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Acute - Sub Acute Toxicity Studies on Siddha Drug Velvanga Parpam

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ABSTRACT

The mineral formulation Velvanga parpam (VP) has been used for the treatment of bleeding haemorrhoids (1st degree haemorrhoids). Bleeding haemorrhoid is caused by rupture of blood vessels in the rectum and anus due to the high internal pressure. Siddha medicine VP is a good remedy for bleeding haemorrhoids. As a mandatory steps were taken to evaluate safety and efficacy of trial drug VP in wistar albino rats. According to OECD guidelines, acute toxicity single dose of 5mg, 50mg, 300mg and2000 mg VP were administered, and monitor for 14 days. Sub acute toxicity studies were carried out in two groups of 10 animals (5 male & 5female)VP administered to rats at the dose 30mg and 60 mg/kg/day for 28 days. Detailed hemotological, biochemical, histopathological evaluation of different organs was performed in all animals. Histo-pathological analysis revealed that the liver, lung, kidney, heart of treated groups did not show any signs of toxicity. The results obtained, up to the dose level of 300mg/kg/bodyweight, and the P value is(<0.05). VP was non-toxic both acute and sub-acute toxicity study. Previously some article published in the same name VP, But my preparation is entirely different and indication also different. The text reference is Agathiyar paripooranam 400.

INTRODUCTION

Haemorrhoids arise from congestion of the internal or external venous plexuses around the anal canal. They are associated with constipation and staining, and also may develop in pregnancy. First degree piles bleed, while second degree piles prolapsed but retract spontaneously. Third degree piles are those which require manual replacement after prolapsing. Bright red rectal bleeding occurs after defecation. Other symptoms include pain, pruritis and mucus discharge.[1]

Now- a- days people inconsiderate symptoms like constipation, which later on may produce, haemorroids, prolapse and fissure-in-ano. The prevalence of the disease in the United Kingdom [UK], haemorrhoids were reported to affect 13% - 36% of the general population. Independent variables include baseline characteristics, sociodemographic data, and health status. Out of 976 participants, 380 patients (38.93%) were suffered from hemorrhoids. In 277 patients (72.89%), hemorrhoids were classified as Grade I, in 70 patients in 2012. A recent prospective study of screening colonoscopy patients revealed the presence of haemorroids in 38.9% with 44.7% of those patients suffering from haemorroidal symptoms. In India, Prevalence 10–20% 4.2–7.9%.[3]

Siddha Literatures have prescribed many medicines for the treatment of Rattha moolam. In *Siddha s*ystem, *Parpam* is a calcined form of any herbal, mineral, metal or animal byproducts. Velvanga parpam is an effective calcined form of a mineral. Tin, known as '*Vangam*' in traditional literature has Astringent, Sedative and, Deobstruent properties. So, the mineral formulation *Velvanga parpam* ingredients possess Styptic action which helps in stopping bleeding.

Herbs and minerals have been in use since long time to treat various diseases[3]. However, many issues related to a lack of scientific evidence about the efficacy and safety of the drugs remains unresolved[4]. The Pre-clinical toxicity studies were essential for determining a safe dose for human trials[5].

Velvanga parpam is widely used in the treatment of urino-genital infections and dyspepsia, diabetes, asthma, arthritis, gonorrhoea, blood disorders[6,7].

Previously some article published in the same name Velvanga parpam, but the text reference, preparation and indications are entirely different.

The Siddha drug Velvanga parpam (VP) quoted in the siddha literature Gunapadam thathu seeva vaguppu has been used for the treatment of Rattha moolam (Bleeding haemorroids) Consequently an effort was made to evaluate acute and sub-acute toxicity of the siddha *mineral* formulation VP in laboratory animals.

MATERIALS AND METHODS

STANDARD OPERATING PROCEDURE OF VELVANGA PARPAM

Velvangam (*stannum),* Vaalairasam (*hydragyrum),* Karpoorasilajith (*asphaltum),* Karchunnam *(Limestone*), these drugs are authenticated by Shakila, Resarch officer, Chemistry dept., Siddha Central Research Institute,

Velvangam (*stannum),* Vaalairasam (*hydragyrum),* Karpoorasilajith (*asphaltum*) these ingredients are ground with karchunna theli neer and calcinated .Then it was again pulverized with chunna theli neer for 6 hrs, calcinate the mixture; finally made as a powder form. The drug is stored in clean and dry air tight container.[8]

ANIMALS

Wistar Albino rats of either sex, weighing 150 g to 200 g were purchased from King Institute of Preventive medicine Animal House, Chennai, India. The animals were fed on standard rodent pellet and RO water was provided *ad libitum.* The animals were kept for overnight fasting before experimentation.

