**Research Article** **CODEN: IJRPJK** **ISSN: 2319 – 9563**

**International Journal of Research in Pharmaceutical and Nano Sciences**

Journal homepage: www.ijrpns.com

# STYPTIC ACTIVITY ON SIDDHA DRUG VELVANGA PARPAM AGAINST ADRENOCHROME INDUCED HAEMORRHAGIC RATS

**P. Parthibhan[*1], M. Sudha[2], P. Sathiya rajeswaran[3], K. Nalina saraswathy[1]**

[1*]Department of Pothu Maruthuvam, Government Siddha Medical College, Chennai, Tamil Nadu, India.

[2]PG Scholar, Department of Pothu Maruthuvam, Government Siddha Medical College, Chennai, Tamil Nadu, India.

[3]Department of Pothu Maruthuvam, Siddha Central Research Institute, Chennai, Tamil Nadu, India.

**ABSTRACT**

Haemorrhoids are dilated veins within the anal canal in the sub epithelial region formed by radicals of the superior, middle, inferior rectal veins. They are divided into two types, internal and external Haemorrhoids. The internal haemorrhoids lie beneath the anal mucous membrane. Traditional Siddha system of medicine is widely practised in India. Siddha has thirty two forms of internal and external medicines. Parpam is one among the internal medicine equated to whitecalx. Velvangaparpam is a calcined form of mineral drug. My Aim of the study is to investigate the safety and efficacy of siddha drug velvangaparpam (VP).VP was evaluated for styptic activity against the standard drug Adrenochrome in Wister albino rats. They divided in to four groups, each group with 6 animals. Group 1 is the control, group 2 and 3 receive the trial drugs of dose 30 and 60mg group 4 receive the standard drug Adrenochrome (10mg/kg). Then detailed hemotological study was performed for all animals. The investigation shows a significant reduction in the Bleeding time, Clotting time, prothrombin time, and fibrinogen time. The values of the trial drug Velvangaparpam treated animals were compared with standard drug Adrenochrome 10mg/animal i.p single dose. Good Styptic activity response of trial drug's explored.

**KEYWORDS**

Velvangaparpam, Siddha drug, Styptic activity and Adrenochrome.

**Author for Correspondence:**

Parthibhan P,
Department of Pothu Maruthuvam,
Government Siddha Medical College,
Chennai, Tamil Nadu, India.

**Email:** parniru@gmail.com

## INTRODUCTION

The word ''haemorrhoid" is derived from the Greek word 'haema' (blood) and 'rhoos' (flowing), and it was probably Hippocrates (460 BC) who was the first to apply the name to the flow of blood from the veins of the anus[1]. The more recent study describing them as specialized highly vascular cushions of discrete masses of thick sub mucosa, containing blood vessels, smooth muscles, elastic and connective tissue which may slide down due to

breakage of collagen and anchoring supporting connective tissue causing symptoms like prolapse, pain, bleeding etc[2,3].

The estimates on haemorrhoid and it's relation to people age in 4th to 6th decade states that here in a chance of 50-85%. India approximately 40,723,288 people are reported to have haemorrhoids. One million new cases are reported annually, at the rate of 47 per 1000 and this rate increases with age. Current statistics suggest nearly half of the world's population will experience some form of haemorrhoidal diseases especially when they reach the age of fifth decade[4].

According to recent studies, several theories have been postulated regarding the cause, including prolonged periods of driving, erect posture, chronic constipation and diarrhoea, straining during defecation, low fibrous diet, heredity, eating spicy foods, sitting on cold seats and benches, doing manual labour, lifting heavy weights, being overweight, pregnancy, weakening of the connective tissue in the rectum and anus that occurs with age[5,6].

In ancient Siddha has analogous quoted the aetiology like eating spicy foods, sitting, horse riding, elephant riding in the disease long time of haemorrhoids. In siddha aspect the disease classified in to 21 types. Ratthamoolam. (1st degree haemorrhoids) is one among them.

Siddha Literatures have prescribed many medicines for the treatment of Ratthamoolam. In *Siddha s*ystem, *Parpam* is a calcined form of any herbal, mineral, metal or animal by products. Velvangaparpam 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 *Velvangaparpam* ingredients posess Styptic action which helps in stopping bleeding.

Herbs and minerals have been in use since long time to treat various diseases[7]. However, many issues related to a lack of scientific evidence about the efficacy and safety of the drugs remains unresolved[8,9]. The Pre-clinical toxicity studies were essential for determining a safe dose for human trials[10]. The interventional Siddha drug *Velvangaparpam*(VP) quoted in the siddha literature Agathiyarparipooranam 400 has been used for the treatment of Ratthamoolam (1st degree haemorrhoids)[11]. Consequently an effort was made to evaluate pharmacological activity of the herbo-mineral based siddha formulation VP in laboratory animals.

