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Full Length Research Paper

Evaluation of the antitrypanosomal activity of C*ucumis metuliferus* pulp extract in rabbits

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The antitrypanosomal efficacy of different doses of *Cucumis metuliferus* pulp extract was investigated in rabbits. Parasitaemia, Packed Cell Volume (PCV), percentage organ/body weight ratio and survival period were monitored. Treatment was by oral administration and it commenced at the establishment of infection that lasted between 1 to 3 weeks. Parasitaemia fluctuated and was kept low in all the treated groups. However, the results showed significant increase in both PCV and body weight ($P<0.05$) in rabbits treated at 500 and 1000 mg/kg body weight. Treatment with 500 mg/kg body weight for 7 consecutive days gave the highest survival period of 47 days beyond 28 days for the untreated control group. Post mortem examination (following the termination of the study) generally revealed pale carcasses, hepatomegaly and splenomegaly. However, treatment for three weeks with 500 and 1000 mg/kg body weight alleviated hepatomegaly and splenomegaly significantly ($P<0.05$). Consequently, a more detailed investigation of the potentials of *C. metuliferus* for antitrypanosomiasis drug discovery is recommended to pave way for the development of drugs for the effective treatment of African trypanosomiasis.

Key words: *Cucumis metuliferus, Trypanosoma brucei,* chemotherapy, trypanosomiasis.

INTRODUCTION

The causative agent of African trypanosomiais, which is the protozoan parasite *Trypanosoma brucei*, is transmitted to humans and domestic farm animals through the bite of the tsetse fly. As at 1990, an estimated 500,000 people were infected with *T. brucei*, almost all of whom live in sub-Saharan Africa. Moreover, the infections become fatal if not treated early (Barrett, 1990). However, in 2009, the number of reported cases dropped to less than 10,000 for the first time in half a century with an estimated actual cases put at 30, 000 (World Health Organization, 2010).

T. brucei is known to invade the connective tissues causing foci of degeneration and necrosis of interstitial and parenchyma cells, as well as induces the extensive infiltration by lymphocyte macrophages and plasma cells (Losos and Ikede, 1972). Since it has not been possible to develop an effective vaccine against this disease, due to the problem of antigenic variation, trypanocides play a major role in its management and control. However, the limited availability, high cost, toxicity (Onyekwelu, 1999) and resistance (Brun et al*.,* 2001) of existing drugs have necessitated the search for alternatives. Medicinal plants have long been known as a good source of drugs for the

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effective treatment of many ailments. *C. metuliferus* is an herbaceous annual climbing or trailing herb belonging to the family of Cucurbitaceae, indigenous to tropical Africa. The fruit pulp from this plant has been widely used in Jos and its environs for the treatment of typhoid, malaria and human immunodeficiency virus infections. In the present investigation, we report the use of *C. metuliferus* pulp extract at dose levels of 500 to 1000 mg/kg body weight for the management of *T. brucei* infection, including induced hepatomegaly and splenomegaly in addition to improving anaemia and promotion of weight gain.

MATERIALS AND METHODS

Animals

Adult male rabbits (1.26 to 2.05 kg) were purchased from Jos, Plateau state, Nigeria. The animals were maintained according to the CIOMS guidelines (1985).

Trypanosome

Trypanosoma brucei brucei, Federe strain was isolated from cattle at Federe, in Plateau state, Nigeria and preserved in liquid nitrogen, in Veterinary and Livestock studies Division, NITR, Vom.

Plant material

Ripe *C. metuliferus* fruits were collected within and around Kaduna Vom, Plateau state, Nigeria between January and March. It was identified at the National Institute for Pharmaceutical Research and Development (NIPRD) Idu, Abuja, Nigeria and a specimen with a voucher number NIPRD/H/6172 deposited in NIPRD herbarium.

Preparation of fruit pulp extract

C. metuliferus fruit was cut into two and the pulp containing the seed was squeezed out. The seed was separated from the pulp using a wire mesh to obtain the pulp extract. The extracted pulp was dried in an oven at 50°C for 36 h and the yield was 32.11%. The dried pulp extract was kept inside a dessicator until it was required.

