The Effect Of Lime *(Citrus Limetta)* Fruit Extract On Haematological Profile Of Rabbits Infected With *Trypanosomes*

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Abstract: An in vivo study was carried out to determine the effect of lime fruits (Citrus limetta) aqueous extract on the hematological profile of Trypanosoma brucei brucei-infected rabbits. After ten (10) days post-infection, the extract was administered orally for three (3) weeks after dose determination and toxicity testing. Sixteen male rabbits were used for this study; they were placed into four (4) groups. Some of the rabbits were not infected but treated with the extract (Test I & II), infected and not treated with the extract (Control II) and not infected but treated with the extract(control I). All statistical analyses were done using Statistical Package for Social Sciences (SPSS) version 17.0. The result obtained showed that there is a significant difference ($p<0.05$) between the parameters of extract treated rabbits and non-extract treated rabbits. Hence the calls for more trails on this extract.
[Nuhu M, Lynn M, Bala Z, Jantiku J, Johnny I and Vivian A. **The Effect Of Lime *(Citrus Limetta)* Fruit Extract On Haematological Profile Of Rabbits Infected With *Trypanosomes*.** *Rep Opinion* 2015;7(11):1-10]. (ISSN: 1553-9873). http://www.sciencepub.net/report. 1. doi:10.7537/marsroj071115.01.

Keywords: Trypanosomes, Haematological parameters, Toxicity and Citrus limeta

1. Introduction

In tropical Africa protozoan parasites cause several diseases of social and economic importance, and one of the most important is Trypanosomasis. In America trypanosomasis is known as chagas diseases while in Africa is divided in to Human Africa trypanosomasis (HAT) or sleeping sickness and African Animal Trypanomasis (AAT) (WHO, 2006).

The prevalence of the disease differs from one country to another as well as in different parts of the country. In 2005, WHO reported major outbreaks in Angola, the Democratic Republic of Sudan, Central African Republic, Chad, Congo, Coted'ivoire, Guinea, Malawi, Uganda and Republic of Tanzania. Sleeping sickness remain an important public health problem in countries like Burkinafaso, Cameroon, Equatorial Guinea, Gabon, Kenya, Mozambique, Nigeria, Rwanda and Zimbabwe where about 50 new cases are reported every year. Sleeping sickness (Trypanosomiasis of man) in tropical Africa has been responsible for mortality and serious ill-health. While nagana (trypanosomiasis of livestock) in Africa has greatly contributed to the widespread of malnutrition in Africa by decreasing the amount of protein (meat) available to man. The world health organization in listing the ten major health facing mankind placed the trypanosomiasis of man and his domestic animals high on the list long side malaria.

Table 1: The mean(×) ± S.D. at Day 0 of control and test groups.

Parameters	Control I	Control II	Test I	Test II
PCV(%)	36.77±5.3	35.45±3.1	34.58±2.8	37.65±1.2
Hb(g/L)	340.75±5.1	341.0 ±8.57	339.0±25.53	339.25±13.94
WBC($\times10^9$/L)	66.05±7.4	73.10±8.51	81.48±2.9	78.2±24.0
RBC($x10^{12}$/L)	3.90±0.26	4.43±0.60	3.78±0.29	4.03±0.15
PLT ($\times10^9$/L)	403.75±9.6	348.0±33.71	397.50±30.35	387.75±57.21

Two-third of the world population has been estimated to suffer from varying degree of malnutrition due to deficiency in protein. Therefore increased production of livestock has been advocated as a most reliable approach to meeting the protein demands of the growing world population. Though, there are potentials for appreciate increasing livestock production in many part of Africa, particularly in Nigeria, these are hampered by lack of manpower, and animals diseases such as tse-tse fly transmitted trypanosomiasis which affects both man and his livestock (WHO, 1979).

1.2 Aims And Objectives

- To determine the effect of lime fruit (*Citrus limetta*) aqueous extract on haematological profile of rabbit infected with Trypanosome.

- To determine the dosage effect of lime fruit (*Citrus limetta*) aqueous extract on haematological profile of rabbits infected with Trypanosome.

Table 2: The mean(×) ± S.D. at week 1 of control and test groups.

Parameters	Control I	Control II	Test I	Test II
PCV(%)	36.27±3.37	31.40±1.37	37.15±0.9	38.07±1.40
Hb(g/L)	340.00±20	322.25 ±7.42	338.75±13.86	377.75±1.37
WBC($\times10^9$/L)	69.15±5.15	82.65±8.51	87.28±0.89	79.15±1.09
RBC($x10^{12}$/L)	4.43±0.50	3.82.±1.76	4.15±0.16	4.05±0.50
PLT ($\times10^9$/L)	329.25±5.74	410.50±15.8	397.50±15.17	372.0±20.24

2. Literature Review

Trypanosomes are haemoflagelllates of the family *Trypanasomatidae* that live in the blood and tissue of their host; they have elongated flattened and lanceolated-shaped bodies. There is great interest in the study of these parasitic flagellates due to the fact that they have been discovered to be the causative agents of major disease of man and his domestic animals; trypanosomes infect both man and other animals thereby causing several kinds of Trypanosomiasis varying from one region to another (Lynne & David, 1997).

