ANTIBACTERIAL AND HAEMOSTATIC ACTIVITIES OF A SIDDHA FORMULATION – *PAVALA PARPAM*

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Summary

Background: Pavala Parpam (PP) is a traditional Siddha medicinal preparation. This marine sourced medicine is synthesized through calcination of Corals as narrated in the classical Siddha literature – *Anuboga Vaidhya Navaneetham*. This literature evident shows that *Pavala Parpam* has astringent action and becomes evident in arresting bleeding. The primary objective of this work was to validate the safety and haemostatic efficacy of PP. *Methods:* The raw *Pavalam* were procured from country drug store at Marthandam, Tamilnadu and purified by the traditional procedure by soaking in lemon juice for 24 h and the test drug PP was prepared by the process of *Pudam* (Calcination) described in Anuboga Vaidhya Navaneetham 3rd part, page no: 132-133. Adrenochrome and other analytical grade chemicals were procured from Sigma chemicals, U.S.A and S.D fine chemicals Ltd, Mumbai. The experiments include preliminary biochemical studies by standard methods, quantitative analysis of Calcium by AAS, antibacterial studies by paper disc diffusion method, acute oral toxicity study under OECD 423 guidelines, and the haemostatic effects of PP in Albino mice including shortened bleeding and clotting time efficacy on by the method described in Ogle *et al*.,1977. *Results:* The qualitative and quantitative analyses of PP show that it has the contents of 37.48% of calcium, ferrous iron, tannin, and tannic acid. These compounds have the property of Haemostatic action. *In vitro* studies, *Pavala Parpam* has good anti microbial activity at the dilution of 25 microlitre/disc against the bacterial strains such as *S.mutans, S.aureus, E.coli, K.pneumoniae and P.aeruginosa*. Animals were found to be safe up to a maximum dose of 2000mg/kg body weight in acute toxicity studies. The experimental studies done on animal model, *Pavala Parpam* shows potent Haemostatic action by exhibiting significant reduction ($P<0.001$) in bleeding time and clotting time of blood compared with control group. *Conclusion: Pavala Parpam* is the safest and efficacious haemostatic drug comparable to Adrenochrome – a standard drug.

Keywords: Siddha, *Pavalam*, *Parpam* preparation, Calcium, Haemostatic, Adrenochrome, Albino mice.

Introduction

The fundamental subjects of Siddha methodologies are *Vadham* (Alchemy),*Vaithiyam* (Medicine), *Yogam*(Yoga), *Gnanam* or *Thathuvam* (Philosophy). Siddhars, spiritual scientists explored and explained the reality of nature and its relationship to man by their yogic awareness and experimental findings. They postulated the concept of spiritualism for self improvement. From that, the different practices were evolved to be known as the "*Siddha System*". [(1)] As per Siddha Materia Medica, every drug is made up of 5 *Boothas* and has got the following properties such as *Suvai*(Taste), *Gunam* (Character), *Veeriyam*(Potency), *Pirivu*(Bio-Transformation) and *Magimai*(Special Property). The last property is a special one which is present only in certain drugs like Lemon.[(2)] These drugs can be obtained for medicinal purposes from the natural sources viz., 1. *Mooligai*(Herbal origin) 2.*Thathu*(Mineral origin) 3.*Jeevam*(Zoological origin).[(3)] There are so many potent medicines, available in the Zoological origin which is practiced by our Physicians. On comparing with mineral origin, zoological origin is having lesser toxicity, almost nil. Siddha drugs are classified under internal and external medicines. Each type consists of 32 forms.[(3)] Among 32 internal medicinal forms, *Parpam* is a well known potent preparation having shelf life of 100 years. *Parpam*s are inorganic preparations produced by the process of *Pudam* (process of burning using dung cakes), burning, frying, blowing and grinding the *Ulogams*(Metals), *Uparasams*(Secondary minerals), *Paadanams*(Arsenic compounds) with juices, *Ceyaneer*(pungent liquid), etc., which convert them into white ash powder.[(3)] It is made up of carbonates, oxides, etc.

