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Full Length Research Paper

Evaluation of immunostimulatory effects of viracomb(Rx) in rabbits

A. Y. Tijani[1*], O. E. Ojo[2], D. K. Oladepo[3], O. A. Salawu[1], S. B. Mohammed [3], A. U. Osunkwo[1], K. S. Izebe[3] and Y. Yaaba[3]

[1]Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria.
[2]EOL limited, Ado - Ekiti, Ekiti State, Nigeria.
[3]Department of Microbiology and Biotechnology National Institute for Pharmaceutical Research and Development (NIPRD) Idu, Abuja, Nigeria.

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Viracomb is a herbal based preparation used in management of conditions associated with immuno-supression. The present study was undertaken to evaluate the immunostimulatory effect of Viracomb in rabbits. Acute toxicity study was carried out in rabbits using acute toxic class method. In the immunostimulatory study, healthy adult rabbits were randomized into five groups of five rabbits per group. Group I rabbits served as the positive control and received 10 ml normal saline/kg while rabbits in groups II, III, IV and V received 50 mg cyclophosphamide/kg body weight on days 0, 2 and 4 and thereafter, rabbits in groups II, III and IV were given daily doses of 75, 150 and 300 mg Viracomb/kg body weight for 28-days while group V served as the negative control. The effect of viracomb on feed and water intake, body weight changes, CD4+ count, haematological and biochemical parameters were evaluated. The oral median lethal dose was estimated to be greater than 5000 mg extract/kg body weight. Viracomb exerted significant decrease in feed intake at 75 mg/kg while slight increase in feed intake was observed at 150 and 300 mg /kg body weight. Significant ($p < 0.05$) decrease in water intake was observed at all dose levels used for the study. There was no remarkable change in body weight of rabbits at all doses used for the study. There was significant ($p < 0.05$) increase in CD4+ counts of rabbits given 150 mg / body weight kg while unremarkable changes were observed in CD4+ counts of rabbits given 75 and 300 mg/kg body weight respectively at the end of the 28-days study. Significant dose dependent increase in haematological profiles was observed while biochemical parameters remained normal. These findings indicate that viracomb is safe acutely and possess immunostimulatory activity and thus, provide evidence for its acclaimed effect in the management of immuno-suppressed related disorders.

Key words: Viracomb, cyclophosphamide, immuno-suppressed, rabbits, immunostimulatory.

INTRODUCTION

The World Health Organization (WHO) estimates that 1500 people die each hour from infectious diseases including HIV/AIDS (WHO, 2008). The Acquired Immune Deficiency Syndrome (AIDS) is caused by the human immunodeficiency virus (HIV) and AIDS is one of the leading causes of death in the world (Seema et al., 2006). In some countries, the prevalence of HIV infection among adult has grown as high as 35%, with life expectancy decreasing by 20 years.

Herbal medicine plays an important role in the management of HIV/AIDS in developing African countries due to inadequate access, lack of adherence and compliance caused by adverse and toxic reactions and limited treatment options associated with the available antiretrovirals. Approximately 80% of the world's population still relies mainly on phytomedicines for primary health care and the remaining 20% use plant products as

*Corresponding author. E-mail: ade_dat@yahoo.com. Tel: +234-8121923668.

ingredients for several drugs (Farnsworth, 1990). Ethno medicines provide a diverse range of natural products with antimicrobial and immune modulating potential. These agents promote positive health and maintain organic resistance against infection by re-establishing the body's equilibrium and conditioning the body's tissues (De P et al., 1998). 'VIRACOMB' is one of such herbal preparation that is widely used in the management of HIV/AIDS and other viral related infections in Western part of Nigeria. This study was carried out to evaluate and provide information on the safety and immuno-stimulatory profile of Viracomb in rabbits. Data generated will form part of the preclinical dossier required by the World Health Organization (WHO) and national agency for food, drug administration and control (NAFDAC) in medicinal plant product development prior to clinical trials in human.

MATERIALS AND METHODS

Supply of viracomb

The sample of Viracomb used for this study was supplied to the Department of Pharmacology and Toxicology, NIPRD as scored (250 mg) tablet by Mr. Emmanuel Oluwaseyi Ojo of EOL Limited, Akure-Nigeria.