The disease include Human Trypanosomiasis (sleeping sickness), commonly found limited to the tse-tse fly belt of Central, West, Eastern, and Southern Africa, which is caused by *T. b. gambiense*. Chagas disease (Human Trypanosomiasis) in America is caused by *Trypanosoma cruzi* (transmitted by the triatomal bug) and "nagana" (African Animal Trypanosomiasis) cause by *Tyrpanosoma congolense, Trypanosoma vivax* or *T.b. brucei.*

Table 3: The mean(×) ± S.D. at week 2 of control and test groups.

Parameters	Control I	Controll II	Test I	Test II
PCV(%)	38.6±4.65	31.4±0.95	36.7±1.97	38.07±0.91
Hb(g/L)	346.50±6.35	312.25 ±5.62	337.75±26.72	340.50±0.57
WBC($\times10^9$/L)	64.20±9.95	82.65±4.86	85.33±1.79	79.77±3.61
RBC($x10^{12}$/L)	4.73±1.02	4.43±0.42	4.15±0.33	4.17±0.15
PLT ($\times10^9$/L)	320.75±7.76	401.50±19.0	316.25±83.92	364.25±99.98

2.1 Incubation Period

The incubation period for *T. congolense* varies for 4-24days: for *T. vivax* 4-40 days and for T b. brucei 5-10 days (Mare, 2000).

2.2 Pathogenesis

Initial replication of trypanosomes is at the site of inoculation in the skin, this causes swelling and sore (Chancre), the trypanosome then spread to the lymp node and blood and continue to replicate. *Trypanosoma congolense* localizes in the endothelial cells of small blood vessel and capillaries. *T.b. brucei* and *T. vivax* localize in the tissue antibody developed to the glycoprotein coat of the trypanosome kills the Trypanosome and result in the development of immune complexes. Antibody, however, does not clear the infection for the trypanosome has genes that can code for many different surface-coat glycoprotiens and change it surface glycoprotein to evade the antibody. In this way there is a persistent infection that results in a continuing circle of trypanosome replication, antibody production, immune complex development and changing surface coat glycoprotein.

2.3 Clinical Signs And Symptoms

Simultaneously infections with more than one trypanosomes species are very common (Nyeko *et. al.*, 1990), and also co-infection with trypanosomes and other haemoparasite (*Babesia* species, *Theilaria* species, *Anaplasma* species and *Ehrlichia* species) frequently occurs, it is difficult to conclude which clinical sign are attributable to a giving parasite. Few adequately controlled studies have been made, and thus a "Typical" clinical response to each *Trypanosome* is difficult to reconstruct. What follows is a summation of the syndrome observed in the field and experimental cases of trypanosomiasis cause by each of the three African animal trypanosomes.

The cardinal clinical sign observed in African trypanosomiasis is anaemia within a week of infection with haematic trypanosomes (*T. congolense* and *T.vivax*) there is usually a pronounce decrease in the parked cell volume (PCV), Haemoglobin (Hb), Red blood cell (RBC), and white blood cell (WBC) levels, and within two months this may drop to below 50 percent of their pre-infection values, (McCorie *et. al.,* 1980). Also invariably present are intermittent fever,

oedema, and lost of weight. Abortion may be seen and infertility of males and females may occur. The severity of clinical response is dependent on the species and breeds of the affected animals and the dose and virulence of the infecting trypanosomes. T. congolense is a haematic trypanosomes found only in the blood vessel of animals it infects, it does not localize and multiply outside blood vessels. Its infection may result in pre-acute, acute, or chronic disease in cattle, sheep, goats, horses, and camels. Pigs often developed a milder disease, while chronic disease is common in dogs. In addition to the above mentioned signs, infections with *T. congolense* is usually associated with salivation, lacrimation, and nasal discharge. As the disease progresses lots of condition like hair colour changing from black to metallic brown are seen. The back if often arched and the abdomen "tucked up", accelerated pulse and jugular pulsation occur and breathing is difficult.

Table 4: The mean(×) ± S.D. at week 3 of control and test groups.

Parameters	Control I	Control II	Test I	Test II
PCV(%)	39.35±4.99	31.87±2.10	37.65±1.23	38.47±0.45
Hb(g/L)	345.25±16.35	291.75±5.68	341.00±28.97	342.50±4.35
WBC($\times10^9$/L)	66.82±8.39	82.65±8.51	91.03±5.85	86.10±4.35
RBC($x10^{12}$/L)	4.15±0.77	2.98±0.42	4.03±0.16	4.17±0.09
PLT ($\times10^9$/L)	308.00±10.09	202.25±51.30	328.00±67.90	301.25±84.62

2.4 Diagnosis

Physical findings and clinical history are very important in establishing diagnosis. Diagnosis symptoms in

humans includes irregular fever, enlargement of the lymph node (particularly those of the posterior triangle of the neck, which is known as Winterbottum's signs), delay sensation to pains (kerandel's signs). Definite diagnosis depends upon demonstration of trypomastigote in blood. Lymphnode aspirates, sternum bone marrow and CSF.

