



**ANTI-TUMOR ACTIVITY OF VELVANGA PARPAM (OFFICIAL SIDDHA DRUG)  
AGAINST DALTON'S ASCITES LYMPHOMA IN RODENTS**



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**Abstract**

**Accepted Date:**

**13/04/2013**

**Publish Date:**

**27/04/2013**

**Keywords**

Velvanga Parpam,  
BPH,  
Dalton Ascitis Lymphoma,  
Anti-Tumour Activity

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Benign prostatic hyperplasia (BPH) is a proliferation of nonmalignant stromal and epithelial cells in the prostate. BPH is the 5th most prevalent non-cancer-related disorder among men aged 50 years and older. The DAL (Dalton Ascitis Lymphoma) cancer cell line proliferation in animal models to ascertain the efficacy of the interventional drug Velvanga parpam (VP) in the reduction of cell multiplication rate with respect to the prostatic hyperplasia. VP was evaluated for anti-tumor activity against DAL bearing Swiss albino mice, administrated at the doses of 1.5 and 3 mg /kg body weight per day for 9 days. The present study deals with the effect of VP on the growth of solid tumor, life span of DAL-bearing mice, hematological profile. VP caused significant decrease in tumor volume and it prolonged the life span of DAL-tumor bearing mice. Hematological profile converted to near normal levels in drug-treated mice. The Velvanga Parpam can be used as a novel potential agent for BPH, and the area of cancer chemotherapy.

## **INTRODUCTION:**

Benign prostatic hyperplasia (BPH) is a proliferation of nonmalignant stromal and epithelial cells in the prostate, which may leads to nodular formation in the periurethral area of prostate and subsequent partial or complete obstruction of the urethra <sup>[1]</sup>. Clinically, BPH is distinguished by the progressive development of LUTS (Lower Urinary Tract Syndrome). These symptoms are variable, range from nocturia, incomplete emptying, urinary hesitancy, weak stream, frequency, and urgency to the development of acute urinary retention. Such symptoms can have a significant negative impact on quality of life, leading many men to seek treatment <sup>[2]</sup>. According to NIH (National Institute of Health), there are more than 7.8 million BPH diagnoses made <sup>[3]</sup>. BPH is the 5<sup>th</sup> most prevalent non-cancer-related disorder among men aged 50 years and older <sup>[4]</sup>. Histological evidence of BPH emerges after age 30, with 50% prevalence in men age 50-61 and 90% prevalence by the age 90<sup>[5]</sup>. Economic burden of BPH accounts for seventh highest 1-year disease specific medical costs <sup>[6]</sup>. Direct and indirect costs to the private sector related to BPH treatment

are estimated to be 3.9 billion dollars annually <sup>[7]</sup>. Many herbal and herbo-mineral <sup>[8]</sup> siddha drugs have been evaluated in tumoricidal actions against various cancers <sup>[9]</sup>. The interventional drug velvanga parpam is quoted in the Veeramamunivar vaagadathirattu, and the drug efficacy is not known. The chosen drug is quoted for the purpose of Neerpai Durbalam <sup>[10]</sup>, which is an analogous with Ukkara Soolai. The disease Ukkara Soolai correlated and symptoms are similar to BPH. It is defined in Yugi vaithiya sinthamani as some abnormal growth developed in lower abdomen, and it compress the lower urinary tract and produce LUTS <sup>[11]</sup>. The cell multiplication is the etiology in BPH. The drug intervention is designed in such a way that the trial drug inhibits the proliferation. With this logic this investigation was correlated in the Dalton Ascitis Lymphoma (DAL) cancer cell line proliferation in animal models to ascertain the efficacy of the Velvanga parpam in the reduction of cell multiplication rate with respect to the prostatic hyperplasia.

## **MATERIALS AND METHODS**

### **SOP of Velvanga Parpam**

The official siddha medicine velvanga parpam (VP) has the ingredients of velvangam(Tin), kattrazhai (Alovevera) and muttai odu (The egg shell). The velvangam is one of the helpful mineral in treating the urogenital ailments<sup>[12]</sup>. Velvangam is taken in an iron bowl and powder of egg shell is poured on the melted velvangam and stirs it thoroughly until the velvangam completely merge. Add juice of aloe vera and grind it for 12 hours (4samam) and keep it in calcinations process<sup>[13]</sup>.

The study on Velvanga Parpam (VP) (XIII/VELS/PCOL/22/2000/CPCSEA/IAEC) was approved by Vel's College of Pharmacy, Chennai-117 on 11.08.2012.