Chemicals

Cyclophosphamide (Korea United Pharm., Batch number-E659703, Manufactured-date; 01, August, 2007.Expiry date; 31 July, 2010) was obtained from Alpha Pharmacy and stores Lagos, Nigeria.

Animals

Healthy adult rabbits of either sex weighing (1.20 - 1.5 kg) obtained from the animal facility centre (AFC), Department of Pharmacology and Toxicology, of the national institute for pharmaceutical research and development (NIPRD), Idu, Abuja, Nigeria, were used for the study. They were housed in stainless steel cages at a temperature of 25 ± 2°C and observed under a 12 h light/dark cycle in a well ventilated room for 2 weeks before the experiment. They were fed with grower's mash and water *ad libitum.* Animals used in this study were housed and cared for in accordance with the "National Institute for Health(NIH) guide for care and use of laboratory animals" NIH publication No. 5 revised (1978) and national institute for pharmaceutical research and development (NIPRD) -Standard operating procedures (SOP) on immunostimulatory studies.

Acute toxicity study

The oral acute toxicity study was carried out using acute- toxic class method described by Schlede et al. (1994). The study was carried out in two phases. In the first phase 300 mg/kg of the preparation was administered to three adult nulliparous non-pregnant female rabbits. These rabbits were observed for signs of toxicity (which include but not limited to paw-licking, salivation, stretching, lachrymation, diarrhoea, lethargy, sleep, convulsion, and coma) and mortality for the first 4 critical hours, thereafter for 72 h and subsequently daily for 7 days. In the second step of the first phase, the procedure was repeated as above in another set of three adult non-pregnant female rabbits given 300 mg/kg due to the absence of death in the group treated with 300 mg/kg in the first step. In the second phase of the study, 2000 mg/kg of the preparation was given to a new set of three adult nulliparous non-pregnant female rabbits. These were observed for signs of toxicity and mortality as described earlier on. The same procedure was repeated at 2000 mg/kg in another set of three adult nulliparous non-pregnant female rabbits. Acute limit test was carried out at the end of the second phase. Three adult nulliparous non-pregnant female rabbits were given 5000 mg viracomb/kg. These rabbits were observed closely for signs of toxicity and mortality in the first 4 critical hours, thereafter for 72 h and subsequently daily for 7 days.

Immuno stimulatory study

Immuno-stimulatory activity of 'viracomb' was evaluated using the method described by (Ziauddin et al., 1996). Thirty adult rabbits of either sex were divided into five groups of six rabbits per group. Group I which served as the normal control was given normal saline. Groups II, III, IV and V were given 50 mg cyclophosphamide /kg body weight on days 0, 2, and 4, respectively. Thereafter, rabbits in group II were given 10 ml normal saline/kg body weight while those in groups III, IV and V received 75, 150 and 300 mg viracomb/kg body weight of the preparation daily for 28days. After every 7 days, that is, 7, 14, 21 and 28 day of the study, blood samples were withdrawn from the ear vein of rabbits in each treatment group for CD4 count determination using flow cytometer and haematological parameters using Sysmex KX21 N haematology analyzer. Another blood portion was dispensed into plain bottles and allowed to clot and centrifuged at 1500 rpm for 10 min. The sera were separated and stored at - 20°C and these samples were assayed for alanine transaminase (ALT) and aspartate transaminase (AST) levels using colorimetric method of Reitman and Frankel (1957), while alkaline phosphatase (ALP) levels were estimated by (King and Armstrong, 1954). Total and conjugated bilirubin levels were also estimated using the colorimetric method described by (Jendrassik and Grof, 1938), and renal function parameters at the on-set and on the 28th days of the study.

Clinical signs and mortality

During the four week treatment period, all the rabbits were observed daily for signs and symptoms of toxicity and mortality during and up to 6 hours after administration of extract

Feed and water intake

The quantity of feed and the amount of water consumed by rabbits in each group were measured daily as the difference between the quantity of feed and water supplied and the amount remaining after 24 h, respectively.