Phytochemistry and pH determination

The phytochemical analysis of the pulp extract was carried out as described by Odebiyi and Sofowora (1978). The pH of the pulp extract and a standard trypanocide, samoricide plus was determined using a pH meter.

Inoculation of parasites

Thirty-six adult male rabbits were weighed and divided into twelve groups (A to L) of 3 animals each. A stabilate of *T. b. brucei* taken from liquid nitrogen was inoculated into the donor rats. Heavily infected blood samples from donor rats were collected through ocular vein puncture and immediately diluted with physiological saline to give 1 x 10^7 parasites per ml to obtain inocula. Healthy rabbits (A to K) were then inoculated with 0.1 ml of the diluted blood sample. Group L was not infected; as such, it served as control. Infection was monitored periodically by wet film examination of blood sample taken from marginal ear veins of infected animals.

Administration of pulp extract

At the establishment of infection, animals were treated orally with the pulp extract at 250, 500 and 1000 mg/kg body weight each for 1, 2 and 3 weeks respectively. A group of infected animal was treated with samoricide plus at the manufacturers' recommended dose of 3.5 mg/kg body weight. A control group was infected but remained untreated while another control group was neither infected nor treated.

Screening of extract for bioactivity

Evaluation of extract for therapeutic potential was done by monitoring the body weight, packed cell volume (PCV) and parasitaemia of all the animals. PCV was determined using the haematocrit centrifugation technique, while parasitaemia was estimated as described by Herbert and Lumsden (1976). Post mortem examination, following the death of animal or termination of the study for gross pathology and weighing of liver, kidneys, heart and spleen was also carried out. The percentage organ/body weight ratio was then calculated and compared between treated and untreated controls using students' t-test at 5% confidence limit.

RESULTS

The parasitaemia for different weeks of pulp extract administration are presented in Figures 1 to 3. The pre-patent period of the infected animals ranged from 4 to 7 days. The parasitaemia of the pulp extract treated groups were kept low, while a relapse of infection occurred after 25 days for samoricide treated group which survived for 16 days beyond the untreated control. Treatment at 500 mg/kg for 1 week resulted to prolongation of life by 18 days beyond that of the control, while 1000 mg/kg for 2 and 3 weeks prolonged life by 10 and 12 days, respectively beyond that of the untreated control group. Changes in body weight and PCV are presented in Tables 2 and 3, respectively. All the treated animals suffered less loss in body weight and PCV as compared to the untreated group. Figures 4 to 6 show the percentage of the organ/body weight ratio. Treatment with samoricide$^+$ and *C. metuliferus* pulp extract prevented hepatomegally and significantly reduced splenomegally at 500 to 1000 mg/kg body weight for 2 to 3 weeks. Gross pathology revealed pale mucous membrane and carcass, enlargement and congestion of spleen, liver with various pulling and kidneys in infected and untreated rabbits. However, evidence of organ congestion was not pronounced in treated groups. The pH of the pulp is 3.38 ± 0.08 and the phytochemicals present are shown in Table 1.

DISCUSSION

The results obtained from this study have provided

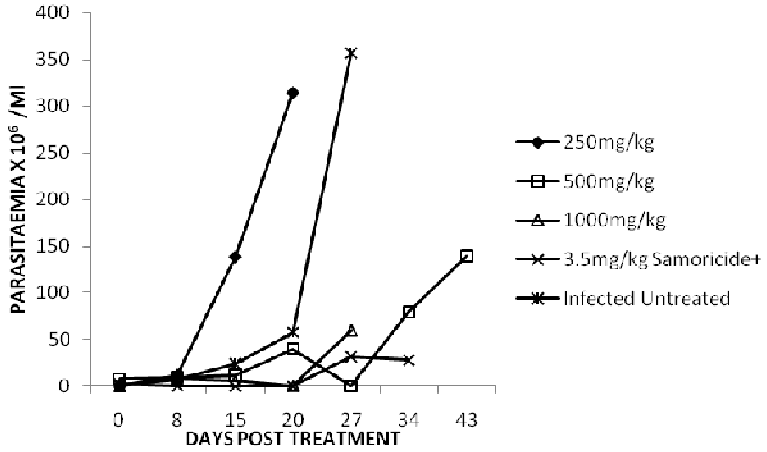

Figure 1. Parasitaemia (x10^6 /ml) of infected rabbits treated with different doses of *C. metuliferus* for one week.