General method of *Parpam* preparation:[(4)] 1. *Sarakku thervu:* Selection of good raw materials under the reference of description of the physical properties narrated in Siddha Gunapadam texts (Materia medica). 2. *Suthi seithal* (Purification and detoxification): This process removes impurities or deleterious properties of the raw drugs. If this is not done, these drugs may induce morbid symptoms/diseases. Mostly, these are purified by repeated ashing by *Pudam* or plunging in certain fluids e.g. cow's urine, herbal juices, oil, etc., or frying with or without fluids and fine pulverization. Each drug has different appropriate purification methods as narrated in the classical Siddha texts. These processes are claimed to change toxic properties and make it potential and safe. 3. The purified ingredients are coarsely powdered and again it should be grounded into a fine powder in a *Karuppu Kalvam* (Agate-black stone mortar). This mortar often preferred as it will not release particles and thus, the medicine may be obtained without impurities.[(3)] 4. *Araitthal* (Trituration) with live fluid: Into the mortar containing fine powder, concerned herbal juices or pungent liquids are added little by little and triturated well for a determined period as per the procedure adopted for different types of *Parpam*. 5. *Villai seithal* (Cake preparation): The obtained semisolid substance is made into small cakes of even dimension and dried under sunlight. The complete dried cakes are set for *Pudam* process. 6. *Maranam* (Incineration): The dried villai are placed in a spreader manner in a mud pan and the mouth of the pan is closed with another mouth of the mud pan. The adjoining portion of the mouth of the pan is sealed by enrolling upto seven layers of *Seelai* – Soil smeared cloth using finely ground soft sand or wheat flour or black gram flour or lime with egg white, depending upon the nature of medicine.[(3)] This sealed apparatus is kept under sunlight until it completely dries well. This sealed mud pan containers subjected to *Pudam* in a pit which is dug in the ground in a spherical size having varied widths and depths from 20 cm to 90 cm depending upon the quantity of medicine to be prepared. *Pudam* is a process of burning using dung cakes. This *Pudam* are described under different types eg. *Kaadai Pudam* – using one dung cake, *Kaudhari Pudam* – using three dung cakes, *Kukkuda Pudam* – using ten dung cakes, *Varaga*

Pudam – using fifty dung cakes, *Manal maraivu Pudam* – using ninety dung cakes, *Gana Pudam* – using hundred dung cakes, *Gaja Pudam* – using thousand dung cakes depending on the size of the pit and the number of dung cakes used. These indicate the amount of heat required and the period of burning for different *Parpams*.[3] The finished form of *Parpam* should fulfill the traditional quality control criteria as mentioned in table 1.

Precautions during *Parpam* preparation:[4] 1. Trituration should be done uniformly and constantly for the prescribed duration. 2. Villai should be free from moisture. It should not be placed as heap in mud pan. 3. Appropriate size of mud pan should be chosen depending upon the size and numbers of villai. 4. The process of *Pudam* should be done in a closed environment with adequate air flow. 5. In most of the *Pudam* process, cow dung cakes have to be used for burning. But for some preparation of *Parpam*s, barks, goat dung cakes are used, especially when high temperature is needed. 6. If there is any contamination in dung cakes such as soil and straw, etc., it will reduce the output of energy. So, proper practical knowledge will help for deciding the quantity of dung cakes. Therefore, Siddhar's described many types of *Pudam*s. 7. The quality and quantity of the ingredients are also involved for the determination of numbers of dung cakes. For example, Gandhagam (Sulphur) and Thalagam (Yellow Arsenic trisulphide) are unstable at high temperature. When these drugs are involved in the preparation *Manal maraivu Pudam* should be used or instead of burning with cow dung cakes, the sealed mud pan should be immersed fully in hot ash. 8. The finished product should be taken out only after complete cooling *Pudam*.