Body weight change

Rabbits in all the groups were weighed twice every week during the period of treatment.

Statistical analysis

All numerical data are expressed as the mean ± standard error of mean (SEM). Statistical analysis was carried out using One way

Table 1. The effect of viracomb on feed intake.

Group	Week 1	Week 2	Week 3	Week 4
I	234.73 ± 25.88	307.93 ± 2.80	321.33 ± 5.07	323.5 ± 16.14
II	160.70 ± 38.68*	144.53 ± 8.07*	176.88 ± 39.03*	181.78 ± 34.07*
III	209.25 ± 15.43*	291.33 ± 27.54*	328.55 ± 39.05*	293.9 ± 15.55*
IV	213.63 ± 49.51	280.63 ± 10.48*	236.88 ± 11.17*	238.45 ± 6.24*
V	222.78 ± 27.45	233.25 ± 26.03	343.48 ± 39.25	298.03 ± 8.58

*Significantly different from the control at $p < 0.05$; n = 5.

Table 2. The effect of viracomb on water intake.

Groups	Week 1	Week 2	Week 3	Week 4
I	911.45 ± 151.08	1055.00 ± 16.54	1035.55 ± 41.84	1045.00 ± 66.39
II	415.75 ± 149.39*	489.00 ± 93.07*	570.30 ± 26.23*	382.05 ± 88.57*
III	553.25 ± 54.64*	708.00 ± 43.51*	629.48 ± 36.09*	585.60 ± 25.42*
IV	803.50 ± 212.11*	718.30 ± 58.88*	531.88 ± 56.84*	506.70 ± 28.54*
V	917.00 ± 165.73	932.95 ± 44.18	1362.25 ± 54.66	1121.25 ± 110.29

*Significantly different from the control at $p < 0.05$; n = 5.

analysis of variance (ANOVA) followed by student's t-test and differences between means were considered to be significant when $p < 0.05$.

RESULTS

Acute toxicity study

All the animals were alive, healthy and active during the observation period. There were no signs of toxicity and mortality at the doses used for the study. The oral median lethal dose of viracaomb was therefore determined to be greater than 5000 mg Viracomb/kg body weight in rabbits.

Immuno-stimulatory study

Clinical signs and mortality

All the treated rabbits were alive, healthy and active during the observation period. Mortality was not recorded at all dose levels used for the study.

Effect of viracomb on feed intake

The preparation produce significant ($p < 0.05$) decrease in feed intake at doses of 75 and 150 mg/kg body weight throughout the 4 weeks study and on the 2nd and 3rd week of the study at 300 mg/kg body weight. It produce significant ($p < 0.05$) increase in feed intake at 300 mg/kg body weight in the 4th week of the study (Table 1).

Effect of viracomb on water intake

The preparation produced significant ($p < 0.05$) decrease in water intake at all doses used throughout the 4 weeks study.

Effect of viracomb on body weight

There was no significant ($p > 0.05$) change in body weight of rabbits at all doses used for the study (Table 3).

Effect of viracomb on cd4+ count

Significant ($p < 0.05$) decrease in the CD4 +count at 75 mg/kg body weight was observed in the 2nd week of the study. However, at 150 mg/kg body weight, the preparation produce significant ($p < 0.05$) increase in the CD4+ count in the 1st and the 4th weeks of the study. At 300 mg/kg unremarkable change in CD4+ count was observed throughout the 4-weeks study.

Effect of viracomb on haematology

The blood cells counts, haemoglobin concentration and haematocrit remains normal at 75 and 150 mg/kg body weight throughout the duration of the study. However, a significant ($p < 0.05$) increase in the red blood cell counts,

Table 3. The effect of viracomb on body weight of rabbits.

Groups	Week 1	Week 2	Week 3	Week 4
I	1.26 ± 0.13	1.37 ± 0.08	1.46 ± 0.05	1.43 ± 0.06
II	1.43 ± 0.11	1.39 ± 0.04	1.41 ± 0.12	1.43 ± 0.02
III	1.24 ± 0.09	1.00 ± 0.09	1.22 ± 0.09	1.22 ± 0.09
IV	1.33 ± 0.15	1.50 ± 0.09	1.40 ± 0.06	1.42 ± 0.10
V	1.43 ± 0.13	1.39 ± 0.11	1.41 ± 0.12	1.44 ± 0.11

*Significantly different from the control at $p < 0.05$; n = 5.