## MATERIALS AND METHOD

### Sop 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 karchunnathelineer and calcinated (Table No.1)[12]. Then again ground the mixture with chunnathelineer for 6 hrs, calcinate the mixture finally makes it as a powder form. The drug is stored in clean and dry air tight container[13].

### Aim

Aim of the study is to evaluate the safety and efficacy of the siddha drug Velvanga Parpam.

### Materials Used

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.

### Chemicals

Adrenochrome is a byproduct of oxidized adrenaline. Its chemical name is 3-hydroxy-1-methyl-5, 6-indoline-dione. Adrenochrome can refer to two things a metabolite of endogenous epinephrine or a product of stabilized pharmaceutical epinephrine. The drug purchased from Ciron drugs and Pharmaceuticals Pvt Ltd.

### 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.

**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 carboxy methyl cellulose).The drug purchased from sytho pharmaceuticals Pvt Ltd.

**Administration of doses**

Velvanga Parpamin 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.

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.

**EVALUATION OF STYPTIC ACTIVITY**

**Procedure**

Animals were randomized into four groups of six animals each. Group I received vehicle, group II and III received Trial drug at the dose of 30 and 60mg/kg. Group IV served as standard. The animals were administered the trail drug orally and the blood sample were collected periodically for evaluation (Table No.2).

**Clotting time**

The tail of the animal warmed for 1 min in water at 40°C, dried and cut at the tip with a razor blade. A 25 µl sample of capillary blood was collected into a microhematocrit glass capillary. The chronometer was started when the blood first made contact with the glass capillary tube. The blood left to flow by gravity between the two marks of the tube, 45 mm apart, by tilting the capillary tube alternately to +60° and -60° angles with respect to the horizontal plane until blood ceased to flow (reaction end point).

**Bleeding time method (BT)**

The tail of the rat warmed for 1min in water at 40°C and then dried. A small cut was made in the middle of the tail with a scalpel. Bleeding time started and noted when the first drop touched the circular filter paper and checked at 30 s intervals until bleeding stops.

**Prothrombin time (PT)**

0.1 ml of plasma mixed with 0.2 ml of pt reagent(calcium thromboplastin) maintain $37^oC$,and absorbed the animals until formation of the fibrin clot. The time should be noted.

**Activated Partial Thromboplastin time (APTT)**

0.1 ml of plasma with 0.1mi of APTT reagent (cephalin-karolin suspension) incubated $37^oC$ for 5 minutes and then adds 0.1ml of 0.025ml $cacl_2$ solution, until formation of the fibrin clot visually detected. The time should be noted.

**Fibrinogen time**

0.25ml of animal blood plasma add 0.05 ml of saline, and incubated $37^oC$. After 30's add 0.1ml of streptokinase solution, wait for 30's, then add 0.1ml of bovine thrombin added. Start the stopwatch. 30 are later which time the fibrinogen clot formed.

**Animal blood collection**

For the remaining blood coagulation variables like prothrombin time etc, animals were anaesthetized with chloral hydrate (4% solution, 7 ml/kg) prior to blood withdrawal. Arterial blood was collected by aspiration from the abdominal aorta which provided an abundant sample free of hemolytic. The blood sample immediately emptied into a plastic tube containing 0.11 M sodium citrate at a ratio of 1:10 anticoagulant blood, gently mixed and centrifuged at 2500 *g* at 4°C for 10 min. Plasma was separated and maintained in ice bath throughout its processing.

**Method**

After, one hour of treatment to the above respective groups, the following parameters such as BT, CT, Prothrombin time, Activated partial Thromboplastin time, Thrombin time and Fibrinogen were screened.

**Statistical analysis**

All experimental results were expressed as mean ± SEM statistics was determined by student t test followed by Dunnet's T Test. By using Grap PAD Prism 5.

## RESULTS AND DISCUSSION

In the toxicity study, No specific signs of toxicity were seen in any of the animals. Anti-haemorrhoidal drug velvanga Parpam is expected to arrest or control bleeding. The test results of bleeding time, clotting time, prothrombin time, thrombin time, fibrinogen time values shows good result while using the trial drug (Table No.3, 4 and 5) (Figure No.1 and 2). The animal's body weight gained during the administration of Velvanga Parpam dosage of 200mg in the period of study (Table No.6). The values are expressed as mean ± SEM analysis was done by student t test followed by Dunnet's T Test (Table No.7 and 8). Test for significance is *P < 0.05, **P < 0.01, ***P < 0.001.

