

# **Panchvalkal: A Monograph**

*Experimental & clinical studies on the use of  
modified Panchavalkal (an Ayurvedic  
formulation) in Leucorrhoea*  
**CCRAS Monograph**

*Based on a series of clinical  
& preclinical studies*

**Eds: Dr Jayashree Joshi, Dr Rama Vaidya**

**Medical & Research Centre, Kasturba Health Society & ICMR  
Advanced Center for Research in Reverse Pharmacology of  
Traditional Medicine,  
Mumbai**

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## **Panchavalkal (Modified) for the Treatment of Leucorrhoea**

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Panchavalkal (an Ayurvedic formulation) in  
Leucorrhoea*

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**# National Institute for Research in Reproductive Health, Mumbai**

**Published by CCRAS, Ministry of Health & Family  
Welfare, Delhi, Government of India.**

# Panchavalkal: A Monograph

**Vata**  
(*Ficus benghalensis* L.)



**Peepal**  
(*Ficus religiosa* L.)



**Udumbar**  
(*Ficus infectoria* L.)



**Plaksha**  
(*Ficus infectoria* Roxb.)



**Shirish**  
(*Albizia lebbek* L.)



**CCRAS, Ministry of Health & Family Welfare, Delhi, INDIA**

*Dedicated to the memory of Late Dr Ranjan Bhatt & Late Dr Vanita Rege*

## PREFACE

*“.....the definition of Ayurveda would be a Shastra /Science that is with ‘the times of life’ and about life and health. As a science, it would be open to change and growth.”*

- Sri Babubhai P. Vaidya on Ayurveda University: Debate in Gujarat Legislative Assembly (1963)

This CCRAS Monograph on Panchavalkal (modified) for the treatment of Leucorrhoea, edited and authored by Dr. Jayashree Joshi, Dr. Rama Vaidya and a team of scientists, is an illustrious example of how with the openness to grow alongwith changing times a conservative traditional healthcare therapy can be developed into evidence based modern healthcare formulation for wider and precise clinical application. The Indian healthcare sector is uniquely positioned; it has adapted to the most advanced biomedical sciences & technologies and at the same time it has preserved and encouraged the rich traditional healthcare sciences & practices. These studies on Panchavalkal for Leucorrhoea has involved a multidisciplinary team of experts from Ayurveda, stri-roga vinyana, reproductive research, gynecology, medicinal chemistry, phyto-pharmacology, microbiology, cytology, clinical biosciences and formulation development. This volume on clinical and experimental work is also an example of unique collaborative efforts between government, academia, and industry.

This compilation of scientific work of the team for over a decade consists of six chapters. The first chapter gives brief introduction to the subject and explains the genesis of the study. Second chapter covers a brief relevant review of literature of Ayurvedic as well as modern concepts of leucorrhoea, and also the Dravyagunavidnyana, phytochemistry and phyto-pharmacology of Panchavalkal. The third chapter elaborates on the clinical study of traditional Panchavalkal kwath with objective evaluation. The fourth chapter takes a detailed account of Panchavalkal vaginal cream formulation development, whereas the fifth chapter provides details of animal safety studies of the vaginal formulation. The last sixth chapter gives thorough details about the comparative clinical study for safety and activity of Panchavalkal vaginal cream & oral Ayurvedic formulation with that of standard allopathic treatment in uncomplicated leucorrhoea. The Appendix A includes a list of bibliography and Appendix B includes reprints of six scientific papers published on based on the initial project with Panchavalkal Kwath. The data from the last 3 chapters is published for the first time in this monograph.

Integrative drug development based on traditional knowledge practices and conventional modern evidences is always a challenging one. However with continuous efforts and engagements in the drug development of modern as well as Ayurvedic medicines over several decades Dr. Ashok Vaidya has shown novel approach of ‘Reverse Pharmacology’. The Reverse Pharmacology which was proposed and defined by Dr. Ashok Vaidya in late 1990’s was adapted as a formal path for a drug development from Ayurveda by CSIR-NMITLI project during 2002 to 2007. Eventually in 2007 ICMR established an advanced centre of Reverse Pharmacology at MRC-KHS under the guidance and leadership of Dr. Ashok Vaidya.