Table 5a. The effect of viracomb on red blood cells, haemoglobin and haematocrit of rabbits.

Groups	Week 1			Week 2		
	RBC/10^6	HGB	HCT	RBC/10^6	HGB	HCT
I	4.39 ± 0.80	9.63 ± 1.77	31.27 ± 5.73	5.76 ± 0.25	12.40 ± 0.55	39.82 ± 1.88
II	4.89 ± 0.31	9.93 ± 0.48	33.50 ± 1.72	4.06 ± 0.23	9.30 ± 0.78	36.70 ± 2.69
III	5.10 ± 0.28	10.97 ± 0.52	84.60 ± 2.16	5.29 ± 0.11	11.34 ± 0.20	36.16 ± 0.59
IV	5.70 ± 0.38	11.70 ± 0.78	40.50 ± 2.50	6.09 ± 0.18	12.43 ± 0.27	40.63 ± 0.52
V	4.82 ± 0.00	11.10 ± 0.71	36.25 ± 1.52	5.28 ± 0.37	11.36 ± 0.60	36.06 ± 2.08

*Significantly different from the control at $p < 0.05$; n = 5.

Table 5b. The effect of viracomb on red blood cells, haemoglobin and haematocrit of rabbits.

Groups	Week 3			Week 4		
	RBC/10^6	HGB	HCT	RBC/10^6	HGB	HCT
I	5.48 ± 0.15	11.44 ± 0.4	37.10 ± 1.01	6.02 ± 0.35	12.6 ± 0.80	40.82 ± 2.48
II	3.95 ± 0.26	38.27 ± 0.47*	25.43 ±1.30	4.13 ± 0.13	8.70 ± 0.57	27.4 ± 1.44
III	4.33 ± 0.33	9.42 ± 0.40	30.16 ± 2.00	4.40 ± 0.37	9.85 ± 0.53	31.53 ± 2.09
IV	5.77 ± 0.10	12.03 ± 0.07*	38.88 ± 0.29*	5.37 ± 0.11	11.20 ± 0.30	36.50 ± 0.88*
V	3.78 ± 0.90	8.44 ± 1.98	26.04 ± 6.06	4.35 ± 0.45	9.90 ± 0.67	30.032.32

* Significantly different from the control at $p < 0.05$; n = 5.

Table 6a. Effect of viracomb on white blood cell counts and differentials.

Groups	Week 1				Week 2			
	WBC/10^3	LYM	MXD	NEUT	WBC/10^3	LYM	MXD	NEUT
I	7.55 ± 1.54	5.32 ± 0.99	0.53 ± 0.10	3.13 ± 1.15	8.9 ± 0.60	4.02 ± 0.35	4.55 ± 0.63	0.43 ± 0.06
II	13.00 ± 2.1*	10.75 ± 2.0*	0.25 ± 0.04	0.50 ± 0.0*	7.45 ± 1.03	2.65 ± 0.12*	4.50 ± 0.85	0.3 ± 0.07
III	10.97 ± 1.3*	10.18 ± 1.2*	0.27 ± 0.08	0.52 ± 0.1*	11.40 ± 0.82	3.92 ± 0.43	6.00 ± 0.96	0.52 ± 0.10
IV	9.46 ± 1.12	8.90 ± 0.99*	0.34 ± 0.10	0.4 ± 0.08*	7.03 ± 0.80	1.87 ± 0.58*	4.73 ± 0.83	0.43 ± 0.03
V	7.20 ± 0.14	1.15 ± 0.60	5.95 ± 0.74	0.10 ± 0.00	8.16 ± 1.20	3.32 ± 0.53	4.32 ± 0.59	0.52 ± 0.09

*Significantly different from the control at $p < 0.05$; n = 5.

haemoglobin concentration and haematocrit levels were observed at the 300 mg Viracomb/ kg body weight in the 3rd week of the study (Tables 5a and b).