Various *Parpam*s are under practice for long time such as *Sangu Parpam*, *Muthuchippi Parpam*, *Palagarai Parpam*, *Naga Parpam*, *Thanga Parpam*, *Pavala Parpam,* etc.[4] These *Parpam*s claimed some advantages such as deep penetration, rapid action, efficacy in minute quantities, long shelf period, no adverse interaction with herbal drugs, usefulness in obstinate and incurable diseases, wide spectrum of therapeutic indications and rejuvenating action, lack of adverse effects if properly made. In *Anuboga Vaidhya Navaneetham,* attributes the *Pavala Parpam* as *Sanjeevi* drug particularly in the management of bleeding from the organs. So, we selected the drug *Pavalam* (*Corallium rubrum*) of marine origin belonging to the phylum Cnidaria. Such marine product *Pavalam* is the major ingredient of *Pavala Parpam*. It has validated its safety and efficacy as Haemostatic drug. Various procedures for the preparation of this *Parpam* are mentioned in Siddha Materia Medica such as *Theran yemaga* method, processing with latex of Erukku *(Calotropis gigantea)*, leaf poultice of Ilanthai *(Zizyphus jujuba)* or Thaivelai *(Gynandropsis pentaphylla)* or Keezhanelli *(Phyllanthus niruri)* or Rabbit's blood or honey or sugarcane candies. Each preparation has different therapeutic benefits. They vary with adjuvant, but all the *Pavalam* included formulations are found to possess diuretic, laxative, astringent, nervine tonic and spermatogenesis properties and useful in the treatment of Azhal aggravated diseases, excessive phlegm and eye disorders. [3]

Material and Methods

Raw materials

The *Pavalam*s, obtained from Calcareous-shells of Red Coral, were procured from the country drug store at Marthandam, Kannyakumari dt. Tamilnadu. Such *Pavalams* were free from dents and perforation. Elumitchai pazha chaaru is a fresh lemon juice obtained from the fruits of *Citrus lemon* (Family: Rutaceae). The lemon juice was filtered using cotton cloth. *Karkandu* – Sugar candy were procured from a local market of Chennai.

Plate 1: Raw Pavalam-Before Purification

Plate 2: Pavalam-After Purification

Preparation of PP

PP was prepared under the guidance of authenticated Siddha Clinical Pharmacologist (Doctor of medicine- Siddha - Gunapadam) as per the method described in Siddha literature (*Anuboga Vaidhya Navaneetham* 3rd part, pg no.132 – 133)[5]. The process of synthesis of this *Parpam* involves two stages. I. Purification *(Suthi seithal)*: The *Pavalam*s were taken in the mud pot and lime juice was added into that mud pot until the *Pavalam* got immersed and the mouth of the mud pot was closed with the other proper mud plate. It was kept without disturbance for 24 hours and then, *Pavalam* was taken from the mud pot and washed with luke warm water and allowed to dry well. The dried *Pavalam* stored in the air tight container. II. Calcination (*Maranam)*: The Ingredients are Purified *Pavalam* 35g, powdered sugar candy (Karkandu) 105g. In earthen vessels, half of the part of Sugar candies was taken. Above the Sugar candies, the entire quantity of purified *Pavalam* was spread to get even layer. Then, another half of the Sugar candies was placed over the purified *Pavalam*. This preparation should be kept tightly with no spaces above it. The mouth of the bowel was closed with another earthen bowel and the joining space between the two bowels were closed with the five rolls of mud pasted fine cotton gauze roll and dried under sunlight. This well dried apparatus was set for *Pudam* (Calcination) with 100 No's of cowdung cakes. For this *Pudam* process, a spherical pit was dug in the ground with width and depth of 90cm. In the pit, 75 No's of cow dung cakes were placed and arranged in circular manner. Over this, the dried apparatus was kept and covered with remaining 25 No's of cow dung cakes in conical manner. Now, the process of *Pudam* was done by burning the dung cakes until it burnt completely. After this process of *Pudam*, the apparatus was allowed to cool and it was opened carefully. The Sugar candies present inside the apparatus got evaporated and the *Pavalam* present as the residue in the apparatus as a white ash material. The white coloured *Pavalam* was taken and ground well in the Kalvam(Stone mortar), until it got into very fine powder. This fine powdered *Pavalam* was said to be *Pavalam*

Parpam and subjected for traditional tests (Table 1) to confirm whether it is properly finished or not. The finished form of PP was stored in an air tight glass container.