All experimental procedures described were reviewed and approved by the Institutional Animal Ethical Committee of K.K College of pharmacy, Chennai-122.and the IAEC approval no. **KKCP/2013/013.**

ACUTE ORAL TOXICITY OECD 425 GUIDELINES

Acute toxicity studies were carried out according to the OECD (Organization of Economic Co-operation and Development) guidelines 423. Healthy female rats, weighing 150–200 g, were selected and oral administration of the single doses of Velvanga Parpam were done especially by

suspending in 1% SCMC (Sodium carboxymethyl cellulose).The drug purchased from Sytho pharmaceuticals Pvt Ltd.

Administration of doses:

Velvanga parpam in 1% SCMC was administered as a single oral dose by gavage using a feeding needle. Animals were fasted prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously observed as per the guideline after substance administration. An oral (p.o) dose of 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg was administered step by step according to the guidelines. The general behaviors of rats were continuously monitored for 1 h after dosing, periodically during the first 24 h (with special attention given during the first 4 hours) and then daily thereafter, for a total of 14 days. Changes in the normal psychomotor activity and external morphology and their body weights were monitored periodically before dosing and the time at which signs of toxicity or mortality were recorded.[9]

The visual observations included skin changes, mobility, aggressively, sensitivity to sound and pain, as well as respiratory movements. They were deprived of food, but not water 12 h prior to the administration of the test substance. Finally, the number of survivors was noted after 24 h and these animals were then maintained for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

REPEATED DOSE 28-DAY SUB-ACUTE ORAL TOXICITY STUDY (OECD – 407 GUIDELINES)

Sub-acute toxicity studies were carried out according to OECD 407 and rats were divided into 2 groups of 10 animals (5 male and 5 female). Velvanga Parpam was administered to rats at the dose of 30 & 60 mg/kg/day for 28 days. The toxic symptoms such as signs of toxicity, mortality and body weight changes were monitored. Rats were anesthetized with ether at the end of the treatment period. All rats were sacrificed after the blood collection.[10]

TABLE I

Test Substance	:	Velvanga Parpam
Animal Source	:	Animal house of King Institute of Preventive Medicine
Animals	:	Male and Female Wistar Albino Rats
Age/Weight	:	20 gms
Acclimatization	:	Seven days prior to dosing.
Veterinary examination	:	Prior to and at the end of the acclimatization period.
Identification of animals	:	By cage number, animal number and individual marking on fur.
Diet	:	Pelleted feed supplied by Sai meera foods Pvt Ltd, Bangalore
Water	:	Aqua guard portable water in polypropylene bottles *ad libitum.*
Housing & Environment	:	The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	:	Between 20 & 24°C,
Relative humidity	:	Between 30% and 70%,
Dark and light cycle	:	Each of 12 hours.

Justification for Dose Selection:

The result of acute toxicity studies in Rats indicated that *Velvanga Parpam* was non toxic and no behavioral changes were observed up to the dose level of 2000 mg. The oral route was selected for use because oral route is considered to be a proposed therapeutic route.

Preparation and administration of dose:

Velvanga Parpam at three doses respectively was suspended in of 1% SCMC in distilled water. It was administered to animals at the dose levels of 30 and 60 mg/kg. The test substance suspensions were freshly prepared every day for 28 days. The control animals were administered vehicle only. Administration was by oral (gavage), once daily for 28 consecutive days.

Laboratory Investigations:

Following laboratory investigations were carried out on day 29 in animals' fasted over-night. On 29th day, the animals were fasted for approximately 18 h, then slightly anesthetized with ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of haematological parameters, the other without any anticoagulant and

was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

RESULTS

Statistical analysis:

Findings such as clinical signs of intoxication, body weight changes, food consumption, hematology and blood chemistry were subjected to One-way Anova. Followed by dunnett's test using a computer software programme. (Graph Pad Prism 5.0)

DISCUSSION

The trail drug Velvanga Parpam was administered to wistar albino rats at the dose of 300 mg/kg. Treatment with 2000mg/kg has produced mortality in 2 out of three animals treated with Velvanga Parpam. Based on OECD 423 the drug is considered to be non toxic upto the dose of 300mg/kg.