### Experimental Animals

Swiss albino mice (20-25g) were used throughout the study. They were housed in standard microlon boxes and were given standard laboratory diet and water ad libitum.

### Tumour cell lines

Dalton Ascitis Lymphoma (DAL) cells were obtained through the courtesy of Amala Cancer Research Centre, Thrissur, Kerala. DAL cells were maintained by weekly

interaperitoneal (i.p) inoculation of 1 x 10<sup>6</sup> cells/mouse.

### Effect of Velvanga Parpam on survival time

Animals were inoculated with 1x10<sup>6</sup> cells/mouse on day '0' and treatment with Velvanga Parpam started 24h after inoculation, at the doses of 1.5 and 3mg/kg/day orally. The control group was treated with same volume of 0.9% sodium chloride solution. All treatments were carried out for 9 days and observation was carried out for 45 days.

The animals were subjected for the analysis of median survival time (MST) of each group (n=6) and changes in body weight. The anti-tumour efficacy of Velvanga Parpam was compared with that of 5 Fluorouracil (20mg/kg/day i.p. for 9 days)<sup>[14]</sup>. MST was noted with reference to control. Survival times of the treated group (T) were compared with those of the control groups (C) using the following calculation.

$$\text{Increase of life span} = \frac{\text{T}-\text{C}}{\text{C}} \times 100$$

Where T = number of days treated animals survived and C = number of days control animal survived.

### Effect of Velvanga Parpam on hematological parameters

In order to detect the influence of Velvanga Parpam on the hematological status of DAL bearing mice, comparison was made amongst three groups (n=6) of mice on the 14th day after inoculation. The three groups comprised (1) tumour bearing mice, (2) tumour bearing mice treated with Velvanga Parpam respectively (1.5 and 3mg/kg/day p.o. for first 9 days) and (3) control mice. Blood was drawn from each mouse in the conventional way and the white blood cell count, red blood cell count, haemoglobin, protein and packed cell volume were determined<sup>[15,16,17]</sup>. The ascitic fluids were collected on 14th day and smeared. The smear was stained with Giemsa stain for cytological studies.

### Effect of Velvanga Parpam on solid tumour

Mice were divided into two groups (n=6). Tumour cells (1x10<sup>6</sup> cells/mice) were injected into the right hind limb of all the animals intramuscularly. Mice of group I were tumour control. Group II received

Velvanga Parpam respectively (1.5 and 3mg/kg) orally for 5 alternate days. Tumour mass was measured from 11th day of tumour induction and was repeated every 5th day for a period of 30 days. The volume of tumour mass was calculated using the formula  $V = \frac{4}{3} \pi r^2$  where is the mean of r1 and r2 which are two independent radii of the tumour mass<sup>[18]</sup>.

### Statistical analysis

All the values were expressed as mean  $\pm$  SEM. The data was statistically analyzed by one-way ANOVA followed by Dunnett's test. P values < 0.05 were considered significant.

## RESULTS AND DISCUSSION

### Anti-tumour studies

#### Mean Survival Time (MST)

Any potential anticancer drug is expected to increase the mean survival time and thus increasing life expectancy. Mice transplanted with DAL in our studies have MST of 22 days, which was increased to 26 and 34.32 days by Velvanga Parpam 1.5 and 3mg/kg respectively. These results are almost comparable to that of 5-FU, the

standard drug for which the MST was 41.21 days.

### **Hematological Parameters**

In malignancy there is always an alteration of various hematological parameters which increase in a few and decrease in others. There is a decrease in Hb, RBC and lymphocytes in malignancy accompanied by an increase in WBC especially Neutrophils, protein and PCV. These changes are due to iron deficiency or due to haemolytic of myelopathic conditions induced by malignancy. Velvanga Parpam have very well reverted the above haematological parameters altered by the transplantable tumour of DAL. Velvanga Parpam may have direct tumoricidal effect and thereby maintain normal haematological profile.

### **Solid tumour volume**

Estimation of solid tumour volume is a direct method of evaluation of anticancer activity. It is indeed a suitable method, which does not involve sacrificing the animal. In the study, the tumour mass was directly measured after implantation intramuscularly. The solid tumour volume was increased by  $6.63 \pm 0.13$  DAL bearing mice, treatment with Velvanga Parpam

decreased significantly ( $P < 0.01$ ) the tumour volume to  $4.15 \pm 0.09$  ml respectively on dose dependent manner at the end of 30 days.

The reliable criteria for evaluating an anticancer drug are prolongation of lifespan of the animal and decrease in WBC count of blood [19]. Our results show an increase in life span accompanied by a reduction in WBC count in Velvanga Parpam treated mice. These results clearly demonstrate the anti-tumour effect of Velvanga Parpam against DAL.

