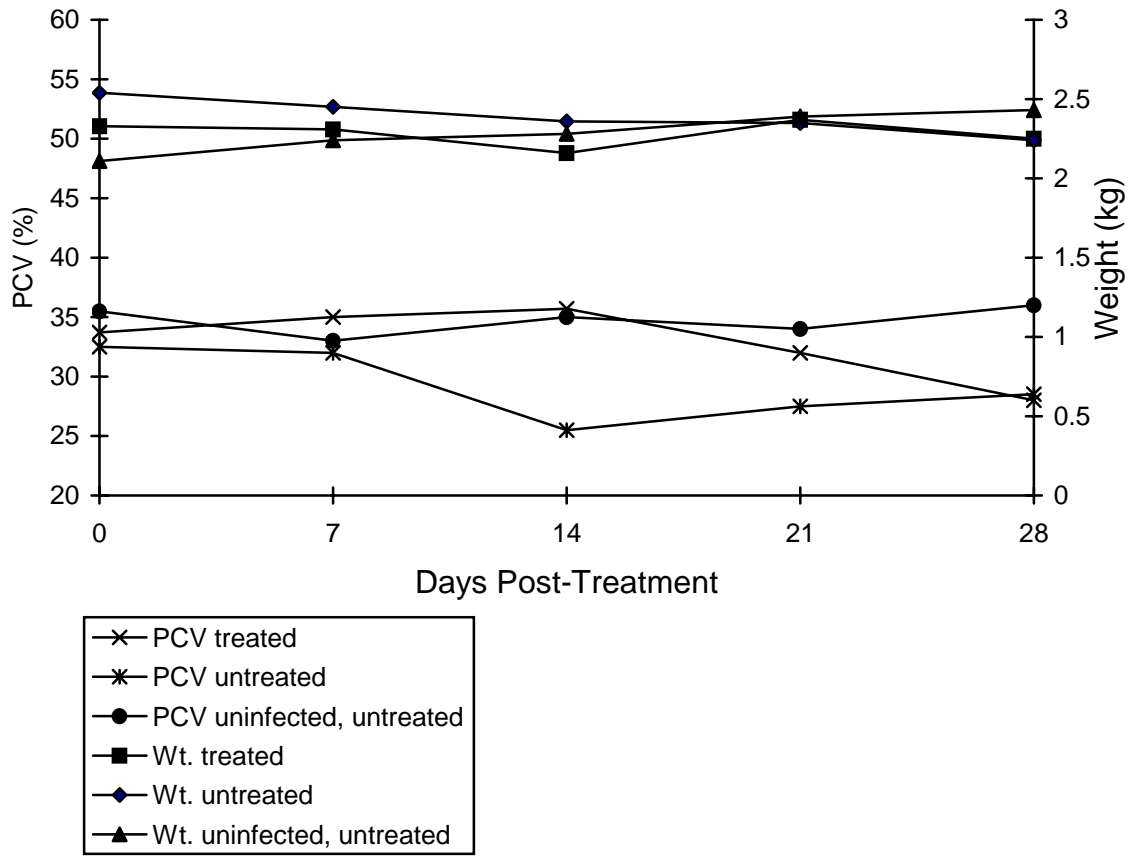


Figure 5: PCV and weight of infected rabbit and treated at 24 hours post inoculation with *M. balsamina* pulp extract.