All animals from control and the entire treated dose (30 and 60 mg/kg) groups survived throughout the dosing period of 28 days for sub acute toxicity study. The results for body weight determination of animals from control and different dose groups show comparable body weight gain throughout the dosing period of 28 days.(TABLE III)

Haematological Investigations:

Blood samples of control and experimental rats was analyzed for hemoglobin content, total red blood corpuscles (RBC), white blood corpuscles (WBC) count, haemoglobin, packed cell volume (PCV)platelets, lymphocytes, neutrophils were analyzed. The results of haematological investigations revealed no significant changes in the values when compared with those of respective controls. (TABLE IV)

Biochemical Investigations:

Serum and Urine was used for the estimation of biochemical parameters. Samples of control and experimental Rats were analyzed for protein, bilirubin, urea, uric acid, creatinine, triglyceride, cholesterol and glucose levels was carried using standard methods. Activities of glutamate

oxaloacetate transaminase/ Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/ Alanine amino transferase (GPT/ALT) and alkaline phosphatase were estimated as per the colorimetric procedure. Results of Biochemical investigations conducted on days 29 revealed the following significant changes in the values of different parameters studied when compared with those of respective controls Urea, SGOT, SGOT, Bilirubin were within the limits. LDL level was elevated in animals of 60 mg/kg dose group ($P<0.05$) total cholesterol level was slightly increased but these were within the normal limits. (TABLE V)

Histopathology:

Histopathological investigation of the vital organs was done. The organ pieces (3-5µm thick) of the highest dose level of 400 mg/kg were preserved and were fixed in 10% formalin for 24 h and washed in running water for 24 h. Samples were dehydrated in an auto technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin.

The organs included brain, heart, kidneys, liver and lungs of the animals were preserved they were subjected to histopathological examination. (Figure no: I)

Table No. II: Dose finding experiment and its behavioral Signs of Toxicity for VP formulation

Dose mg/kg	**Day-1**	**Day-2**	**Day-3**	**Day-4**	**Day-5**	**Day-6**	**Day-7**	**Day-8**	**Day-9**	**Day-10**	**Day-11**	**Day-12**	**Day-13**	**Day-14**
300	+	+	+	+	-	+	+	+	+	-	+	+	+	+

SUB-ACUTE ORAL TOXICITY 28-DAY REPEATED DOSE STUDY IN RATS

Table No. III: Change in body weight

Treatment	0th day	5th day	10th day	15st day	20th day	25th day	28th day	% increase
Control	175.83± 6.84	179.50± 6.28	181.83± 6.46	184.83±6. 31	187.16± 6.01	190.66± 6.46	193.66± 5.70	9.79
100mg/ kg	163.16± 6.38	163.33± 6.60	165.33± 6.39	167.83±6. 610	169.66± 6.99	171.16± 7.32	174.83± 7.37	6.67
200mg/ kg	150.83± 9.58	150.66± 9.09	153.50± 9.58	155.01±9. 56	157.33± 9.54	159.53± 9.31	163.16± 9.67	7.56

Table No. IV: Haematological Parameter

Haematological parameter	Control	*Trial drug*	
		100 mg	200mg
Total R.B.C. count ($\times 10^6$ mm^3).	9.09±0.15	9.11±0.12	9.11±0.16
Total W.B.C. Count ($\times 10^3$ mm^3).	12.67±0.22	12.12±0.758	11.23±0.012
Haemoglobin (Hb) (g/dl)	15.61±0.36	15.67±0.275	15.78±0.78
Hematocrit (%).	44.21±1.01	42.42± 0.952	38.7±1.07
Platelets (×103 mm^3).	834.91±24.01	845.21±16.55	863.58±16.25
Lymphocytes(%).	84.7±1.32	79.28±2.63	72.8±5.49
Neutrophils (%).	20.6±0.65	19.6±1.252	18.952±0.65