The common problems encountered in cancer chemotherapy are myelosuppression and anaemia [20]. Anaemia occurring in tumour bearing mice is mainly due to reduction in RBC or hemoglobin production, and this may occur either due to iron deficiency or due to haemolytic or other myelopathic conditions [21]. Treatment with Velvanga Parpam brought back the hemoglobin content, RBC and WBC counts to near normal. This indicates that Velvanga Parpam have a protective effect on the haemopoietic system.

Further, analysis of haematological parameters showed minimum toxic effect in mice treated with Velvanga Parpam. In DAL bearing mice, haematological parameters were reversed to normal by Velvanga Parpam administration (9 days).

Cytological studies of ascitic fluid on the 14th day in DAL bearing mice revealed that the tumour cells are large in size showed binucleation. In Velvanga Parpam 1.5 and 3mg/kg treated animals bearing DAL, the cells, showed plasmacytoid feature with varying degree of degeneration and cytoplasmic vacuolation and also showed active mitosis. All these cytological studies indicate the cytotoxic effect of Velvanga Parpam.

In DAL bearing mice, there was a regular and rapid increase in ascitic fluid volume. Ascitic fluid is the direct nutritional source for tumour growth; it meets the nutritional requirement of tumour cells <sup>[22]</sup>. Velvanga

Parpam treatment decreased the volume of solid tumour as well as ascites volume, viable cancer cell count and increased the life span. It may be concluded that Velvanga Parpam decrease the nutritional fluid volume and thereby arrest the tumour growth and increase the life span.

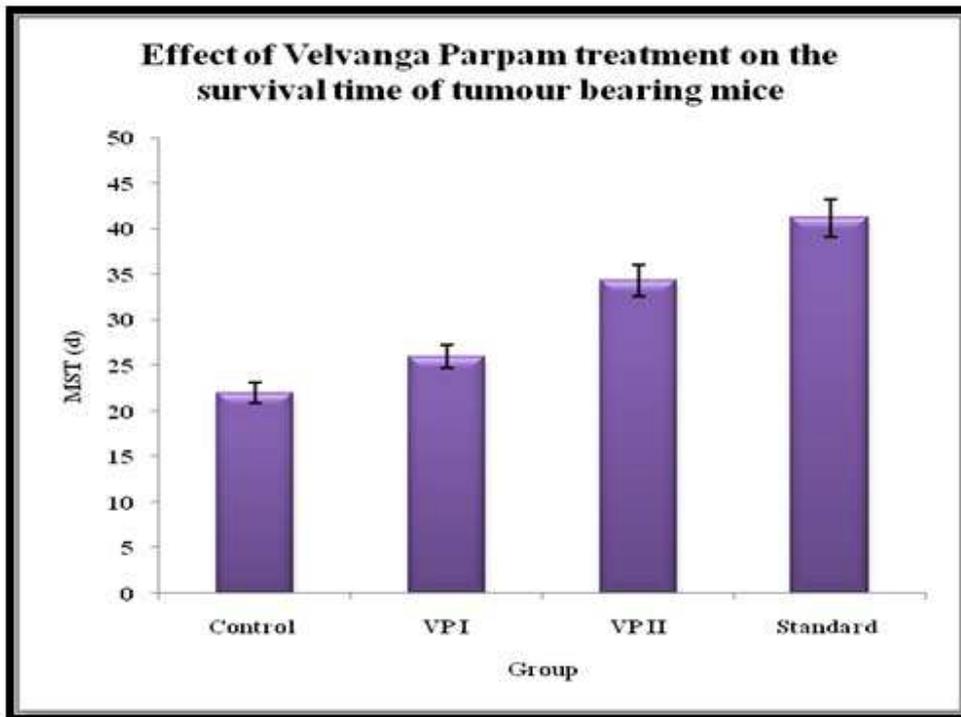
### **CONCLUSION**

In the present study the Velvanga Parpam was studied for its anti-tumour effect against transplantable tumour. The anti-tumor effect of the Velvanga Parpam is evident from the increase in lifespan of drug treated animals, reduction in solid tumour volume and also the altered haematological parameter comes to normal. All these data confirms that the Velvanga Parpam can be used as a novel potential agent for BPH, and the further study may conform on the area of cancer chemotherapy.