Qualitative and Quantitative analyses:
5 g of *Pavalam Parpam* was taken in a 250 ml clean beaker and 50 ml of ghee was added to it. Then, it was boiled well for about 20 min. Then, it was allowed to cool and filtered in a 100 ml volumetric flask and made up to 100 ml with ghee. This preparation was used for the qualitative analysis of acidic/ basic radicals and biochemical constituents [6] and quantitative estimation of calcium content in it. For the Quantitative analysis of Calcium present in *Pavalam Parpam* was done at Mattex Laboratories, Chennai by using the equipment: Atomic Absorption Spectrometer (AAS) – Make: Varian, Australia.

Anti bacterial activity by paper disc diffusion method:
The sterilized (autoclaved at 120 ^{o} C for 30 min) medium (40-50 ^{o} C) was inoculated (1 ml / 100 ml of medium) with the suspension (10 ^{5} cfu mL $^{-1}$) of the microorganism (matched to Mc Farland barium sulphate standard) and poured in to a petridish to give depth of 3-4 mm. The paper impregnate with the test compound PP as10, 25, 50, 100, 500 and 1000 µg mL^{-1}in dimethyl farmamide was placed on the solidified medium. The plates were pre incubated for 1 h at room temperature and incubated at 37degree Celsius for 24 and 48 hr for anti bacterial activities respectively [7]. Preparation of standard bacterial suspensions: The average number of viable *Streptococcus mutans, Staphylococcus aureus, Escherchia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* organisms per ml of the stock suspensions were determined by means of the surface viable counting technique (Miles and Misra, 1938). About 10^{8}-10^{9} colony-forming units per ml will be used. Each time, a fresh stock suspension was prepared; the experimental conditions were maintained constant so as to obtain suspensions with very close viable counts. Sample concentration: 4 gm – 400 ml of solvent in 10 µl, 25 µl, 50 µl, and 100µl / disc. Standard against Bacteria: Ciprofloxacin HCl, 50 mcg / disc. Zone of inhibition: 14 mm – Low sensitive, 15 mm – Moderate, above 16 mm – Highly sensitive

Safety and efficacy studies
These studies were carried out at Research and Development, CL. Baid Metha College of Pharmacy, Chennai.

A. Preparation of drug for dosing:
PP, used for the study, was suspended each time with ghee until attaining good solubility and the other drugs used were suspended each time with 1% (w/v) solution of Sodium Carboxy methyl cellulose before administration.

B. Drugs and Chemicals:
Adrenochrome and fine chemicals used in these experiments were obtained from Sigma Chemicals Company, U.S.A. Other analytical grade chemicals were obtained from S.D Fine Chemicals Ltd., Mumbai.

C. Experimental animals:
Swiss strain albino mice of weighing 25-30g of either sex were used for the pharmacological and toxicological studies. The animals were kept under standard conditions 12:12 (day/night cycles) at 22^0C room temperature, in polypropylene cages. The animals were fed on standard pelleted diet (Hindustan Lever Pvt Ltd., Bangalore) and tap water *ad libitum*. The animals were housed for one week in polypropylene cages prior to the experiments to acclimatize to laboratory conditions. The experimental protocol was approved by the Institutional Animal Ethical Committee (Ref No: IAEC/XIV/18/CLBMCP/24-07-07) of CL. Baid Metha College of Pharmacy.

D. Acute oral toxicity study:
Acute oral toxicity was conducted as per the OECD guidelines (Organization of Economic Cooperation and Development) 423 (Acute Toxic Class Method). The acute toxic class method is a stepwise procedure with 3 animals of a single sex per step. Depending on the mortality and /or moribund status of the animals, on the average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure results in the use of a minimal number of animals while allowing for acceptable data based scientific conclusion. The method uses four doses of 5, 50, 300, 2000 mg/kg body weight and the results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of chemicals which cause acute toxicity. Swiss strain albino mice of either sex weighing 25-30g were fasted overnight, but allowed water *ad libitum*. Since the formulation is relatively non toxic in clinical practice the highest dose of 2000 mg/kg/p.o (as per OECD guidelines "Unclassified") was used in the acute toxicity study. The animals were observed closely for behavioral toxicity, if any by using FOB (Functional observation battery).