This work of Panchavalkal in Leucorrhoea has followed the path of Reverse Pharmacology which facilitates multidisciplinary integration while demonstrating desired conventional evidence in drug development.

Experiential domain of knowledge is available for use of Panchavalkal in women's diseases through classical Ayurvedic literature, traditional clinical practices and few clinical studies with subjective parameters. However the objective evidence of activity through i) semiquantitative symptom scoring, ii) cervical cytology, iii) colposcopy and iv) vaginal pH alongwith v) safety using organ function tests before and after treatment, was for the first time demonstrated through this exploratory clinical study of local use of Modified Panchavalkal in cases of Leucorrhoea. The Team's background work at National Institute of Reproductive Health Research (NIRRH of ICMR) in reproductive tract infections (RTI's) and engagements with family planning clinics including earlier studies in high risk HIV positive women has immensely helped the group to develop the protocols for diagnostic methods and instruments of semi quantitative clinical evaluations for this study. Ayurvedic expertise from Sion Ayurveda Mahavidyalaya and from MRC-KHS has added components of assessment of Deha-Prakruti and Doshaprakopa-Vikruti. Interestingly Prakruti analysis of participants in this study indicated that majority of the subjects in the study had Pitta dominating dwandwaj Prakruti. This finding corroborated with the classical description of Panchavalkal indication. The first open clinical study with Panchavalkal douche alone for 2 weeks has demonstrated clinical symptomatic improvement as well as objective improvement in cytological, colposcopic and vaginal pH estimation. Subsequent comparative clinical study with local Panchavalkal douche and simultaneous oral Ayurvedic treatment also showed significant symptomatic as well as objective clinical improvement. These results were comparable with the parallel group treated with conventional modern medical management which included systemic and/ or local antimicrobial therapy.

Panchavalkal kwath douche has few shortcomings such as preparing the fresh kwath every day, need for the patient to visit doctor for taking douche, and the effective contact of medicine with the cervicovaginal lesion is also for limited time. To overcome this drawback the team has developed vaginal cream with the help of Dr. Dilip Mehta of Viridis Bio-Pharma. Classical Ayurvedic literature and practices use different alternative methods to douche (Yonidhavan) viz. Kalka (application of paste), Pichu (application of medicated tampons), & Varti (medicated vaginal pessary). Vaginal drug delivery formulations of modern drugs like miconazole, clotrimazole, clindamycin, mycostatin, lactate gel preparations etc are widely available and are extensively used by clinicians. Also V-gel (Himalaya) and Pentaphyte-5 cream (Dr Paleps Research Foundation) are the two Ayurvedic products based on Panchavalkal which are available in the market. However our team of scientists have followed methodical integrative path of Reverse Pharmacology to develop vaginal cream with preclinical studies and a globally acceptable polymer gel base.

The process of Panchavalkal vaginal formulation development included pharmacognosy, passport data of each of the five plants of Panchavalkal which included primarily the source of collection, method of transportation, system of storage etc., phytochemical profiling and standardization of different extracts, purity testing for heavy metals, pesticides residue, microbial load, and aflatoxin contents as

per WHO norms. This vaginal formulation was prepared by using vaginally compatible gelling agent carbopolymer viz. carbopol 940 (IP). This is superior to the petroleum jelly used in many vaginal/ dermal formulations as it does not weaken the latex in the condoms which are used by husbands of thousands of women in the child bearing age. This extracts were also studied for in vitro antimicrobial activity against organisms commonly observed in vaginitis. Most importantly selected safety studies in animals were undertaken viz. vaginal tolerability in rabbits and dermal tolerability in rats. These studies demonstrated local, systemic, hematological, and organ safety in two different animal species studied over 28 days.

After having a well developed standardized vaginal cream for local application a controlled study in comparison with conventional modern medical treatment was planned with the primary objective to study tolerability, effectiveness and safety of Panchavalkal vaginal cream for first 10 to 12 days. To complete the syndromic treatment these patients were given a standardized oral Ayurvedic formulation consisting of extracts of Tinspora cordipholia, Triphala, and Trikatu for next 2 weeks. This group with Ayurvedic treatment was compared with the treatment group of standardized modern medical therapy recommended by CDC and National guidelines of 2002 and 2006. Results of this study established the clinical safety of Panchavalkal vaginal cream. Clinical and biochemical safety was also demonstrated for the local as well as systemic Ayurvedic treatment. The efficacy was comparable with that of conventional modern medical therapy.