Because of greater frequency of higher parasiteamia, there is a better chance detecting organisms in the body fluids in infections cause by *T. b. rhodesiense* than *T. b. gambianse.* Because of priority parasite number in the blood may vary therefore multiple specimen should be collected and a number of technique should be used to detect the trypomastigote (Lumsden *et. al.,* 1981).

Health care personel most adhere to universal percussions when handle specimen from patients suspected of having African trypanosomiasis because the trypomastigoteis highly infectious. Blood can be collected from either finger prick or venepuncture. Venous blood should be collected in a tube containing EDTA. Multiple slides should be made for examination. And multiple blood examination should be done before trypanosomiasis is rule out. Parasite will be found in large number in the blood during the febrile period and in small number when the patient is afebrile.

Serological techniques, which have been widely used for epidemiological screening, include Indirect Flourescent Antibody Technique (IFAT). ELISA, the Indirect Haemaglutination Test and Card Agglutination Trypanosomiasis Test (CATT) (De Raadt, and Seed 1997, and Kakoma *et, al*., 1985). *T. b. gambianse* isolation in small laboratory animals is usually unsuccessful; in contrast, *T. b. rhodesiense* readily infect animals. Cultivation is not practical for most diagnostic laboratory but is more successful than animal inoculation (Aerts *et al*, 1992).

2.5 The Use Of Lime Juice In The Treatment Of Parasitic Infections

Over the years, medical plants have been recognized to be of great importance to the health of individuals and communities. In many developing countries, herbal medicines are assuming greater primary care and their international trade has increased. However the markets in these countries are not adequately regulated and many herbal products in circulation are unregistered by national regulatory bodies (WHO, 1996).

A survey was carried out using a structure questionnaire among 500 residents, aged 18-80 years, living in an urban community in Port Harcourt in South Eastern Nigeria, to determine their use of medical plants as home remedies in the treatment of malaria; of the 500 questionnaires for this study, 308 were completed and returned by respondents. Of this number of respondents 40.3% use medical plants for the treatment of malaria in their homes in addition to synthetic anti-malaria drugs. The plants commonly used by respondents are lemon grass (*Cymbogon citrates;* 21.2%). Lime (*Citrus limetta;* 17.6%), neem (*Azadirechta indica;* 17.2%) guava (*Asidium guajara;* 11.2%), pawpaw (*Carica papaya;* 8.6%) bitter leaf (*Vernonia amygdalina,*7.8%), sweet orange (*Citrus sinensis;* 5.3%), mango (*Mangigera indica;* 3.5%), and other herbal remedies 7.6%. the plants were either used as orally administered decoctions or employed in steam baths (Ebong *et al,* 1999).

It has been reported that mange is treated by scrubbing the skin lesion with the fibrous palm kernel

fruit waste with the addition of lime, kitchen salt, lime juice and palm oil for a couple of weeks. (Okolo and Unaigbe, 1984).

Lime juice and red potash, was reported to be used in the treatment of helminthiasis in poultry by adding the mixture into their drinking water in Nigeria (Fajimi *et al,* 2005).

Table 5: Comparing base-line parameters with other parameters in control 1.

Parameter (Base-line)	Difference	t-Value	P-Value
PCV cI – PCVcI wk I	3	0.151	0.890
PCV cI – PCVcI wk 2	3	0.435	0.435
PCV cI – PCVcI wk 3	3	0.583	0.601
Hb cI – Hb cI wk 1	3	0.650	0.950
Hb cI – Hb cI wk 2	3	2.644	0.077
Hb cI – Hb cI wk 3	3	0.501	0.650
Wbc cI – wbc cI wk 1	3	0.598	0.598
Wbc cI – wbc cI wk 2	3	0.161	0.882
Wbc cI – wbc cI wk 3	3	0.136	0.901
Rbc c1 – Rbc c1 wk 1	3	1.492	0.233
Rbc c1 – Rbc c1 wk 2	3	1.351	0.269
Rbc c1 – Rbc c1 wk 3	3	0.545	0.624
Plt c1 – Plt c1 wk 1	3	1.460	0.081
Plt c1 – Plt c1 wk 2	3	1.797	0.099
Plt c1 – Plt c1 wk 3	3	1.564	0.134

P<0.05 is significant.
Key;
c1= Control 1
wk = Week

2.6 Control

In areas where African sleeping sickness is endemic, the trypanosome has been controlled by eliminating the parasite through regular population screening programs. This approach effectively reduced the prevalence of the disease to low level, however, with interruptions in surveillance, resurgence of the disease usually occur.