E. Study on Haemostatic action of PP in albino mice:
Swiss albino mice of either sex, 20-25 g were randomly distributed into 3 groups of 6 animals each and the following regimen of treatment was instituted. Group I animals were treated with mixture of ghee and water at 1ml/100g,b.wt/p.o as control. Group II animals were treated with test drug PP at the dose of 500 mg/kg/p.o suspended in ghee 2ml.Group III animals were treated with standard drug Adrenochrome 10 µg/animal/i.p. One hour after the respective treatment, the animals were anesthetized with ether. The abdomen was opened and portion of liver was cut off with fine scissors from the left lobe. A blotting paper was used to measure the time at which the profuse bleeding was stopped. The time to stop the bleeding (no more staining of blotting paper with blood) was recorded and compared with control and standard groups. For calculating Clotting time, blood was collected in Capillary glass tube by with drawing blood through retro orbital puncture. The time of fibrin thread appearance at the breaking point of capillary tube was counted and compared with the groups. [8]

Statistical analysis:
All *In vivo* experimental results were expressed as mean ± standard deviation followed by Dunnett's test.

Results

Finished form of *Pavala Parpam* (PP)

The PP was prepared following strictly the method mentioned in the Siddha text. The finished PP gave positive results to all tests for *Parpam* as mentioned in Siddha Gunapadam literature (Table 1).

Plate 3: Test Drug-Pavala Parpam

Qualitative and Quantitative evaluation:

The acid radicals present in PP are Chloride and Carbonate. The basic radicals present in PP are Calcium and Ferrous iron. The biochemical properties present in PP are Tannic acids, Unsaturated compounds, Reducing sugar (trace), Alkaloids, Proteins, Tannins, and Amino acids(Table 2). The Quantitative analysis of Calcium present in PP is 37.48 %.

Antibacterial evaluation:

In-vitro antibacterial activity of PP was screened against five bacteria strains. The results of table 3 show PP exhibits good antibacterial activity in 25µl / disc itself when compared to standard drug Ciprofloxacin.

Acute oral toxicity studies and dose determination:

The results of table 4 shows PP at the dose of 2000mg/kg/po did not exhibit mortality and did not show any signs of acute toxicity and behaviour changes. As per OECD 423 guidelines, the dose is said to be "Unclassified" under the toxicity scale. Hence, further study with higher doses was not executed. So, the drug PP falls under class 4 (LD50 > 2000mg/kg).

Haemostatic activity:

The results of table 5 for Haemostatic studies show that PP produced significant reduction of bleeding and clotting time when compared to the control and standard group.

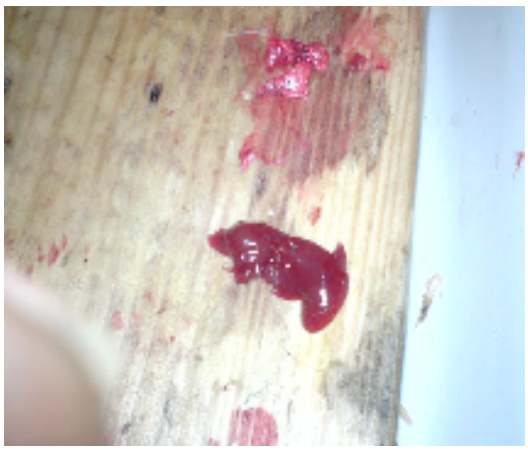

Plate 4: Dissected Liver from Albino Mice

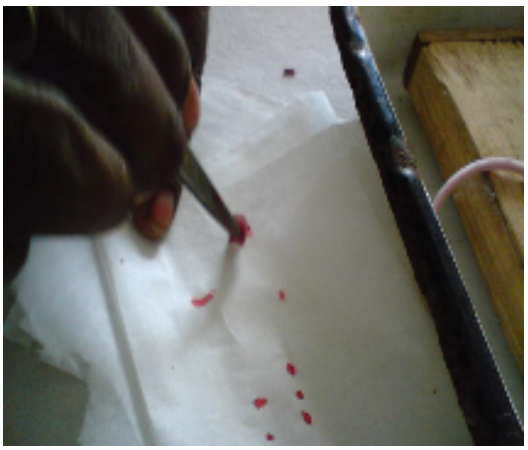

Plate 5: Estimating the bleeding time on blotting paper using the portion of liver