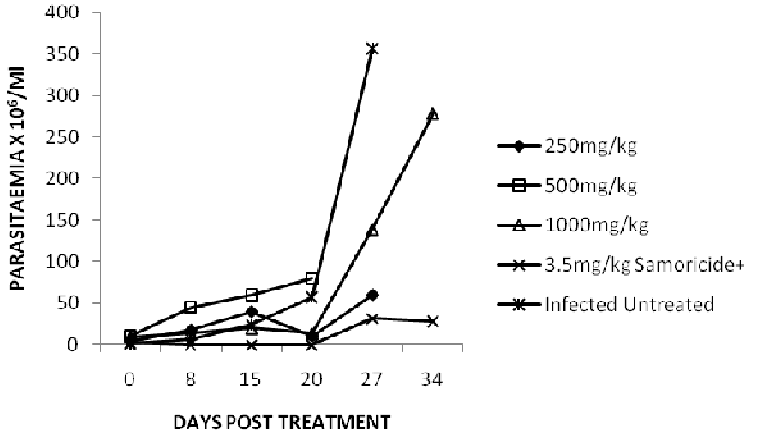

Figure 2. Parasitaemia (x10^6 /ml) of infected rabbits treated with different doses of *C. metuliferus* for 2 weeks.

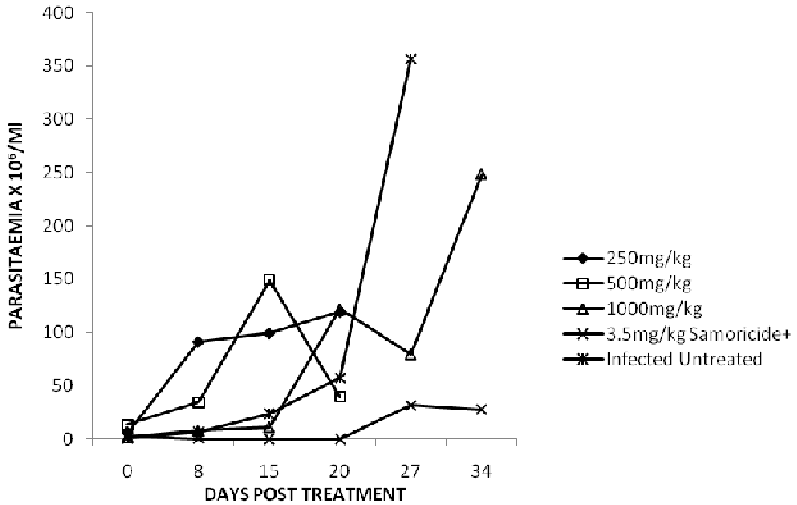

Figure 3. Parasitaemia (x10^6 /ml) of infected rabbits treated with different doses of *C. metuliferus* for 3 weeks.

Table 2. Weight (kg) of infected rabbits treated with *C. metuliferus* at different doses.

Dose (mg/kg)	Days post treatment				
	0	7	14	21	28
250	1.69±0.1*	1.53±0.1	1.62±0.1	1.47±0.1	1.16±0.0
	1.26±0.3**	1.44±o.3	1.43±0.3	1.37±0.3	1.32±0.3
	1.53±0.1***	1.48±0.1	1.46±0.1	1.44±0.1	1.32±0.01
500	1.56±0.4	1.66±0.4	1.75±0.5	2.30±0.0	2.29±0.0
	1.45±0.4	1.35±0.4	1.30±0.4	1.55±0.6	1.30±0.5
	1.57±0.5	1.53±0.5	1.46±0.5	1.64±0.3	1.27±0.0
1000	1.80±0.5	1.91±0.4	1.86±0.5	1.34±0.3	2.21±0.0
	1.66±0.1	1.71±0.02	1.68±0.03	1.72±0.1	1.72±0.03
	1.81±0.1	1.93±0.2	1.71±0.4	1.79±0.3	1.70±0.4
Samoricide+	1.49±0.2	1.48±0.2	1.48±0.2	1.63±0.3	1.59±0.2
Infected, untreated	1.62±0.02	1.62±0.1	1.51±0.1	1.42±0.01	1.30±0.04
Uninfected, untreated	2.05±0.5	2.02±0.3	2.26±0.3	2.16±0.3	2.22±0.3

*----------Treated for 7 days, **----------Treated for 14 days and ***---------Treated for 21 days.