The most effective control measures include (i) an integrated approach to reduce the human reservoir of infection and (ii) the use of insecticides and fly traps (Molyneux, 1983).

In regions where the disease is endemic, natives appear to be more resistant t infection than are new arrivals to the areas, even though there is no evidence of acquired immunity (Murray *et al,* 1984). West Africa sleeping sickness effects primarily rural populations and tourist are rarely infected. Chemoprophylaxis is not recommended because of drug toxicity, and vaccines are unavailable. It may be possible to develop a vaccine, because immunity to re-infection occurs with *T. b. gambiense* (Khonde *et al,* 1995). Other measures used were the reduction of human contact in areas of endemicity, reduction of vegetation around human settlements, insecticides spray, and prophylactic treatment of domestic animals.

Table 6: Comparing base-line parameters with other parameters in control II.

Parameter(Base-line)	Diff	t-Value	P-Value
PCV c2 – PCVc2 wk I	3	2.447	0.920
PCV c2 – PCVc2 wk 2	3	3.600	0.037
PCV c2 – PCVc2 wk 3	3	2.770	0.070
Hb c2 – Hb c2 wk 1	3	2.495	0.088
Hb c2 – Hb c2 wk 2	3	6.898	0.006
Hb c2 – Hb c2 wk 3	3	6.460	0.080
Wbc c2 – wbc c2 wk 1	3	3.350	0.099
Wbc c2 – wbc c2 wk 2	3	4.275	0.023
Wbc c2 – wbc c2 wk 3	3	3.833	0.031
Rbc c2 – Rbc c2 wk 1	3	2.582	0.082
Rbc c2 – Rbc c2 wk 2	3	6.654	0.007
Rbc c2 – Rbc c2 wk 3	3	2.149	0.059
Plt c2 – Plt c2 wk 1	3	2.149	0.121
Plt c2 – Plt c2 wk 2	3	4.785	0.017
Plt c – Plt c2 wk 3	3	4.136	0.026

P<0.05 is significant.
Key: c2 = Control 2.
Wk = week

3. Materials and method

3.1 materials, reagents and equipments

This includes:

Cotton wool, methanol, Xylene, needles, Refrigerator (concord-Deluxed food master), Normal saline, Glass slide, cover-slip, BC-2800VET Auto-Haematology Analyzer, 2ml EDTA container, weighing balance and Microscope (Olympus)

3.2 Methodology

3.2.1 Collection Of Lime Fruits

The lime fruits (*Citrus limetta*) used for this work were plucked from a garden in Bukuru, Jos-Plateau State.

3.2.2 The Climate Of Jos Town

Bukuru is a town in Jos-South Local Government Area of Plateau State. Jos topographically, lies in the highest part of an altitude of 4000ft above sea level with sticking topographical features; it is also an erosion relic which covers about 3000 square miles. The highest surface covers somewhere around Kuru and Gyel district where Rivers, tributaries flow to Lake Chad, Rivers Niger, Benue and Gongola. Its watershed presents rather high ridges of appropriately rocky mountains that feature as water divides. This gives an undulating, swampy plain diversified by the ridges and low granitic inselbergs. They are carved out from narrow rocky valleys that portray picturesque waterfalls and mass gorges (Gwom, 1992).

The climate is generally of rainfall which varies from "2 to 12" with an annual estimate reaching 58ft in April to September. March and early April are the hottest months of the year. July and August are very cold with the mean maximum temperature of about 60^{o}F and the mean minimum temperature of 40^{o}F. Low humidity reaches less than 20% between November and March.

The Vegetation consists of grass and grove of canarium, Oranges and Mango trees. Most parts of land show flat-top lateritic hills and isolated streams which host in natural woods especially bamboo. The federal geological survey of Plateau state found four groups of soil, Alluvial, South belt forest soil, the interior zone of laterite, and the northern zone of sandy soil with Jos and its environs lying in the interior zone of the laterite soils. (Gwom, 1992). The Map of Jos-South Local Government is shown in Appendix ii.

3.2.3 Preparation Of Lime Fruit And Cold Water Extraction

- 2.5kg (2500mg) of the washed fruits was blended using electric blender.
- 4 litres of distilled water was added, mixed and filtered using muslin material.
- The filtrate was poured in plates.
- The plates were oven dried at 50^{0}C for 72hrs.
- The extract was scrap from the plates and grinded in a mortar and stored in desiccators (Emeruah, 1982).

3.2.4 Detection Of Phytochemistry Of Lime Fruit Extract

Resin, tannin, saponin, flavonoids, glycoside were found in the extract while alkaloid was not present.

3.2.5 Determination Of Acute Toxicity (Lethal Dose 50) Of The Fruit Extract

Lethal Dose (LD_{50}) this is a single dose which kills half of an experimental laboratory population.