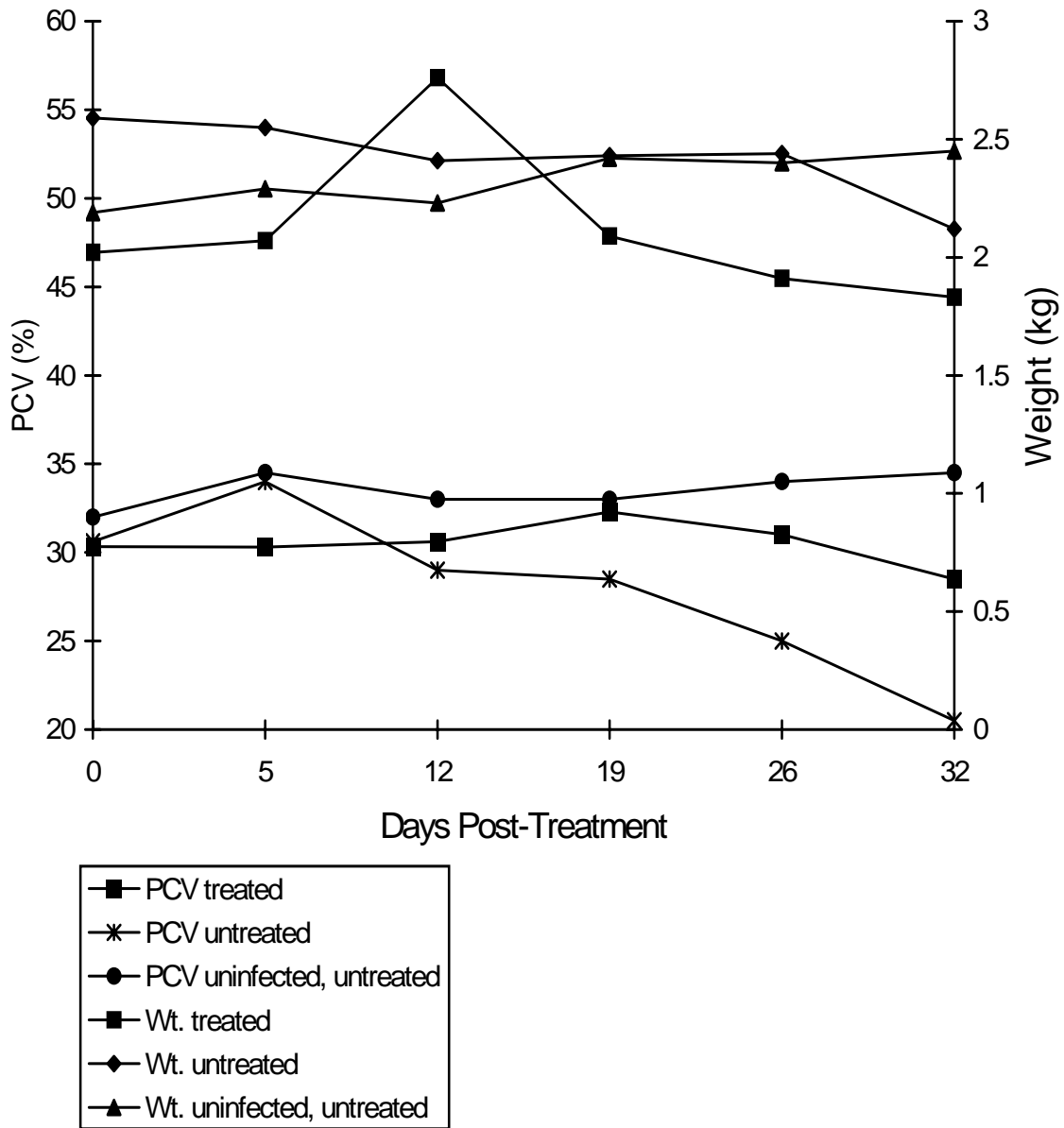


Figure 6: PCV and weight of infected and treated rabbit at the establishment of parasitaemia with *M. balsamina* pulp extract.

Discussion

The result of this study showed that *M. balsamina* pulp extract has potential in the management of African trypanosomiasis due to *T. brucei*. The pulp extract of this plant has shown some level of antitrypanosomal activity by prolonging the prepatent period of animals treated after 24 hours post parasite inoculation. There was also the extension of life of all the treated groups beyond that of the untreated control. However, it was observed that the prolongation of life conferred by the extract was highest in animals treated on the establishment of infection while the pretreated group recorded the least survival

period. What this suggests is that the extract strengthened the host defense, which was already activated because of the presence of parasites in circulation in the rabbits with established infection. This observation is of particular interest because *M. balsamina* pulp extract was earlier reported to boost immune system (Okeke, 2004). It is therefore likely that the pulp was able to elicit the production of antibodies in the presence of parasites in circulation. This, taken together with the longer prepatent period observed in 24 hrs treated animals suggests that the extract may positively influence the defense capacity of the treated animals. It may well be that the extract enhanced phagocytosis. The net effect of all these was the resultant prolongation of the lives of the treated animals as a consequence of the parasites being kept in check.

The observed trypanostatic effect of the extract was accompanied by corresponding increase in PCV. Anaemia is the most outstanding clinical and laboratory feature of African trypanosomiasis (Suliman and Fieldman, 1989) and also the primary cause of death (Losos and Ikede, 1972; Mamo and Holmes, 1975). Anemia as indicated by PCV level is known to worsen with increasing parasitaemia (Ogbadoyi et al., 1999). The prolongation of lives of treated animals may therefore also be associated with the ability of this extract to improve the PCV possibly by reducing the parasite load or neutralizing the toxic metabolites produced by trypanosomes.

The positive effect of the extract particularly on the curative group can further be deduced from the weight status of the animals. At two weeks post treatment, animals in the curative group gained weight by 1%; while the pretreated group and the group treated 24 hrs post parasite inoculation lost weight by 12.3% and 7.9% respectively. This observation indicates that the animals in the curative group were in a better physical state to eat more than those in the other groups. They were therefore more able to resist weight loss that is usually associated with trypanosomiasis.

M. balsamina pulp extract has been reported to have anti-tumor, anti-HIV, and antiplasmodial effects (Bourinbaiar, 1995; Bot, 2004; Benoit-Vical et al., 2006). These reports are of interest to us in two respects. The anti-tumour activity is particularly exciting because all four drugs currently used clinically in the treatment of sleeping sickness are known to have some level of anti-cancer activities (Barret and Barret, 2000). Hitherto, drug developers are reluctant to invest in research and development of drugs for the treatment of African trypanosomiasis alone for lack of profitable market.

Therefore, the antiplasmodial and anti-HIV activities taken together with the anti-tumour activity may generate the much needed interest in potential investors to invest in research and development of ethnomedicine and/or synthesis of modern pharmaceuticals from lead compounds that may be obtained from extracts of this plant.

In conclusion, this study has provided evidence that *M. balsamina* fruit pulp extract is trypanostatic, reduces anemia and promotes weight gain in experimental African trypanosomiasis. It is potentially a useful antitrypanosomal agent. Further work is in progress to determine the full effect it has on larpine trypanosomiasis.

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