Table 3. Packed cell volume (%) of infected rabbits treated with *C. metuliferus* at different doses.

Dose (mg/kg)	Days post treatment				
	0	7	14	21	28
250	29.50±2.5*	29.67±3.7	31.50±1.5	30.00±5.0	27.50±0.0
	32.50±0.5**	35.50±3.5	34.50±0.5	33.00±0.0	34.50±0.5
	31.5±0.5***	34.50±1.5	32.50±0.5	33.00±3.0	30.50±3.5
500	31.50±0.5	36.00±4.5	31.00±4.0	32.50±0.0	33.00±0.0
	29.00±0.5	34.00±2.2	31.33±2.6	31.00±3.0	30.50±6.5
	28.00±0.5	31.30±0.9	30.33±4.5	31.00±1.0	31.00±0.0
1000	32.00±2.0	35.33±3.7	26.00±3.7	23.00±2.0	34.00±0.0
	29.00±2.0	36.67±4.8	30.67±6.3	30.50±4.5	35.50±4.5
	27.50±1.5	35.00±6.7	29.00±1.6	29.67±2.1	29.33±6.9
Samoricide+	30.00±0.0	34.00±2.0	34.50±0.5	33.00±3.0	39.00±3.0
Infected, untreated	30.00±1.0	32.67±3.9	31.50±1.5	27.00±0.8	25.30±0.5
Uninfected, untreated	30.67±2.1	32.50±2.5	38.50±2.5	40.30±0.5	41.00±0.8

*----------Treated for 7 days, **----------Treated for 14 days and ***---------Treated for 21 days.

evidence that *C. metuliferus* pulp particularly at 500 to 1000 mg/kg has potential in the management of trypanosomiasis. The treatment kept the parasitaemia at low level, while that of the untreated control group kept rising until the animals died of acute infection. The trypanostatic effect of the extract was highest at 500 mg/kg for one week. The prolongation of life for some treated groups which was even higher than samoricide®