Table 7: Comparing base-line parameters with other parameters in Test I.

Parameter (Base-line)	Diff	t-Value	P-Value
PCV t1 – PCV t1 wk 1	3	1.000	0.391
PCV t1 – PCV t1 wk 2	3	1.431	0.248
PCV t1 – PCV t1 wk 3	3	2.925	0.061
Hb t1 – Hb t1 wk 1	3	1.104	0.350
Hb t1 – Hb t1 wk 2	3	0.038	0.970
Hb t1 – Hb t1 wk 3	3	0.267	0.807
Wbc t1 – wbc t1 wk 1	3	2.679	0.075
Wbc t1 – wbc t1 wk 2	3	1.797	1.700
Wbc t1 – wbc t1 wk 3	3	2.570	0.082
Rbc t1 – Rbc t1 wk 1	3	1.321	0.278
Rbc t1 – Rbc t1 wk 2	3	1.321	0.278
Rbc t1 – Rbc t1 wk 3	3	1.508	0.229
Plt t1 – Plt t1 wk 1	3	1.638	0.200
Plt t1 – Plt t1 wk 2	3	1.710	0.186
Plt t1 – Plt t1 wk 3	3	1.670	0.178

P<0.05 is significant.
Key: t1 = test 2
wk = week

3.3 Preparation Of Extract Solution

20,000mg of powdered extract was dissolved in 100ml of distilled water to give 200mg/ml concentration.

3.4 Procedure For Ld_{50} Determination

Thirteen male Mice were used; the animals were separated in four groups (group 1-4 with 3 mice each and group 4 with 1 mice) and starved for 12 hours before given them the extract suspension orally. After which they were given water and feed and then observed for signs of toxicity for 24 hours and two weeks.

Group 1: animals were given 10mg/kg body weight.

Group 2: animals were given 100mg/kg body weight.

Group 3: animals were given 1000mg/kg body weight.

Group 4: animals were given 5000mg/kg body weight.

Group 5: animal was given no extract solution only feed and water (Akinpelu and Onakoya, 2006).

3.5 Experimental Animals

Adult male rabbits were obtained and housed from the animal house, Federal College of Veterinary and Medical Laboratory Technology NVRI Vom, Plateau State. They were fed on commercially prepared pelletized feed and Distilled water for seven days to acclimatize. Sixteen (16) male rabbits were used for this work.

3.6 Assessment Of Base Line Parameters

The weight of each rabbit was taken after acclimatizing for a week as an index of physical status of the animals. 2ml of blood from each rabbit was collected into an EDTA container and was analyzed using BC-2800VET Haematology Analyzer.

Table 8: Comparing base-line parameters with other parameters in Test II.

Parameter (Base-line)	Difference	t-Value	P-Value
PCV t2 – PCV t2 wk I	**3**	1.00	0.391
PCV t2 – PCV t2 wk 2	**3**	1.00	0.391
PCV t2 – PCV t2 wk 3	**3**	1.73	0.182
Hb t2 – Hb t2 wk 1	**3**	1.73	0.273
Hb t2 – Hb t2 wk 2	**3**	1.33	0.230
Hb t2 – Hb t2 wk 3	**3**	1.55	0.110
Wbc t2 – wbc t2 wk 1	**3**	2.24	0.391
Wbc t2 – wbc t2 wk 2	**3**	1.00	0.204
Wbc t2 – wbc t2 wk 3	**3**	1.61	0.073
Rbc t2 – Rbc t2 wk 1	**3**	2.71	0.390
Rbc t2 – Rbc t2 wk 2	**3**	1.00	0.390
Rbc t2 – Rbc t2 wk 3	**3**	1.00	0.215
Plt t2 – Plt t2 wk 1	**3**	1.57	0.763
Plt t2 – Plt t2 wk 2	**3**	0.44	0.688
Plt t2 – Plt t2 wk 3	**3**	2.511	0.087

P<0.05 is significant.
Key: t2 = test 2
wk = week

3.7 Design Of The Study Groups Of The Animals

The animals were divided in four groups of four rabbit each;

Control I:- Uninfected and not treated.

Control II:- Infected but not treated.

Test I:- Infected and treated with 100mg/kg body weight of extract.

Test II:- Infected and treated with 200mg/kg body weight of extract.

3.8 The Source Of The Trypanosome Used

The parasite used for this work was *Trypanosoma brucei brucei* which was obtained from Nigerian Institute of Trypanosomiasis Research (NITR) Vom-Plateau state.

3.9 Inoculation Of Experimental Animals

The experimental animals were taking to Nigerian Institute for Trypanosomiasis Research (NITR) Vom where they were inoculated with *T.b. brucei* by Rabbit inoculation technique (World Organization for Animal Health, 2000).The blood was diluted 50:50 with normal saline before injecting intraperitoneally into the rabbits, each inoculums contains about 1×10^6 of parasites (Herbert and Lumbsden, 1976).