The key message coming out of all these clinical and experimental studies are as follows;

- Panchavalkal Douche and vaginal cream both are clinically safe and are significantly effective for symptomatic relief.
- Panchavalkal local therapies are predominantly beneficial in bacterial vaginitis and fungal infections but were not so effective in Trichomoniasis, Chlamydia, and viral infections.
- Panchavalkal local therapy showed better response in Pitta dominant Prakruti individuals and with Pitta dominant pathology of the disease.
- Panchavalkal therapy can be an effective alternative to the surgical mode of management for the cervical erosion and persistent vaginal discharge.
- Panchavalkal local therapy along with oral Ayurvedic therapy individualized as per the prakruti and doshaprakopa would enhance syndromic Ayurvedic management for Leucorrhoea.

Panchavalkal is one of the Ayurvedic formulations available for local therapy of Leucorrhoea. There is ample scope to investigate other commonly used traditional Ayurvedic formulations used locally such as Triphala Kwath dhavan, Yashtimadhu-taila Pichu, Nimba-taila Pichu for the conditions where Panchavalkal was not so effective. Concomitant use of other traditional local therapies such as Yoni-Dhoopan (vaginal fumigation) as well as systemic internal medication would add a different dimension to the management of Leucorrhoea. Reverse Pharmacology based integrative research and drug development would bring forward many such complementary or alternative therapies in the management of Leucorrhoea. Finally a concern in the management of Leucorrhoea is about the repetition of infection and

asymptomatic persistence of infection leading to the complications of pelvic inflammatory disorders. Hence it is an ethical obligation of the healthcare providers to provide holistic and integrative healthcare to ensure complete and comprehensive management of Leucorrhoea for preventing secondary complications and restoring health, and addressing the management of the couple when necessary.

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Sd

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Editor

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Foreword

Authority from CCRAS

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**Chapter 1.****Introduction- genesis of the study***Jayashree Joshi, Dilip Mehta , Rama Vaidya*

- 1. Introduction to Panchavalkal**
- 2. Leucorrhoea- current concepts, management strategies by WHO, special studies**
- 3. Leucorrhoea (shwetapradara)- Ayurvedic concepts & management**
- 4. Genesis of the study**

**1. Introduction to Panchavalkal**

In Ayurveda there is a fundamental concept, based on inference and experience, that certain medicinal plants or medicines (*dravyas*) work best in combination. This is similar to the potentiation concept in modern pharmacology and it is indeed amazing how the ancient physicians selected various plants for combining successfully with the idea of either enhancing therapeutic efficacy or reducing side effects. There are therefore several formulations in traditional medicines which use medicinal plants or products in groups of 3, 5, 7 or 10, the common examples being “Triphala, Trikatu, Panchagavya, Panchatikta, Panchalavana, Dashamula etc. “Panchavalkal (PVK)” is one of the common combination therapies described in all standard texts.

Panchavalkal literally means “5 barks”, ie pancha = 5, and valkal = barks, in Sanskrit, and in several regional languages. PVK is prepared from the barks of 5 specified medicinal plants from the ficus family and is extensively used for the treatment of women’s diseases, specially leucorrhoea, uterine diseases and cervical erosions, and also for wounds and ulcers in other parts of the body. The combination of the barks of 5 medicinal plants, namely *Vata (Ficus bengalensis Linn)*, *Udumbara (Ficus racemosa Linn)*, *Peepal (Ficus religiosa Linn)*, *Plaksha (Ficus infectoria/ Ficus lacor)*, and *Parisha (Thespia populnea)* in equal proportions is known as the Classical Panchavalkal and is described in the treatment of women’s diseases, wounds

and ulcers. Its use is described in Ayurvedic texts (Charak, Sharangdhar; Kashyap Samhita; Bharat Bhashajya Ratnakar; Bhava - Prakasha) and in books on traditional practices in recent times by famous Vaidyas (Tewari Premvati; Joshi Nirmala; Gogte VM) as it is used extensively by traditional practitioners in general practice as well as in Ayurvedic Medical Hospitals all over the country.