Significant ($p < 0.05$) increase in white blood cell count was observed at 75 mg/kg of in the 2nd and the 4th week of the study. At 150 mg/kg, the preparation produce significant ($p < 0.05$) increase in white blood cell count in the 1st week and in the 1st, 3rd and 4th of the study at 300 mg/kg body weight (Table 6a and b).The preparation produce significant ($p < 0.05$) dose dependent increase in

Table 6b. Effect of viracomb on white blood cell counts and differentials.

Group	Week 3				Week 4			
	WBC/10^3	LYM	MXD	NEUT	WBC/10^3	LYM	MXD	NEUT
I	8.64±1.61	10.18±6.09	14.66±10.08	0.66 ± 0.50	9.06 ± 1.02	24.48 ± 3.35	23.52 ± 2.49	52.00 ± 3.52
II	9.30±2.29	3.10±0.41*	6.6 ± 2.51*	0.23 ± 0.07	15.03 ± 4.03*	22.23 ± 9.87*	6.63 ± 3.39*	71.13 ± 13.19*
III	8.92±1.32	3.88±3.22*	4.86 ± 0.58*	0.18 ± 0.04	9.08 ± 0.74	28.60 ± 2.26*	11.48 ± 0.61*	59.93 ± 2.70*
IV	11.8±1.85	5.43±1.50*	4.43 ± 0.45*	0.37 ± 0.12	9.88 ± 0.90	20.30 ± 7.54*	16.98 ± 4.50	62.73 ± 7.35*
V	6.28±1.44	3.78 ± 0.29	3.9 ± 0.11	0.18 ± 0.02	8.13 ± 0.29	14.08 ± 0.60	16.45 ± 1.44	49.48 ± 6.35

*Significantly different from the control at $p < 0.05$; $n = 5$.

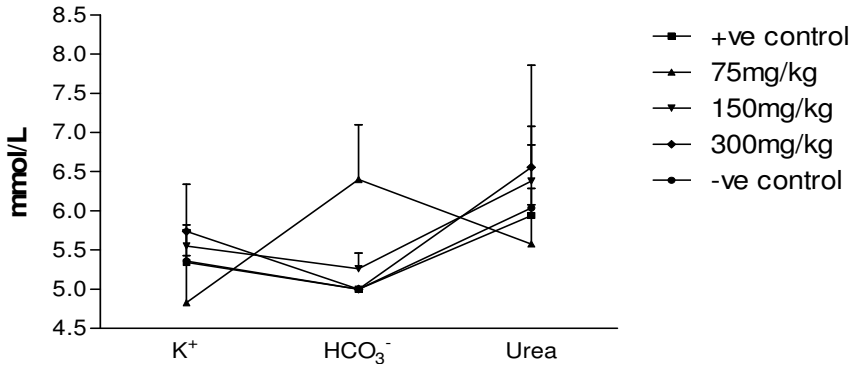

Figure 1a. Baseline values of potassium ion, bicarbonate ion and urea.

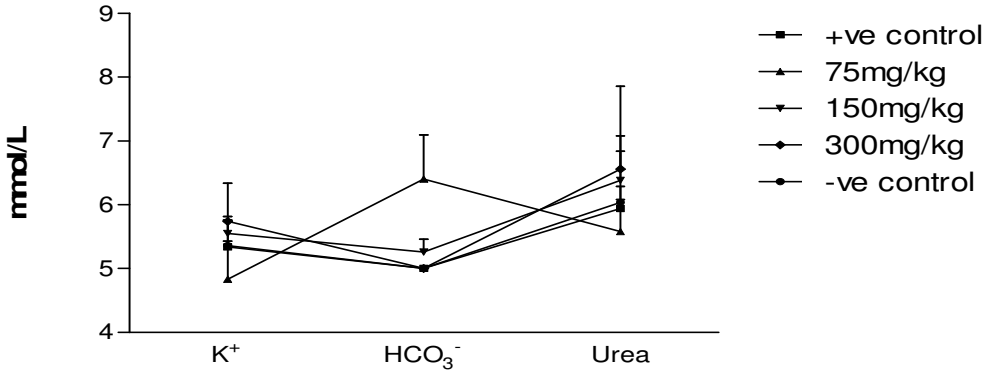

Figure 1b. Serum level of potassium ion, bicarbonate ion and urea concentration after 28 days.

lymphocyte counts in the 1st and the 4th week of the study. At all dose levels used for the study, significant ($p < 0.05$) decrease in neutrophil counts was observed in the first three weeks of the study. However, in the 4th week of the study significant ($p < 0.05$) increase in neutrophil level was observed in the group treated with 75 mg/kg body weight (Table 6b).