3.10 Determination Of Parasitaemia

A week after inoculation the infected animals were taken to Nigerian Institute for Trypanosomiasis Research (NITR) Vom and there they established the existence of the infection (trypanosomiasis) in the rabbits, using wet preparation method and the parasite number was estimated using "A rapid matching method for estimating host parasitaemia at 400 magnifications with *T. b. brucei* (Herbert and Lumbsden, 1976).

3.11 Preparation Of Extract Suspension;

20,000mg of the powdered extract was dissolved in 100ml of distilled water (representing 200mg/ml concentration) and allowed to stay overnight for proper dissolution.

3.12 Administration Of Extract Suspension

The route of administration of extract suspension used in this work was oral (route). The animals were given the extract daily base on their weight seven days after inoculation and establishment of infection;

Group 111:- Infected and treated with 100mg/kg body weight.

Group 1V:- Infected and treated with 200mg/kg body weight.

Collection Of Blood Sample

The animals were bled once in seven days for the period of four weeks.

3.13.1 Venepuncture Method Procedure;

- The animal was restrained.
- The ear was swabbed with cotton wool sucked in xylene.
- 2ml needle was inserted into the vein and blood was collected into 2ml EDTA container and mixed properly. This was done to all the rabbits.
- The blood samples were transported to the laboratory and analyzed within 3 hours of collection (Morton *et. al.*,1993).

3.13.2 Sample Analysis

The samples were analyzed automatedly using BC-2800VET Haematology Analyzer.

3.13.3 Procedure For Operating Bc-2800vet;

- Connect the machine to power source.
- Start up machine and allow it to perform auto-rinsing.
- Go to the menu and select the subject for analysis (rat, goat, horse or rabbit etc).
- Allow the blood to mix well on automatic blood mixer for 15 minutes.

- Slot in the well mixed blood to the sample probe.
- Press the start button to aspirate sample.
- The sample is analyzed within 3 minutes and the result is printed.
- The machine rinse out itself automatically after running each sample.
- Switch off the machine after the auto-rinsing.
- Disconnect it from power source and cover it.

Table 9: Comparing Control 1 and Test I

	Control 1		**Test 1**				
Parameters	**N**	x±S.D.	**N**	x±S.D.	dif	t-value	p-values
PCV(%) wk1	4	36.30±3.37	4	35.15±1.8	3	0.445	0.686
PCV(%) wk2	4	38.60±4.65	4	35.70±1.2	3	1.066	0.364
PCV(%) wk3	4	39.35±4.90	4	37.55±1.22	3	0.847	0.459
Hb(g/L) wk1	4	340.0±20.0	4	348.75±23.7	3	0.529	0.633
Hb(g/L) wk2	4	346.50±6.75	4	337.7±30.6	3	0.529	0.633
Hb(g/L) wk3	4	345.27±16.4	4	341.0±28.9	3	0.337	0.758
WBC($\times10^9$/L) wk1	4	69.15±5.13	4	87.27±1.79	3	6.109	0.090
WBC($\times10^9$/L) wk2	4	67.20±9.90	4	85.33±3.90	3	4.473	0.021
WBC($\times10^9$/L) wk3	4	66.82±8.39	4	91.03±5.85	3	5.251	0.013
RBC($x10^{12}$/L) wk1	4	4.43±0.49	4	4.15±0.33	3	1.614	0.205
RBC($x10^{12}$/L) wk2	4	4.72±1.00	4	4.15±0.33	3	0.871	0.448
RBC($x10^{12}$/L) wk3	4	4.15±0.78	4	4.03±0.15	3	0.277	0.800
PLT ($\times10^9$/L) wk1	4	329.25±5.70	4	397.5±30.35	3	4.134	0.026
PLT ($\times10^9$/L) wk2	4	320.75±7.7	4	316.25±83.9	3	0.101	0.925
PLT ($\times10^9$/L) wk3	4	308.00±10.0	4	328.0±67.9	3	0.692	0.539

Key: P<0.05 is significant.
Wk = week

3.13 Result Analysis

The result obtained was subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) version 17 for statistical difference at 95% confidence limit via student t-test. The results are represented in the tables below:

Table 10: Comparing Control I and Test II

Parameters	**N**	x±S.D.	**N**	x±S.D.	dif	t-value	p-values
PCV(%) wk1	4	36.30±3.37	4	38.07±0.90	3	1.221	0.309
PCV(%) wk2	4	38.60±4.65	4	38.07±0.90	3	0.231	0.832
PCV(%) wk3	4	39.35±4.90	4	38.47±0.45	3	0.371	0.735
Hb(g/L) wk1	4	340.0±20.0	4	337.75±2.70	3	1.720	0.184
Hb(g/L) wk2	4	346.50±6.75	4	340.50±6.57	3	1.720	0.184
Hb(g/L) wk3	4	345.27±16.4	4	342.50±4.36	3	0.372	0.735
WBC($\times10^9$/L) wk1	4	69.15±5.13	4	78.15±2.18	3	5.239	0.014
WBC($\times10^9$/L) wk2	4	67.20±9.90	4	79.78±3.64	3	3.144	0.052
WBC($\times10^9$/L) wk3	4	66.82±8.39	4	86.1±4.89	3	3.237	0.048
RBC($x10^{12}$/L) wk1	4	4.43±0.49	4	4.05±0.10	3	9.667	0.020
RBC($x10^{12}$/L) wk2	4	4.72±1.00	4	4.17±0.09	3	51.494	0.000
RBC($x10^{12}$/L) wk3	4	4.15±0.78	4	4.17±0.09	3	0.067	0.951
PLT ($\times10^9$/L) wk1	4	329.25±5.70	4	372.0±40.4	3	2.118	0.124
PLT ($\times10^9$/L) wk2	4	320.75±7.7	4	364.25±49.9	3	1.568	0.215
PLT ($\times10^9$/L) wk3	4	308.00±10.0	4	301.25±84.6	3	0.158	0.884

Key: P<0.05 is significant.
Wk = week.

Table 11: Comparing Control II and Test I

	Control II		**Test I**			
Parameters	**N**	x±S.D.	**N**	x±S.D.	dif	t-value	p-values
PCV(%) wk1	**4**	33.40±2.74	**4**	35.15±1.8	**3**	1.173	0.326
PCV(%) wk2	**4**	30.80±0.95	**4**	35.70±1.2	**3**	4.508	0.020
PCV(%) wk3	**4**	31.87±2.09	**4**	37.55±1.22	**3**	12.534	0.001
Hb(g/L) wk1	**4**	312.25±14.8	**4**	348.75±23.7	**3**	2.781	0.069
Hb(g/L) wk2	**4**	292.75±5.60	**4**	337.7±30.6	**3**	2.781	0.069
Hb(g/L) wk3	**4**	291.75±5.6	**4**	341.0±28.9	**3**	3.188	0.050
WBC($\times10^9$/L) wk1	**4**	82.62±3.53	**4**	87.27±1.79	**3**	2.557	0.083
WBC($\times10^9$/L) wk2	**4**	89.8±4.80	**4**	85.33±3.90	**3**	1.978	0.142
WBC($\times10^9$/L) wk3	**4**	90.7±4.34	**4**	91.03±5.85	**3**	0.319	0.771
RBC($x10^{12}$/L) wk1	**4**	3.43±0.29	**4**	4.15±0.33	**3**	5.000	0.015
RBC($x10^{12}$/L) wk2	**4**	3.55±0.42	**4**	4.15±0.33	**3**	1.796	0.170
RBC($x10^{12}$/L) wk3	**4**	2.97±0.47	**4**	4.03±0.15	**3**	6.332	0.008
PLT ($\times10^9$/L) wk1	**4**	401.50±31.7	**4**	397.5±30.35	**3**	0.162	0.882
PLT ($\times10^9$/L) wk2	**4**	233.75±19.1	**4**	316.25±83.9	**3**	2.141	0.122
PLT ($\times10^9$/L) wk3	**4**	202.25±51.3	**4**	328.0±67.9	**3**	2.182	0.117

Key: P<0.05 is significant.
Wk = week.

Table 12: Comparing Control II and Test II

	Control II		**Test II**			
Parameters	**N**	x±S.D.	**N**	x±S.D.	dif	t-value	p-values
PCV(%) wk1	**4**	33.40±2.74	**4**	38.07±0.9	**3**	2.631	0.078
PCV(%) wk2	**4**	30.80±0.95	**4**	38.07±0.9	**3**	27.187	0.000
PCV(%) wk3	**4**	31.87±2.09	**4**	38.47±0.45	**3**	22.264	0.000
Hb(g/L) wk1	**4**	312.25±14.8	**4**	337.75±2.7	**3**	16.645	0.000
Hb(g/L) wk2	**4**	292.75±5.60	**4**	340.50±6.57	**3**	16.645	0.000
Hb(g/L) wk3	**4**	291.75±5.6	**4**	342.50±4.36	**3**	12.796	0.001
WBC($\times10^9$/L) wk1	**4**	82.62±3.53	**4**	78.15±2.18	**3**	2.555	0.084
WBC($\times10^9$/L) wk2	**4**	89.8±4.80	**4**	79.78±3.64	**3**	3.202	0.049
WBC($\times10^9$/L) wk3	**4**	90.7±4.34	**4**	86.10±4.89	**3**	1.848	0.162
RBC($x10^{12}$/L) wk1	**4**	3.43±0.29	**4**	4.05±0.10	**3**	4.134	0.026
RBC($x10^{12}$/L) wk2	**4**	3.55±0.42	**4**	4.17±0.15	**3**	4.186	0.025
RBC($x10^{12}$/L) wk3	**4**	2.97±0.47	**4**	4.17±0.09	**3**	4.707	0.018
PLT ($\times10^9$/L) wk1	**4**	401.50±31.7	**4**	372.0±40.4	**3**	1.185	0.321
PLT ($\times10^9$/L) wk2	**4**	233.75±19.1	**4**	364.25±49.9	**3**	11.238	0.002
PLT ($\times10^9$/L) wk3	**4**	202.25±51.3	**4**	328.0±67.9	**3**	1.721	0.184

Key: P<0.05 is significant.
Wk = week.