The traditional system of medicine or Ayurveda, which is in use for centuries in India, underwent a phase of recession in India after the invasion by foreign warriors in the medieval ages and was not investigated or documented with the same scientific rigour as the modern medicine and biological sciences in the last two centuries. Most of the knowledge remained preserved through the Vedic literature and practitioners of Ayurveda, passed on initially by oral mnemotechnic methods and later through the granthas in Sanskrit and the several regional languages born from it. However in the medieval ages some granthas were translated into English and German and also some other languages (Wise T, 1845; Sharma PV, 1983). Many scientists in the twentieth century, with biomedical background, investigated the biological properties of Ayurvedic plants, particularly from the 2<sup>nd</sup> decade of the 20<sup>th</sup> century onwards. Since then a number of biologically active extracts and compounds/ ingredients have been isolated from PVK and related plants and their “in vitro” and for some plants and phytoconstituents, “in vivo” studies have been carried for antimicrobial activity in order to determine the biological plausibility of effectiveness and the putative mechanisms of action (CCRAS, 1990; 1996; 2001; Bhatt RM, 1984, 1985; Patel Saraswati, 1991; Mandal, 1997; Bindu et al 1997, Khan et al, 1998; Gupta, 2004; Palep et al , 2004; Hemaiswarya, 2009).

The traditional PVK treatment is in the form of a decoction prepared from the 5 plants. It had to be prepared fresh everyday. Recently Bhatt RM (1984-1986), Mehta DS et al (Unpublished data 2002) and Mehta AG & Palep et al (1994), Ashar et al (2011,2012), Aswar et al (2008). Vyas et al (Vyas) have demonstrated the antibacterial activity of PVK extracts “in vitro” against pathogenic organisms and have prepared user friendly formulations like a oral tablets, capsules, dermal cream, or liquid or gel hand wash.

## Classical vs Modified Panchavalkal

न्यग्रोधोदुम्बराश्वत्थपारीषप्लक्षपादपा : ।  
पञ्चैते क्षीरिणो वृक्षास्तेषां त्वक् पञ्चवल्कलम् ॥ भा. नि.

क्षीरीवृक्षाः हिमा वर्ण्या योनिरोग व्रणापहाः

The classical Panchavalkal is the combination of barks of 5 trees of the ficus family : *Vata* or *Ficus bengalensis*, *Udumbar* or *Ficus glomerata*, *Plaksha* or *Ficus infectoria* (*lacar*), *Ashwatta* or *Ficus religiosa* and *Parisha* or *Thespesia populnea* Corr. These are extensively used in diseases of women as per the classical texts.

The above 5 plants are grouped together as Classical Panchavalkal. In Ayurveda sometimes substitute plants or alternatives (called as *paryayi vanaspati*) are also described. (Bhava Prakash, 1986; Gogte VM, 2000; Bapalal Vaidya, 1998). These are used if:

- a) The plant does not grow in that region and / or
- b) If the substitution is likely to work better for a particular medical condition or for a particular patient as per Prakriti or doshaprakop or hetu.

The alternatives for Classical Panchavalkal that have been described in texts are *Vetas* (*Calamus rotang*) as per Sharangdhar and *Shirish* (*Albezzia lebeck* Linn) as per Bhava-Mishra and Kaiyadeva Nighantu. When one of these alternatives is used it is called as the Modified Panchavalkal. In vitro studies with single extracts or different combinations, of Classical and Modified PVK, against bacteria and fungi commonly causing leucorrhoea, were carried out by Bhatt RM et al and revealed that Modified PVK with Shirisha had the best antibacterial and antifungal activity. The MIC varied from 0.3 to 0.8 % (Patel, 1993).

क्वचित् कपितनस्थाने शिरीषो वेतसः क्वचित् ।

कैयदेवनिघण्टुः ओषधिवर्गः ॥४३८॥

Apart from Dr Ranjan Bhatt's work on antibacterial activity of Panchavalkal (PVK) extracts against vaginal pathogens she had also carried out preliminary studies on prevention of infections in burns and wounds by PVK extracts in animal models and in a pilot study in burns patients (Bhatt et al, 1984). This work was further extended by Dr Madhuri Gore, Head of Dept of Burns Ward, and Dr Rama Vaidya, former Dean, Bhavan's SPARC, Mumbai, at LTMG Sion Hospital, in the Burns ward in 100 cases of mild to moderate burns with excellent results and Patel DS (Unpublished data 2002) and also by Palep HS (2004).