**Table No.1: Ingredients of Velvanga Parpam**[12+]

| S.No | Ingredients | Chemical name | Actions |
|---|---|---|---|
| 1 | **Velvangam**, | *STANNUM(TIN)*, | Astringent<br>Sedative<br>Deobstruent |
| 2 | **Vaalairasam** | *HYDRAGYRUM(*or) *MERCURY* | Alterative<br>Laxative<br>Diuretic<br>Anti-syphilitic |
| 3 | **Karpoorasilajith** | *ASPHALTUM* | Haemostatic<br>Diuretic<br>Astringent<br>Nutrient |
| 4 | **karchunnathelineer** | *LIMESTONE WATER* | Alterative<br>Astringent<br>Sedative<br>Antidote |

**Table No.2: Grouping**

| | | |
|---|---|---|
| **Group 1** | **Control** | **Distilled water 2ml, po, single dose** |
| **Group 2** | Trial drug | *Velvanga Parpam* (30 mg/kg), po, single dose |
| **Group 3** | Trail drug | *Velvanga Parpam* (60 mg/kg), po, single dose |
| **Group 4** | Standard | Adrenochrome (Sigma) 10 µg/animal/i.p, single dose |

**Table No.3: Results**

| S.No | Parameters | Control | Group I VP (30mg/kg) | Group II VP (60mg/kg) | Group III AC(10µg) |
|---|---|---|---|---|---|
| 1 | Bleeding time (s) | 88.33±1.667 | 97.50±3.819* | 93.33±2.108* | 85.83±2.713*** |
| 2 | Clotting time (s) | 117.5±2.814 | 115.01±2.58* | 103.3±1.667** | 100.8±2.386*** |
| 3 | Prothrombin Time(s) | 25.67±0.954 | 25.33±0.802* | 21.01±1.095** | 19±1.033*** |
| 4 | Activated Thromboplastin time(s) | 25.33±0.714 | 21.83±1.222* | 18.01±0.5774** | 14.02±1.033** |
| 5 | Thrombin time (s) | 30.5±1.285 | 23.33±1.202* | 20.5±0.6191** | 14.33±1.085*** |
| 6 | Fibrinogen (mg/dl) | 192.7±4.372 | 175.02±4.830* | 148.7±3.383** | 117.5±2.141*** |

Values are expressed as mean ± SEM analysis was done by student t test followed by Dunnet's T Test. Test for significance is *P < 0.05, **P < 0.01, ***P < 0.001.

**Table No.4: Bleeding Time (Sec)**

| S.No | Bleeding Time | Con | vp30 | vp60 | Ac10 |
|---|---|---|---|---|---|
| 1 | Number of values | 6 | 6 | 6 | 6 |
| 2 | Minimum | 85.00 | 85.00 | 85.00 | 80.00 |
| 3 | 25% Percentile | 85.00 | 88.75 | 88.75 | 80.00 |
| 4 | Median | 87.50 | 97.50 | 95.00 | 85.00 |
| 5 | 75% Percentile | 91.25 | 106.3 | 96.25 | 91.25 |
| 6 | Maximum | 95.00 | 110.0 | 100.0 | 95.00 |
| 7 | Mean | 88.33 | 97.50 | 93.33 | 85.83 |
| 8 | Std. Deviation | 4.082 | 9.354 | 5.164 | 6.646 |
| 9 | Std. Error | 1.667 | 3.819 | 2.108 | 2.713 |
| 10 | Lower 95% CIof mean | 84.05 | 87.68 | 87.91 | 78.86 |
| 11 | Upper 95% CIof mean | 92.62 | 107.3 | 98.75 | 92.81 |
| 12 | Sum | 530.0 | 585.0 | 560.0 | 515.0 |

**Table No.5: Clotting Time (Sec)**

| S.No | Clotting Time (Sec) | Con | Vp30 | Vp60 | Ac10 |
|---|---|---|---|---|---|
| 1 | Number of values | 6 | 6 | 6 | 6 |
| 2 | Maximum | 110.0 | 105.0 | 100.0 | 95.00 |
| 3 | 25% Percentile | 110.0 | 108.8 | 100.0 | 95.00 |
| 4 | Median | 117.5 | 117.5 | 102.5 | 100.0 |
| 5 | 75% Percentile | 125.0 | 120.0 | 106.3 | 106.3 |
| 6 | Maximum | 125.0 | 120.0 | 110.0 | 110.0 |
| 7 | Mean | 117.5 | 115.0 | 103.3 | 100.8 |
| 8 | Std.Deviation | 6.892 | 6.325 | 4.082 | 5.845 |
| 9 | Std.Error | 2.814 | 2.582 | 1.667 | 2.386 |
| 10 | Lower 95% CI of mean | 110.3 | 108.4 | 99.05 | 94.70 |
| 11 | Upper 95% CI of mean | 124.7 | 121.6 | 107.6 | 107.0 |
| 12 | Sum | 705.0 | 690.0 | 620.0 | 605.0 |