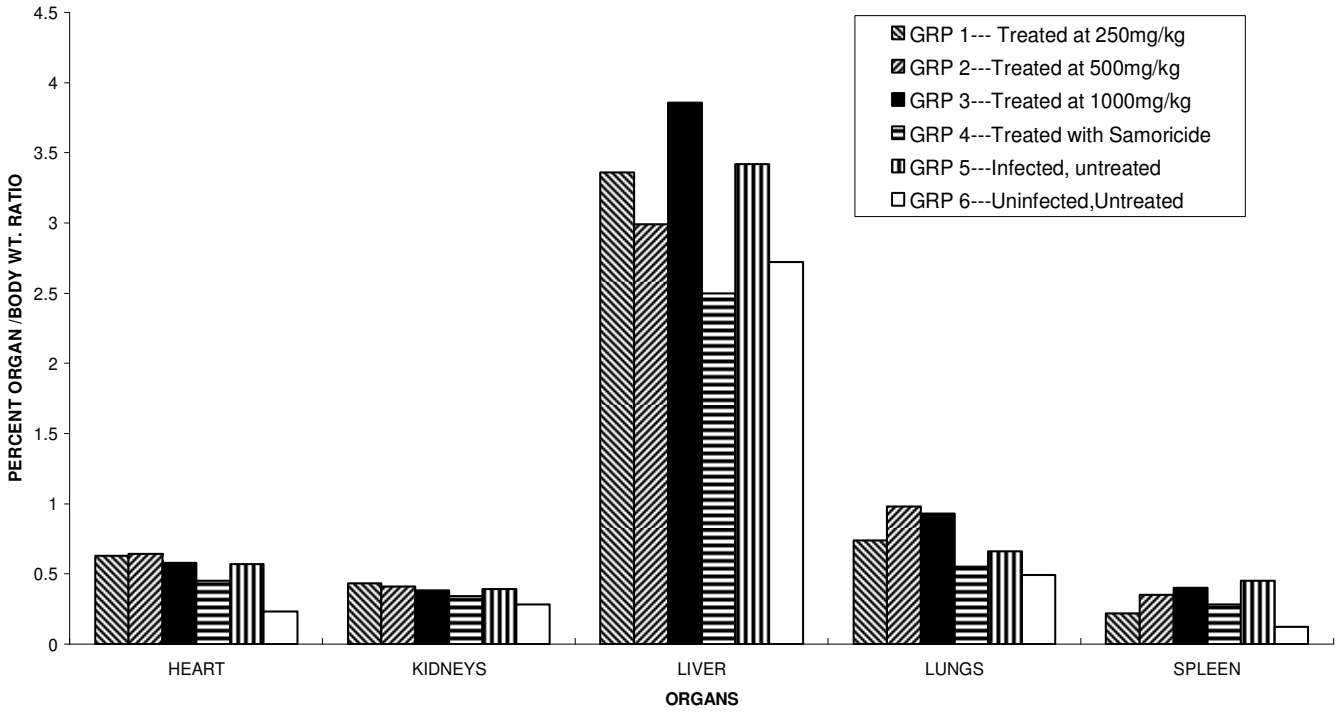

Figure 4. Percent of organ/body weight ratio of infected rabbits treated with *C. metuliferus* for 1 week.