4. Discussion

This study was carried out with the aim at evaluating the effect of lime fruit extract in *Trypanosoma b. brucei*-infected rabbits and to compare their values with uninfected rabbits. All statistical analyses were done using Statistical Package for Social Sciences (SPSS) version 17.0. Data were expressed as Mean±S.D. Differences among the test and control groups were examined using a student t-test at 95% confidence limit. When P< 0.05 the value is considered to be statistically significant. Total number of 16 rabbits were used which were placed in to test and control groups.

Table I shows the Mean ±S.D. Values of PCV, Hb, WBC, RBC and PLT of both the controls and tests groups at day '0'; before the animals were infected. This served as base-line parameter for other parameters within the group.

Table 13: The result of phytochemistry of the lime fruit (*citrus limetta*) extract.

s/no	SUBSTANCE	FRUIT EXTRACTS
1	Resins	+
2	Alkaloids	-
3	Tannin	+
4	Saponin	+
5	Glycosides	+
6	Flavonoid	+

KEY:
+ = Present
- = Absent

Table 2 to 4 show the Mean±S.D values of PCV, Hb, WBC, RBC and PLT at first , second and third week of treatment with the extract for the test groups. It was observed that the mean ±S.D of PCV, Hb and RBC of control II reduced from (36.45±3.1 to 31.4±0.95), (341.0±8.5 to 291.5±5.63) and (4.43±0.60 to 2.98±0.42) respectively when compared with base-line parameters in table I. These values remained almost the same with their respective base-line parameters in control I, Test I and Test II. However, the mean ±S.D of the WBC parameter increased in all the groups like in control I; there was a mean ±S.D increase of (73.10±8.51 to 82.65±81) within three weeks post-infection.

Table 5 shows the Comparison of the base-line parameters with their respective parameters collected at week intervals within the same group. The t- and p-values of the pair of PCV, Hb, WBC, RBC and PLT as shown on this table are not significant as $p>0.05$ in all the parameters in this group. This is because the rabbits in this group (control I) were not infected and so they lived their normal. However, those in control II group (infected but not treated) had statistical difference ($p<0.05$) when compared with their respective base-line parameters. These could be as a result of the infection without treatment as shown in table 6.

Tables 7 & 8 show the t- and p-values of Test I and Test II groups as they are compared with their respective base-line parameters. In all the groups there was no statistical difference as all the parameters showed $p>0.05$ with the exception of PCV at week 3 that had $p<0.05$. This could be attributed to the effect of the lime fruits extract when compared with the infected and not treated group.

Tables 9 &10 show the mean ±S.D, t-values and p-values of the comparisons between (control I and Test I) and (control I and Test II) respectively. As shown on the two tables almost all the parameters had statistical difference ($P<0.05$) in most of the weeks. Tables 11 & 12 show the comparison between (control II and Test I) and that of (control II and Test II); in both tables $p<0.05$.

Table 14: The Toxicity testing result.

Group	Dose per kg body weight	observation after two weeks
1	10mg/kg body weight	_
2	100mg/kg body weight	_
3	1000mg/kg body weight	_
4	5ooomg/kg body weight	_
5	Nil	_

KEY; - = No sign of toxicity observed.

5.1 Conclusions

Trypanosoma brucei brucei-infected rabbit that were not treated with the lime extract had a reduced PCV, RBC, Hb and WBC this is in line with this report; *T. b. brucei* is a tissue parasite which induces anaemia in infected Rabbits as well as other susceptible animals, such as cattle, dogs, rats and mice (Jenkins *et. al*., 1980).

The infected and treated (rabbit) with lime fruits extract showed statistical difference ($p<0.05$) when compared with the infected but not treated which has $p>0.05$. It can then be concluded that the lime fruits had an effect on most of the haematological parameters of T. b. brucei-infected rabbits as suggested by (Fejimi and Taiwo, 2004).

5.2 Recommendations

Base on the findings of this research work, we want to recommend more trials on this extract singly or in combination with other herbs in much higher concentrations. This is because of the positive effect demonstrated by the extract on the haematological parameters of the treated rabbits. The extract was also found to be non-toxic this makes its handling safer and because parasitic infections with severe anaemia are prevalent in the African continent.

Acknowledgement

We want to appreciate all the staff of State Specialist Hospital Gombe for their support.

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11/5/2015