**Table No.6: Change in body weight**

| Treatment | $0^{th}$ day | $5^{th}$ day | $10^{th}$ day | $15^{th}$ day | $20^{th}$ day | $25^{th}$ day | $28^{th}$ 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.7: Hematological parameter**

| S.No | Haematological parameter | Control | *Trial drug* | |
|---|---|---|---|---|
| | | | **100 mg** | **200mg** |
| 1 | Total R.B.C. count (×$10^6$ $mm^3$) | 9.09±0.15 | 9.11±0.12 | 9.11±0.16 |
| 2 | Total W.B.C. Count (×$10^3$ $mm^3$) | 12.67±0.22 | 12.12±0.758 | 11.23±0.012 |
| 3 | Haemoglobin (Hb) (g/dl) | 15.61±0.36 | 15.67±0.275 | 15.78±0.78 |
| 4 | Hematocrit (%) | 44.21±1.01 | 42.42± 0.952 | 38.7±1.07 |
| 5 | Platelets (×103 $mm^3$) | 834.91±24.01 | 845.21±16.55 | 863.58±16.25 |
| 6 | Lymphocytes (%) | 84.7±1.32 | 79.28±2.63 | 72.8±5.49 |
| 7 | Neutrophils (%) | 20.6±0.65 | 19.6±1.252 | 18.952±0.65 |

Data are expressed as mean ± SEM

**Table No.8: Biochemical Parameters**

| S.No | Biochemical parameter | Control | *Trial drug* | |
|---|---|---|---|---|
| | | | **100 mg** | **200mg** |
| 1 | Creatinine (mg/dl) | 0.5890±0.079 | 0.75±0.04 | 0.54±0.11 |
| 2 | Urea (mg/dl) | 15.30 ± 0.47 | 14.33±0.49 | 14.17±1.078 |
| 3 | Triglycerides (mg/dl) | 52.20±1.13 | 50.23±1.08 | 49.17±1.86 |
| 4 | Total Cholesterol (mg/dl) | 46.60±1.21 | 52..460±1.08 | 53.836±1.05 |
| 5 | Total protein (mg/dl) | 4.40±0.26 | 4.12±0.35 | 4.020±0.765 |
| 6 | Albumin (g/dl) | 3.20±0.41 | 3.30±0.35 | 3.29±0.26 |
| 7 | AST (IU/L) | 121.41±2.68 | 118.3±1.67 | 116.76±3.065 |
| 8 | ALT (IU/L) | 69.40±1.57 | 69.012±2.32 | 68.72±3.258 |
| 9 | ALP (IU/L) | 112.6±4.67 | 115.01±1.021 | 116.41±2.108 |