Table 1: Traditional Tests for formation of *Parpam*

S.No	Test
01	White in colour without any shiny appearance
02	Tasteless and odourless
03	Did not regain luster on heating again at same temperature
04	Sample floats on water. Did not immediately immersed in water
05	Not translucent
06	Impinged in the papillary ridges when the sample rubbed in between Index finger and thumb

Table 2: Preliminary biochemical studies

Test for Chemicals	Observation	Inference
Calcium	Formation of white precipitate	+
Ferrous iron	Appearance of blood red colour	+
Chloride	Formation of white precipitate	+
Carbonate	Formation of effervescence	+
Reducing sugar	Mild colour changes	Trace
Alkaloids - *Meyer's method*	Appearance of cream colour	+
Drafendroff Method	Appearance of orange precipitate	+
Amino acids	Formation of violet precipitate	+
Tannic acid	Formation of bluish black precipitate	+
Tannins	Formation of white precipitate	+
Unsaturated compounds	Get decolorized	+
Sugar – *Bendict's method*	Mild colour change	+
Protiens – *Biuret test*	Formation of violet colour	+

The nil inference of other biochemical analyses are not indicated in this above table

Table 3: PP sensitivity against bacterial strains

Organism	**Standard Drug Ciprofloxacin 50 mcg/disc**	**Test drug (PP**µl/disc)		
		Zone of inhibition in mm		
		10µl	25µl	50µl
Strep. mutans	30	15	18	21
Staph. aureus	29	12	17	22
E.coli	30	18	21	24
K.pneumoniae	30	13	17	19
Ps.aeruginosa	29	15	18	20

14 mm – Low sensitive, 15 mm – Moderate, above 16 mm – Highly sensitive

Table 4: Acute oral toxicity – Dose finding experiment and its behavioral signs of toxicity

Parameters	5mg/kg					50mg/kg					300mg/kg					2000mg/kg				
	1h	2h	3h	4h	24h	1h	2h	3h	4h	24h	1h	2h	3h	4h	24h	1h	2h	3h	4h	24h
Appearance	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Activity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	**S**	N	N	N	N
Gait	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	**D**	N	N	N
Reaction to stimuli	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	**2+**	**2+**	3+	3+	3+
Lacrimation	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Salivation	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Pilo erection	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Stimulant	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Depressant	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	**1+**	A	A	A	A
Writhing	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Diarrhoea	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Rearing	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Paw licking	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Convulsion	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Mortality	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A

N- Normal, S – Sluggish, D – Disturbed, 1+ - Present minimum, 2+ - Present medium, 3+ - Present maximum, 4+ - Highly observable, A – Absent

Table 5: Haemostatic effects of *Pavalam Parpam* in albino mice

Haemostatic Indices	Group I Control	Group II Test drug PP	Group III Standard-Adrenochrome
Bleeding time (s)	134.33 ± 29.042	56.66± 2.582***	87.16 ± 3.81***
Clotting time (s)	186.66 ± 68.313	110 ± 31.305 ***	158.3 ± 18.9

Values are expressed as mean ± S.D followed by Dunnett's Test. ***P<0.001, (n - 6)

Discussion

The *Pavala Parpam* processed in sugar candy is used as haemostatic drug in the treatment of excessive and irregular bleeding through vagina *(Perumbadu)* and bleeding through rectum *(kuruthimoolam)* in Siddha medicine. In *Anuboga Vaidhya Navaneetham*, *Pavala Parpam* is said to be a *Sanjeevi* drug for arresting the haemorrhage from the organs. But currently, this medicine has not been practiced for treating haemorrhagic condition. Many of the earliest texts indicate that *Pavala Parpam* has good effects on respiratory disorders such as cough, wheezes and haemoptysis and indicated for chronic bronchitis and bronchiolitis.[9] Generally, *Pavalam* has *Thuvarppu suvai* (Astringent) which has the property of arresting the bleeding arising from the organs. To explore the haemostatic potential of this formulation that the Siddha medicine has intensive drugs for critical conditions also to the global community, these studies were made.