Effect of viracomb on electrolyte and renal function

At the end of the 28-days study, the electrolytes, urea and creatinine concentrations remain normal at the doses used for the study when compared with the baseline values (Figures 1a, b, 2a and 2b)

Effect of viracomb on liver function parameters

The liver function parameters remain normal (Figures 3 and 4).

DISCUSSION

The absence of adverse effects and death following oral

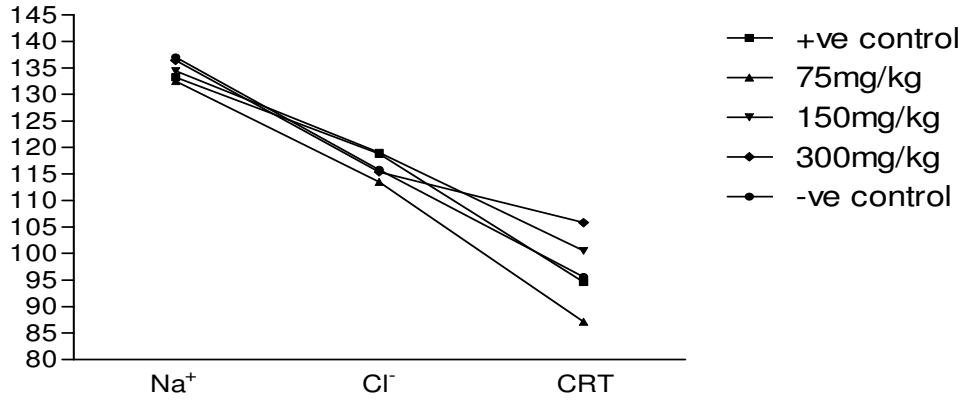

Figure 2a. Baseline values of sodium, chloride and creatinine.

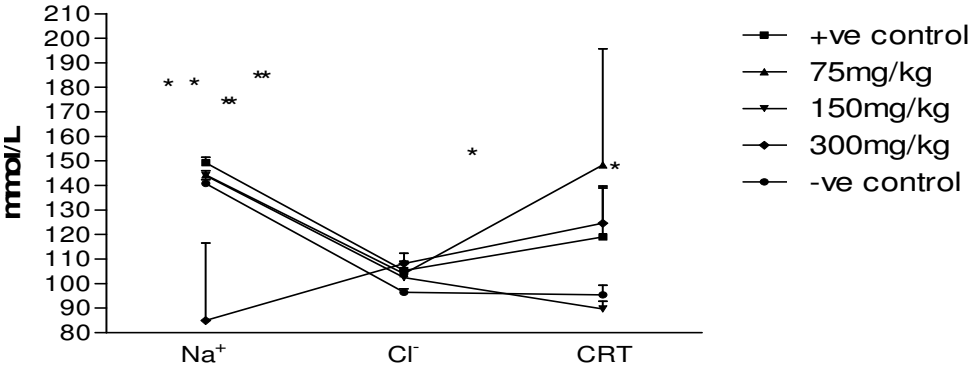

Figure 2b. Serum level of sodium, chloride and creatinine 28 days after treatment.