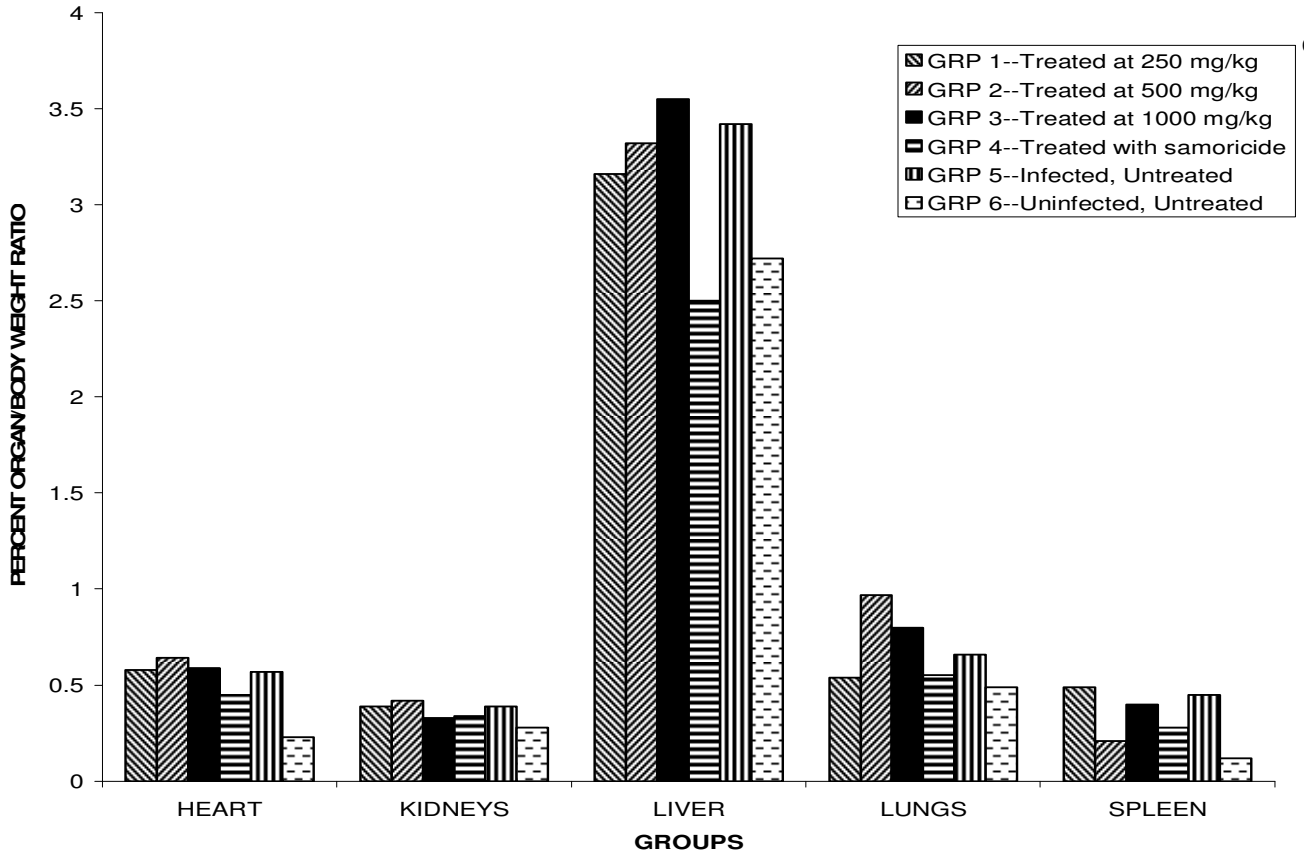

Figure 5. Percent organ/body weight ratio of infected rabbits treated with *C. metuliferus* at different doses for 2 weeks.