The preliminary biochemical study of *Pavala Parpam* reveals the content of calcium, ferrous, chloride, carbonate, tannin, tannic acid, unsaturated compounds and alkaloids. Since the presence of calcium (37.48%), ferrous, tannin, and tannic acid, it has the action of haemostatic. Calcium – Plays the important role in the coagulation of blood. It is the IV factor in coagulation mechanism. It acts by converting prothrombin to thrombin and also necessary for the formation of both intrinsic and extrinsic thromboplastin. It is required for the maintenance of the capillary permeability.[10] Ferrous Iron – In Haemoglobin, iron is present in ferrous form.[10] So *Pavala Parpam* has influence on raising Hb content in the blood.

Now-a-days, many pharmaceutical companies isolate the components and extract from the corals namely Eleutherobin, Sarcophytols A and B, Hydroxyapatite, Calcium.[22] Eleutherobin involves in preventing multiplication of cancerous cells and prevents breast and ovarian carcinoma.[13] Sarcophytols A and B are also found to posses anti tumour promoting character.[11] Hydroxyapatite is used as bone graft to facilitate the regrowth of bone in fracture.[12] Calcium isolated from coral is known as coral calcium. This is used as calcium supplements for several diseases instead of synthetic calcium. Recently, organic materials extracted from the corals are used in the studies of anti-HIV drug.[12]

The anti microbial study reveals that *Pavala Parpam* has significant anti bacterial activity. Most bacterial strains are highly sensitive to *Pavala Parpam*. So, *Pavala Parpam* can be very useful in the treatment of respiratory infections. The LD_{50} of *Pavala Parpam* as per OECD guideline falls under class four with no signs of acute toxicity at the maximum dose of 2000mg/kg. The haemostatic action study on animal model shows that the reverse pharmacology done with the *Pavala Parpam* in bleeding time and clotting time of blood, treated with test drug PP at 500mg/kg.,b.wt/p.o in albino mice showed positive correlation of results. *Pavala Parpam* exhibits significant reduction in both bleeding and clotting time when compared to untreated control animal's blood. The significant reduction in bleeding with *Pavala Parpam* is well comparable to that of Adrenochrome, a standard haemostatic drug. The standard Adrenochrome semicarbazone has lack in reducing clotting time *in vivo* model.[14]

Oral administration of *Pavala Parpam* caused a significant decrease in the template bleeding time confirms that the positive haemostatic action via on effect on the formation of platelet plugs in the site of small vessels injuries (Primary haemostasis). The formation of fibrin involves the cascade coagulation factors and mediators, allowing the platelet plug to be stabilized. So, the clotting time allows easily assessing the function of intrinsic and common pathways of the coagulation cascade.[10] In addition to augmenting primary haemostasis, the haemostatic effect of PP may be related to pathways of the coagulation cascade, as indicated by shortened clotting times in these studies.

Conclusion

In spite of the long usage of *Pavalam* in the system, the confirmation of no toxicity declares the *Pavala Parpam* at the dosage level of 500mg/kg body weight as the safer, potential drug under the Siddha system. We conclude that the indented dosage of PP from 130mg to 260mg narrated in *Anubogha Vaithiya Navaneetham* is a safer, potential therapeutic dose for haemostatic effect. Even though, arising bleeding is an acute condition, the onset of action of *Pavala Parpam* is not known. The study done here is only the preliminary work of *Pavala Parpam*. Further, we can explore this drug in the following aspects viz., Formation of molecular and structural formula, active constituents, onset and mode of action and its half life period, measurement of Prothrombin time, Plasma fibrogen concentration, Platelet aggregation and thrombin induced aggregation, etc.

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