Data are expressed as mean ± SEM

Table No. V: Biochemical Parameters

Biochemical parameter	Control	*Trial drug*	
		100 mg	200mg
Creatinine (mg/dl)	0.5890±0.079	0.75±0.04	0.54±0.11
Urea (mg/dl)	15.30 ± 0.47	14.33±0.49	14.17±1.078
Triglycerides (mg/dl)	52.20±1.13	50.23±1.08	49.17±1.86
Total Cholesterol (mg/dl)	46.60±1.21	52..460±1.08	53.836±1.05
Total protein (mg/dl)	4.40±0.26	4.12±0.35	4.020±0.765
Albumin (g/dl)	3.20±0.41	3.30±0.35	3.29±0.26
AST (IU/L)	121.41±2.68	118.3±1.67	116.76±3.065
ALT (IU/L)	69.40±1.57	69.012±2.32	68.72±3.258
ALP (IU/L)	112.6±4.67	115.01±1.021	116.41±2.108

Table No. VI: Relative Organ Weight

Dose	Relative Organ Weight of rats					
	Liver	Kidney	Brain	Lungs	Heart	Spleen
Control	2.8±0.1	0.66±0.02	0.38±0.22	0.29±0.01	0.29±0.01	0.15±0.01
100mg/kg	2.89±0.1	0.65±0.02	0.42±0.01	0.30±0.02	0.31±0.01	0.17±0.01
200mg/kg	3.01±0.1	0.66±0.03	0.42±0.01	0.31±0.01	0.31±0.01	0.16±0.01

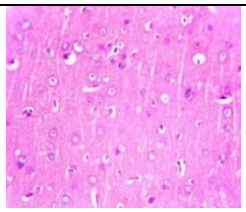
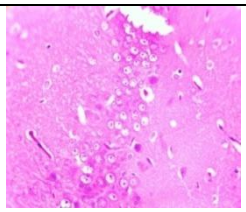
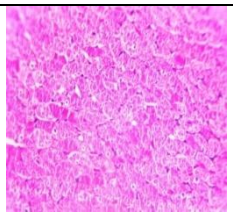
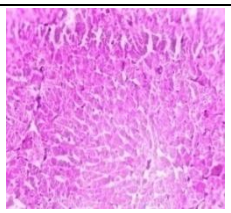
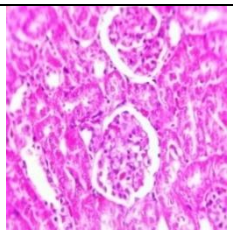
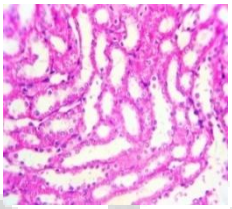
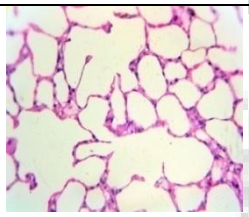
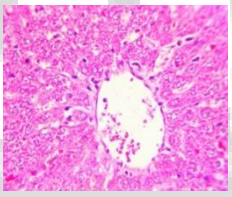
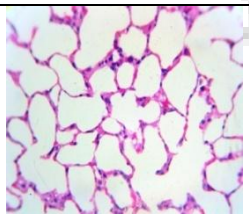
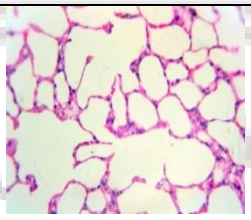
ORGANS	LOW DOSE	HIGH DOSE	FINDINGS
BRAIN			Cerebellum – Normal
HEART			Normal Cardiomyocytes
KIDNEY			Cortex - Normocellular glomeruli
LIVER			Periportalzone– Normal
LUNG			Normal

Figure: I

CONCLUSION

The acute and sub-acute toxicity study of *Velvanga parpam (VP)* revealed no toxicity by oral route over a period of 28 days. So, it can be concluded that the *Velvanga parpam (VP)* prescribed for therapeutic use. Higher dose is 2000mg/kg. In this trial drug VP given animal at the dose of 400 mg it got toxic effect. So the before dose is a active form.

Basically the mineral drug toxicity level is differing from one to another; it depends upon the combination of the metal & minerals. So we start at 5mg and gradually increase the dose till the lethal dose level.

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