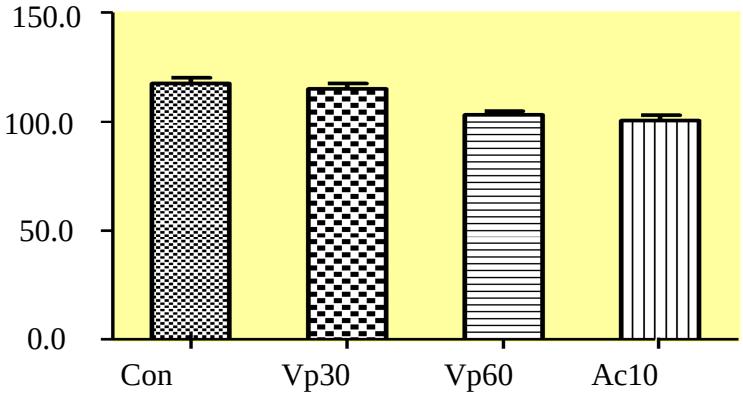


**Figure No.1: Clotting Time**

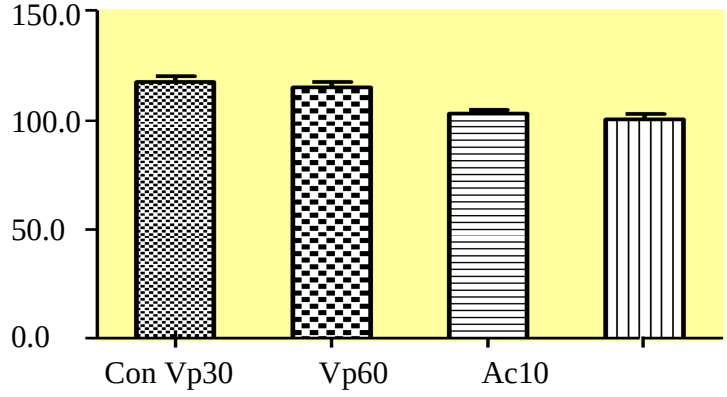


**Figure No.2: Bleeding Time**

## CONCLUSION

The result obtained in the present study, the values of trial drug treated animals were compared with the positive control drug treated animals in Adrenochrome 10 µg/animal/i.p, single dose. Efficacy studies revealed that the drug is good styptic activity as its P value <0.05, and the drug safe up to a dose of 300mg/kg. So, it is concluded that the drug *Velvanga Parpam* proves its efficacy and safety in bleeding haemorrhoids.

## ACKNOWLEDGEMENT

The author P. Parthibhan gratefully acknowledges to Dr. K. Kanagavalli, and Dr. P. Sathiya Rajeswaran, Research officer-scientist-2 and incharge, Siddha Central Research Institute, Arumbakkam. Chennai, they gave valuable suggestions, and also, thank to PG Scholar Dr. M. Sudha, Govt. Siddha Medical College, and Dr. K. Nanina saraswathy, Asst.Medical officer, Chennai, They are helped a lot in the preparation of the article.

## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

## REFERENCES

1. Khubchandani I, Paonessa N, Khawaja A. Surgical treatment of hemorrhoids, *Springer, London*, 2nd edition, 1, 2009, 7-13.
2. Bhandari K S. An evaluation of the effectiveness of rubber band ligation as a modality of treatment of second degree haemorrhoids, Dissertation submitted for M.S. (General Surgery) in the Dept. of General surgery, BMC to RGUHS, Bangalore, Karnataka, 2006.
3. Philip H G, Santhat N. Principles and practices of surgery for colon, rectum and anus, *Quality Medical Publishing Inc, London,* 1st edition, 1992, 10-38.
4. Statistics by country for Haemorrhoids [Internet] cited on 6 Jan 2013. Available from http://www.rightdiagnosis.com/h/hemorrhoids/stats-country.html.
5. Gami Bharat. Hemorrhoids-a common ailment among adults, causes and treatment a review, *International Journal of pharmacy and pharmaceutical sciences,* 3(5), 2011, 5-12.
6. Malik I A, Gopalan S. Use of CAM results indelay in seeking medical advice for breast cancer, *Eur J Epidemiology,* 18, 2003, 817-822.
7. Shekelle P G, Morton S C, Suttorp M J, Buscemi N, Friesen C. Challenges in systematic reviews of complementary and alternative medicine topics, *Ann Intern Med,* 142, 2005, 1042-1047.
8. Rahul B, Patil, Shreya R, Vora and Meena M, Pillai. Protective effect of Spermatogenic activity of *Withaniasomnifera* (Ashwagandha) ingalactose stressed mice, *Annals of Biological Research,* 3(8), 2012, 4159-4165.
9. Anoop A, Jagadeesan M and SubramaniumS. Toxicological studies on *Linga Chendooram*-I, a siddha drug, *Indian J Pharma Sci,* 64(1), 2002, 53.
10. Ramachanthiran S, Agathiyar paripooranam, Thamarai noolagam, Second Edison, 1998, 40.
11. Thiyagarajan R. Siddha Material Medical, *Indian Medicine and Homeopathy,* Second Edition, 2004, 206, 281, 529-536.
12. Saka W A, Akhigbe R E, Akinola A O, Azeez O M. Hematoxicity of amodiaquine in Sprague-Dawley Rats, *ToxInt In press*, 2012, 42.
13. Dacie S I V, Lewis S M. Practical haematology, Livingston, Melborne and New York, *London, J and A Churchill Ltd*, 7thedition, 10, 1991, 67.

**Please cite this article in press as:** P. Parthibhan *et al.* Styptic activity of siddha drug velvanga parpam against adrenochrome induced haemorrhagic rats, *International Journal of Research in Pharmaceutical and Nano Sciences,* 4(4), 2015, 269 - 275.