Since Parisha (*Thespia populnea*) is not widely available and not so well documented and also because of the confirmatory antimicrobial properties of Shirish it is preferable to use the modified Panchavalkal with Shirisha (*Albizzia lebeck*) in practice for women's infections. The anti-inflammatory, antihistaminic and antifungal activity of *Albizzia lebeck* is well documented.

The primary indications for which PVK is used in Ayurveda are women's diseases, particularly leucorrhoea (*shwetapradara* = white discharge) and wound healing (*vranaropana*).

It is important that the disease is understood before the treatment can be defined hence a brief description of leucorrhoea is given below.

## 2. Leucorrhoea- Current concepts, WHO classification, Management strategies

Leucorrhoea (Leucos= white + rrhoea= discharge) or excessive white discharge is a common symptom in women, particularly in the reproductive age group. In earlier days it was ignored as a general symptom of weakness, however with changing lifestyles and advances in diagnostic microbiology and advent of screening for cervical cancer by Papanicolaou smears it soon became apparent that leucorrhoea

could be a forerunner of Reproductive Tract Infections (RTIs) including Sexually Transmitted Diseases (STDs), and Cervical Cancer. Indeed it could be the earliest symptom in some cases, however many cases of early or chronic infection may be totally asymptomatic.

Leucorrhoea or white discharge is the symptom caused by cervicitis or vaginitis and often both are co-existent. Multiple causes are also common. It is important to recognize the common physiological and pathological causes of leucorrhoea.

**Table 1.1. Classification of Causes of Leucorrhoea**

<b>Cause</b>	<b>Examples</b>	<b>Usual Therapy</b>
<b>Physiological</b>	Physiological midcycle mucorrhoea, pregnancy, sexual stimulation, Intrauterine device Sterile	No treatment required Counselling
<b>Systemic Nongenital Causes</b>	Anemia, Protein deficiency, Congestive Cardiac Failure, Uncontrolled Hypertension	Treatment of the Cause
<b>Non-infectious genital diseases</b>	Prolapse, Fibroids, Ovarian tumours	Gynecological Surgery
<b>Non Sexually Transmitted Infectious</b>	Bacterial vaginitis, Pseudomonas infection, Mycoplasma genitalis (STD also), Staphylococcal infections, Streptococcal infections due to poor hygiene or iatrogenic	Specific antibacterial therapy: Local/ and/or Systemic, Plant extracts
<b>Sexually Transmitted Infections</b>	Gonorrhoea, Syphilis, Chlamydia, Trichomonas vaginitis, Herpes Simplex Viral infection etc	Specific antibiotics, Plant extracts; partner treatment essential

It is logical to derive that PVK will act more effectively against infectious causes and not against physiological or other gynecological pathologies.

In the early nineties of the last century the global epidemic of RTIs/STDs was recognized and leucorrhoea was recognized as an important signal symptom within

the syndromic approach to diagnosis. Whilst an etiological diagnosis of the causative organisms with specific diagnostic tests, like culture, immunochemistry or PCR, for various infections is ideal, the tests are expensive and the technology is not available in most countries, especially the developing countries (WHO, 1994, 1998; Aral, 2008; Coleman, 2013). Moreover mixed or multiple infections are common (Bang et al, 1989; Joshi et al, 1991, 1994).

Currently it is estimated that more than 300-500 million women over the world suffer from various STDs like Chlamydia, Trichomoniasis, Gonorrhoea, Syphilis, Herpes Simplex Virus Infection, Human Papilloma Virus infection etc (Aral SO, 2006; Rekart et al, 2008; Da Ros, 2008; Coleman, 2013).