Effect of Velvanga Parpam treatment on the survival of tumour bearing mice

Treatment	MST (d)	Life Span (%)
Tumour control (Saline 3 ml/kg. p.o)	22 ± 0.572	-
Velvanga Parpam (1.5mg/kg p.o)	26± 0.852**	15.38
Velvanga Parpam(3mg/kg p.o)	34.32 ± 0.654**	35.89
5-FU (20mg/kg i.p)	41.21 ± 0.442**	46.61

\*\*P<0.01 Vs Tumour control; Data were analyzed by one way ANOVA followed by dunnet test.  
N = 6

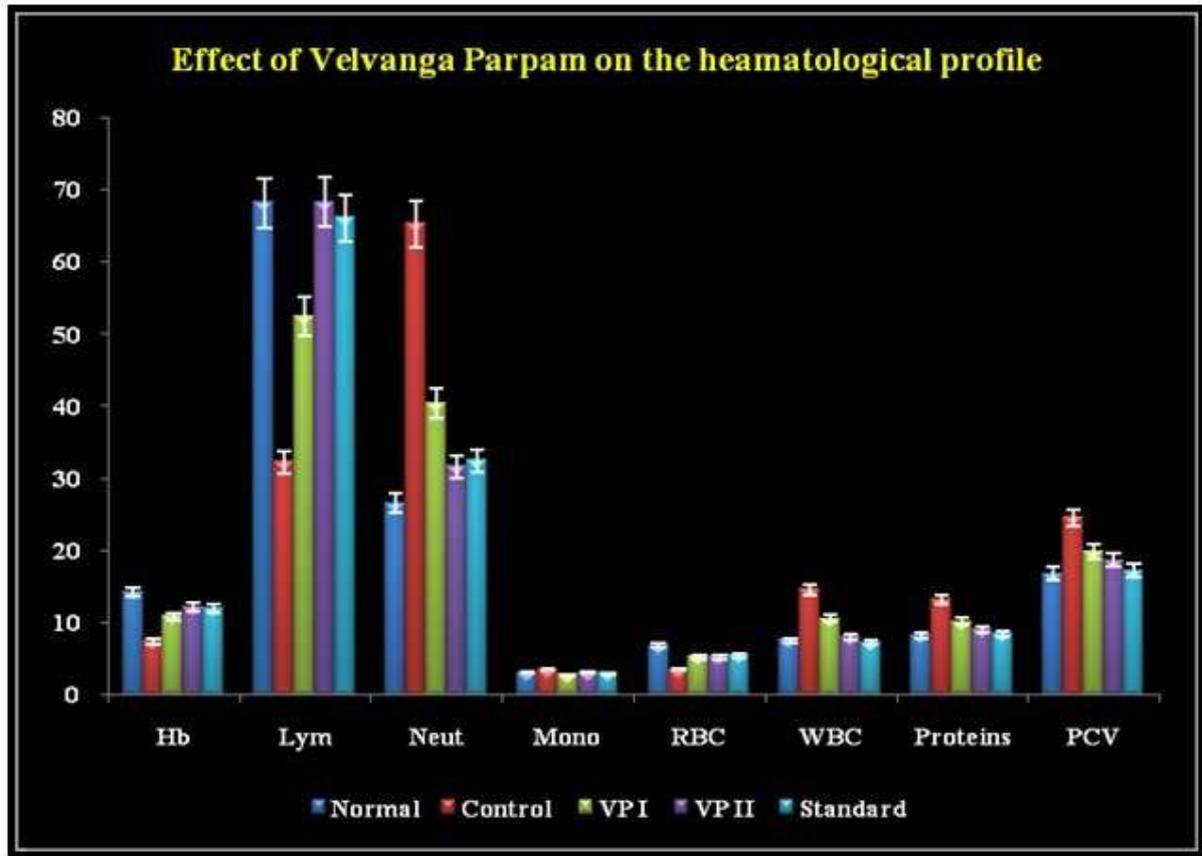


**Effect of Velvanga Parpam treatment on the haematological profile of tumour bearing mice**

Treatment	Hb (g%)	Differential count %			RBC (million/mm <sup>3</sup> )	WBC (10 <sup>3</sup> cells/mm <sup>3</sup> )	Proteins (g%)	PCV (mm)
		Lymphocyte	Lymphocyte	Lymphocyte				
Normal saline  (5 ml/kg)	14.2±0.4**	68.1±1.2**	26.5±1.4**	3.1±0.5	6.8±0.6**	7.5±0.5**	8.2±0.4**	16.8±0.5**
DAL control (1 x 10 <sup>6</sup> cell)	7.4±0.5	32.2±0.2	65.2±1.6	3.5±0.4	3.5±0.2	14.6±1.0	13.1±1.2	24.5±0.3
DAL (1 x 10 <sup>6</sup> cell) +  VP (1.5mg/kg p.o)	10.8±0.4**	52.4±1.4**	40.4±1.5**	2.7±0.6	5.2±0.5*	10.5±0.5**	10.1±0.4**	19.8±0.5**