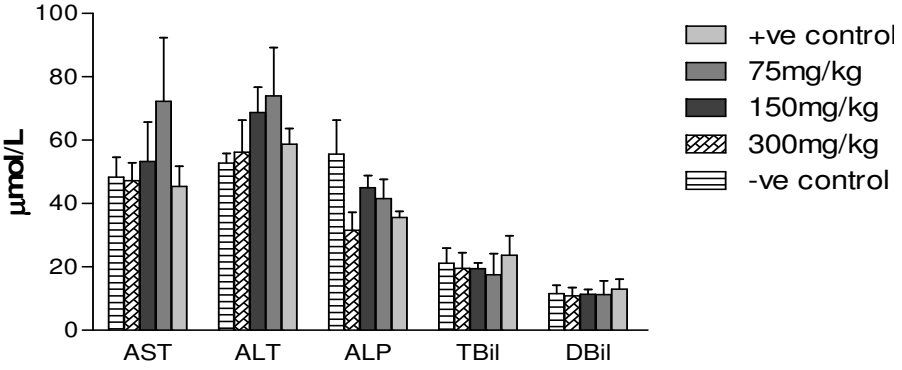

Figure 3. Baseline values of liver function parameter.

administration of Viracomb at a dose of 5000 mg extract/kg body weight observed in rabbits suggests that the preparation is practically non-toxic acutely (Salawu et al., 2010). This safety profile may account for its use in the management of immuno-depressed related disorders. Decrease in body weight is a sensitive indicator of adverse reaction to a toxic test article by a living organism (Peter and Boyd, 1966; Salawu et al., 2008).

There was slight increase in body weight of rabbits at all the doses used for the study. The slight increase in body weight of rabbits at all dose levels used showed that the treatment did not adversely affect the health status of the rabbits and may be due to the inconsistent changes observed in feed and water intake. The results obtained from the study supports the ethnotherapeutic indication of the preparation for management of immuno-supressed

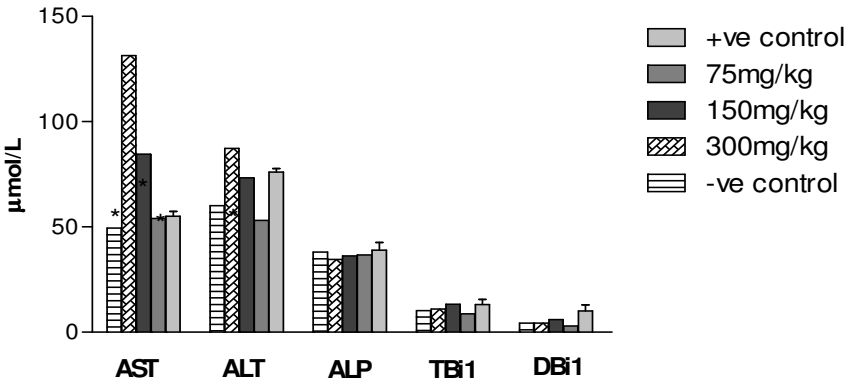

Figure 4. Liver function parameter of rabbits after 28 days.

related disorders. The study affirms that Viracomb is an effective immunostimulatory preparation as evidenced in significant increase in CD4+ count and the Lymphocyte counts of immuno-supressed rabbits given various doses of the preparation. The ability of the viracomb-treated animals to overcoming the side effects of drug-induced immunosuppression characterized by reduced CD4+ count, reduced white blood cell count (WBC) and anemia provides evidence for immunostimulatory effects of Viracomb. Any treatment that results in increased CD 4+ count and the hematological profile will be useful in the management of immuno-suppressed related disorders (Lazzarin et al., 2008). The evaluation of the effect of Viracomb on the liver and renal functions showed that the value of markers of liver and renal functions remains normal. Thus, the preparation can be safely used in the management of protracted conditions associated with depression of immune-system. Herbal medicines have the advantage of an impressive safety profile, and can therefore, be given for long periods without any significant side effects. Herbal medicines can be given independently, or as additional therapy to modern medicines. The current goal in HIV management of prolonging a healthy life at minimum risk and cost, can thus, be achieved admirably with herbal medicines. This study has provided scientific evidence for the first time on the efficacy and safety of Viracomb against chemically induced immunosuppression in rabbits. Additional research is needed to satisfactorily determine the role of Viracomb in healthcare. Well-designed, randomized, controlled clinical trials would best evaluate the efficacy, tolerability, and safety of Viracomb, its comparative efficacy with conventional therapy, and potential drug interactions.

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