#### **Prevalence of RTIs in Indian background:**

The prevalence of gynecological infections has been reported to be high in several studies from 5 % in high socioeconomic groups to 70 % in low socioeconomic groups, particularly in rural areas (Bang Rani, 1989). We have observed an overall prevalence upto 33% of RTIs, including STIs, in women of reproductive age (Joshi JV et al 1991, 1993, 1994; Palayekar et al, 1996, 2004) depending on their age, contraceptive use, and behavioural risk. Our report was the first one to indicate a high prevalence of RTIs in women with Low Risk for STDs, ie from Family Welfare clinics and from antenatal clinics. Most of the earlier studies were from Gynecological Outpatient Departments or STD clinics. Other authors also subsequently reported significant proportion of women with STDs in community based studies or antenatal clinics ( Paul et al 1999; Kulkarni et al 2012; Hawkes & Santhya 2002; Rao S et al 1997; Jindal et al 2009; Ray et al 2008; Chugh & Gaiind 2012). Subsequent some studies in the last decade have shown lower rates (about 3 to 10%), particularly for Chlamydia and Trichomoniasis, probably because there was increased awareness of physicians as well as patients, and extensive use of syndromic approach to treatment (Joyee et al 2004; Rajapure et al 2013), particularly in semiurban and urban areas. There was higher prevalence in the urban slums and tribal areas (Rao VG et al, 2009). However even a 10 % prevalence rate indicates the

possibility of RTI in every 10th woman in the reproductive age and this demands special diagnostic and therapeutic measures.

**Diagnostic aspects:**

We have studied the value of different diagnostic tests in diagnosis of RTIs/ STDs , particularly the Papanicolaou smear. It was observed that the Pap smear is useful in the diagnosis of some 7 or 8 RTIs and this was first reported by us in the All India Conference in 1985 and later published as shown in the Table below (Joshi et al 1994; Mali et al 1986, 1987; Palayekar et al 1996). The Pap smear may not be very sensitive but the specificity is acceptable and since the Pap test is widely used in screening programmes for cervical cancer on a mass scale concurrent infections if detected in the smear are reported as per the International Bethesda Guidelines (Bethesda System, 2001). A suspected diagnosis of RTI by a cytologist should be adequately followed up by the clinician for further investigation and management including partner treatment when needed.

**Table:1. 2. Value of cytology in RTIs**

## a) Immunocytochemistry In cytologically positive cases

ICC test	% Positive
HSV (IF)	92
HPV (PAP)	72.6

## b) Wet vaginal smear/culture

Test	Sensitivity%	Specificity%	PPV%	NPV%
TV	100	89.6	66.7	100
Fungal	62.5	100	100	90
BV	85.7	54.5	54.5	85.7

## c) Pap smear vs Immunocytochemistry for Chlamydia trachomatis

Test	DFA	EIA	Pap Smear
No Positive	60	29	37
%	16.8	8.1	10

**Key:** ICC= Immunocytochemistry;PPV= positive predictive vaue; NPV= Negative predictive value; HSV= Herpes simplex virus; HPV=Human Papilloma Virus; IF= Immunofluorescence; PAP= Peroxidase Antiperoxidase; TV=Trichomonas vaginalis; Fungal=Fungal infection; BV= Bacterial vaginitis Actinomyces (DFA and culture), Chlamydia and Mobilucus (Oil immersion) were also studied (Adapted from Mali, BN 1986; Joshi JV,1991; Palayekar V,2000)

Needless to say this should be followed by counseling. This will go a long way in controlling the RTI/STD epidemic. The sensitivity and specificity of Pap smears for RTIs is fairly good in research studies as observed by us (Table 1.2). However in a mass screening program where the quality of the Pap smear collection and screening may be variable the reliability may be relatively less.

We (Palayekar, Joshi et al, 1994, 1996, 2000) compared 4 nonculture tests for the diagnosis of cervical infection with *Chlamydia trachomatis*. Endocervical smears stained with Geimsa stain, Papanicolaou stain were compared with local Antigen detecting ELISA (Enzyme Linked ImmunoSorbent Assay) with the Direct Antigen Detecting Immunofluorescent assay (DFA) in different groups of women (>1000 women). It was observed that whilst the DFA was most sensitive, Geimsa was least sensitive for the cervical sample and the Pap smear when collected by trained personnel was more sensitive than ELISA or Geimsa stain. It is important to maintain the quality of staining and screening in Pap smears (Joshi et al, 2001).