DAL (1 x 10 <sup>6</sup> cell) +  VP (3mg/kg p.o)	12.1±0.5**	68.2±2.3**	31.6±2.5**	3.1±0.4	5.2±0.3*	8.0±0.4**	9.0±0.2**	18.6±0.2**
DAL (1 x 10 <sup>6</sup> cell) +  5FU (20 mg/kg i.p)	12.0±0.5**	66.0±2.0**	32.4±2.4**	2.9±0.4	5.4±0.2**	7.2±0.6**	8.4±0.3**	17.2±0.3**

\*\*P<0.01 Vs Tumour control; Data were analyzed by one way ANOVA followed by dunnet test.

N = 6

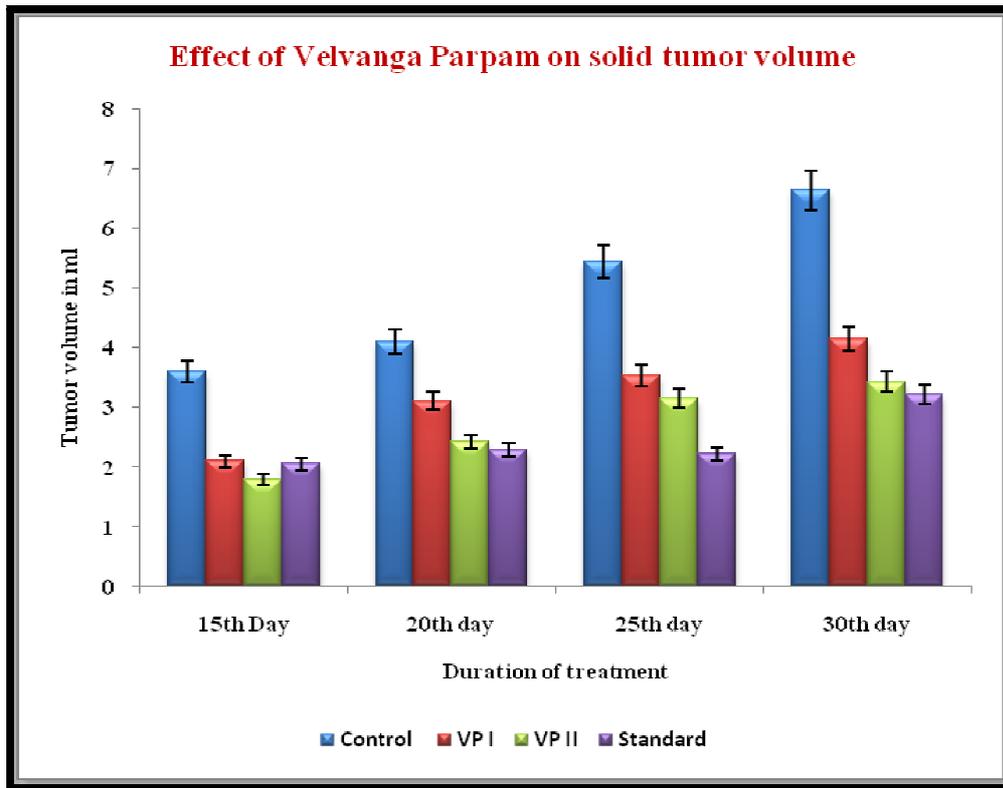


**Effect of Velvanga Parpam on solid tumor volume**

Treatment	Dose (mg/kg)	Solid tumor volume (ml)			
		15 <sup>th</sup> Day	20 <sup>th</sup> day	25 <sup>th</sup> day	30 <sup>th</sup> day
Tumor control	-	3.60 ± 0.131	4.10 ± 0.090	5.44 ± 0.210	6.63 ± 0.131
Velvanga Parpam	(1.5 p.o)	2.10 ± 0.052**	3.11 ± 0.062**	3.53 ± 0.091**	4.15 ± 0.094**
Velvanga Parpam	(3 p.o)	1.79 ± 0.044**	2.42 ± 0.054**	3.14 ± 0.066**	3.42 ± 0.082**
5-FU	(20 i.p)	2.05 ± 0.035**	2.28 ± 0.035**	2.22 ± 0.052**	3.21 ± 0.060**

\*\*P<0.01 Vs Tumour control; Data were analyzed by one way ANOVA followed by dunnet test.

N = 6



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