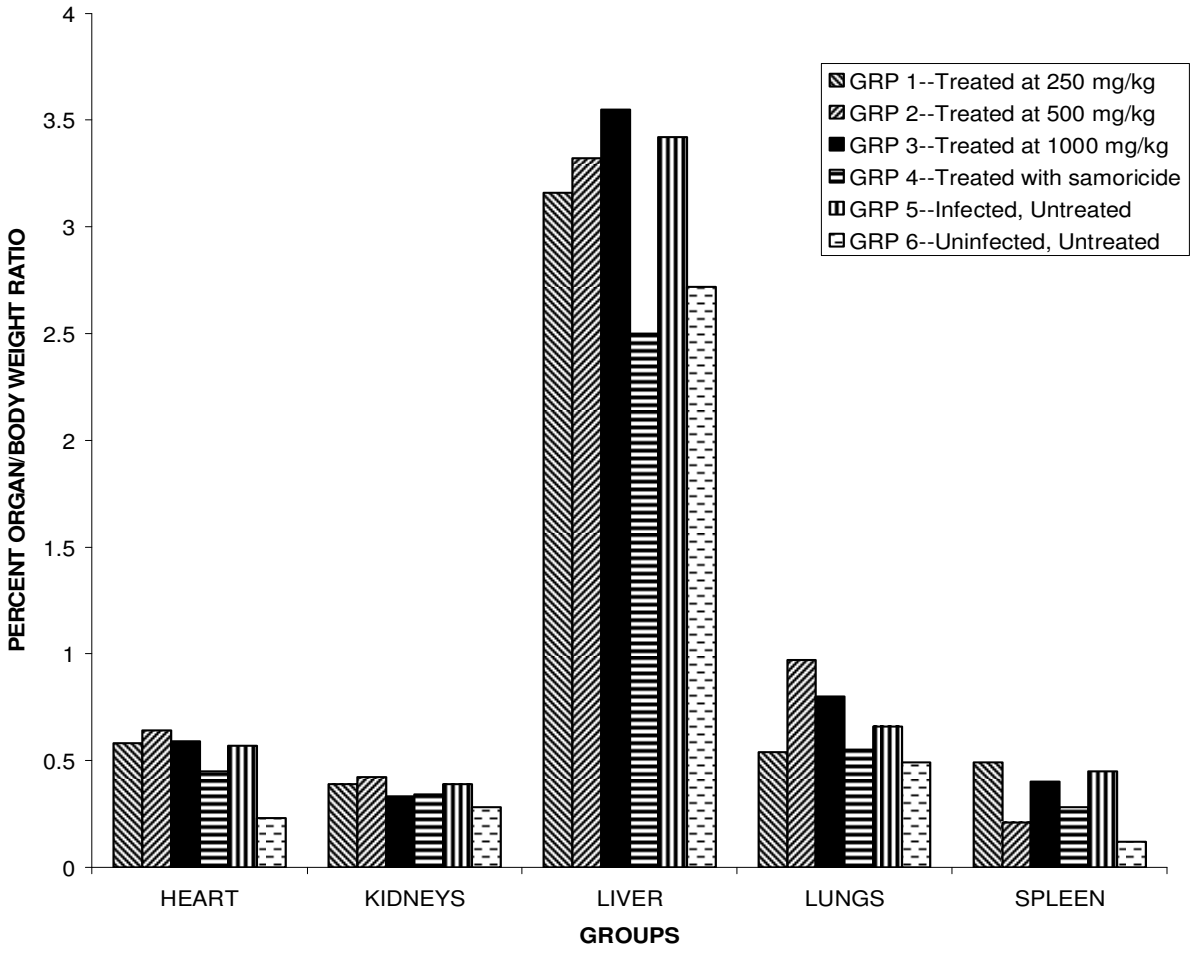

Figure 6. Percent organ/body weight ratio of infected rabbits treated with *C. metuliferus* at different doses for 3 weeks.

Table 1. The phytochemicals present in the pulp extract of *C. metuliferus*.

Phytochemicals	Observation
Tannins	-
Resins	+
Alkaloids	-
Glycoside	+++
Flavonoid	+
Saponin	++

Key: +++ = highly present; ++ = moderately present; + = faintly present; --- = not present.

treated may therefore suggest the ability of the extract to enhance the body's ability to phagocytose the parasite, thereby leading to low parasitaemia. In addition, the result also implies that treatment at 500 to 1000 mg/kg body weight is sufficient to elicit enough response against *T. brucei* infection. Therefore, since immune suppression is a common feature in African trypanosomiasis, treatment with *C. metuliferus* pulp could probably be responsible for the elevation of immunity, which led to the observed prolongation of life. The observed prolongation of life for the treated group is also accompanied by the improvement in PCV values and body weights. It has been well established that the measurement of anaemia and loss of weight gave a reliable indication of the disease status (Murray, 1979) and productive performance (ILCA, 1986) of trypanosome in infected

animals. The result revealed therefore that treatment with *C. metuliferus* can lead to increase in productive performance, an indication that the rise in parasitaemia was controlled. This was due to improvement in PCV values. Since *T. brucei* infection has been associated with enormous production of hydrogen peroxide (Meshnick et al., 1977), which resulted to increased susceptibility of erythrocyte toward *in vitro* peroxidation, the improvement of PCV of the treated groups could therefore be due to antioxidant effect of the pulp or its ability to reduce the free fatty acid levels which are reported to lyse red cells if they are not bound to albumin (Akanji, 1985). The cells are therefore constantly under attack from free radicals or reactive oxygen species (ROS). Furthermore, the weight gain observed indicates that the animals are in a better position to eat more, and thus makes them more able to contain certain weight loss usually associated with trypanosomiasis. The presence of phytochemicals such as glycoside, resin and saponins could also be responsible for these observations.

Hepatomegally and splenomegally are consistent observations in African trypanosomiasis especially in laboratory rodents (Greenwood and Whittle, 1980; Mansfield, 1982). Significant reduction ($P<0.05$) in the percentage of organ/body weight ratio of spleen and liver of treated groups at 500 to 1000 mg/kg body weight was observed. This indicated the protective role of the pulp on these organs while the heart, lungs and kidneys were not affected by the treatment ($P>0.05$). In extravascular sites, the *T. brucei* sub group is reported to cause cellular infiltration and tissue cell degeneration and death (Mwambu and Losos, 1978).

Although, the extract could not kill the trypanosomes, it appears to have trypanostatic activity, and can boost the capacity of the host to control anaemia, protect liver and spleen probably by detoxifying the toxic metabolite produced by the parasite. This makes further work on this plant (alone or in combination with other plant preparations) worthwhile.

Conclusion

C. metuliferus has antitrypanosomal properties at 500 to 1000 mg/kg body weight. It promotes weight gain, reduces anaemia and controls hepatomegaly and splenomegaly in *T. brucei brucei* infection. The extract is potentially useful in the management of anaemia, which is a major pathological feature of African trypanosomiasis and other pathological features such as hepatomegaly and splenomegaly.

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