### **Therapeutic aspects:**

The World Health Organisation (WHO) , after several studies, surveys and meetings outlined the syndromic approach to the treatment of RTIs/STDs (1995, 1996, 1998) so that the meager resources are utilized for treatment rather than diagnosis without treatment. Many more cases receive the treatment with the syndromic approach. However it is not always effective. The Center for Disease Control (CDC), Atlanta also has issued guidelines for the treatment of STDs and RTIs (1996, 2006, 2010). Management involves treatment of both partners in case of STIs and includes the use of local or systemic antibiotics like azithromycin, doxycyclin, antiparasitics, or antifungals like nystatin or forcanazole.

We have been involved in the formation of National guidelines for the treatment of RTIs/ STIs at the Primary Health Care level in 2006 (NIRRH, 2006) and used the same guidelines for syndromic treatment of leucorrhoea alongwith partner treatment.

The allopathic treatment of leucorrhoea depends on the symptoms, signs and causative organisms. Resistance to antibiotics is well known phenomenon and may occur in 5 to 15 % of cases (Banntyne & Smith,1998; Sobel, 1999; Somani et al ,2000; Secor, 2012).

In view of the cost, side effects and possible drug resistance with antimicrobials, a scientific evaluation of Ayurvedic therapies assumes prime importance. Complementary medicine has been evaluated in other parts of the world also. Incompletely treated, chronic infections can lead to a number of complications in women, men, and in the newborn also if a woman happens to conceive with an infected cervico-vaginal tract (Paavonen et al, 1999; Ross & Holmes , 2011; Bang et al , 1989, Mutua, Cochrane Review 2012, Sutcliffe, 2012). Complications of RTIs/STIs are listed below in Table 1.3:

#### **Major consequences of incomplete treatment**

1. Drug resistance
2. Chronic PID
3. Life threatening conditions- ectopic gestation, septicaemia
4. Horizontal transmissions & vertical transmissions

Hence it is necessary to evaluate complementary therapies for the management of RTIs/STIs with objective criteria.

**Importance of Partner Treatment** : Male partners of women with STDs may have minimal or no symptoms. However until they are also simultaneously treated chances of reinfection remain constant. This can lead to chronic PID and other complications like intrauterine or neonatal infections if women become pregnant with an untreated genital infection. Hence partner treatment is always advised when essential and it has been our practice to treat the couple as a whole. We also advise abstinence (preferably), or the use of the mechanical barrier method like condom during the treatment period. CDC and other organizations have also accepted PT or prescription treatment of partners (to be given to the women) since sometimes the males are inaccessible and do not visit the clinic (CDC, 2010, Shiely 2010).

We have always insisted on partner treatment whether it is Ayurvedic therapy or Standard Allopathic treatment of leucorrhoea or other STI related symptom. If it is ayurvedic treatment for the woman then we insist on allopathic syndromic treatment for the male partner as there is no evidence based published data on Ayurvedic treatment for male RTIs/STIs in the males.

Major Consequences of Reproductive Tract Infections , particularly if untreated or incompletely treated are given in Table 1.3:

**Table 1.3 : Possible consequences of untreated RTIs/STIs in men, women & neonates**

Women	Men	Neonates/ Offspring
Pelvic pain	Urethral stricture	Low Birth Weight
Menorrhagia	Pelvic pain	Neonatal sepsis
Irregular menses	Urinary frequency/ burning	Long term developmental problems
Dysmenorrhoea	Arthritis/ skin lesions/ conjunctivitis	Jaundice
Dyspareunia	Infertility	Laryngeal papillomata
Leucorrhoea	Itching	
Itching	Anogenital Warts/ cancers	
Urinary frequency/ burning	Epididymitis	
Arthritis/ skin lesions/ conjunctivitis	Orchitis	
Fever / nausea /Vomiting		
Infertility		
Ectopic gestation		
Postpartum/ postabortal sepsis		
Cervical cancer/ Warts/ other genital cancers		
Abortion		
Premature delivery		

( Modified from WHO, Holmes, Hillier et al)

### 3. Leucorrhoea (*shwetapradara*)- Basic Ayurvedic background

Leucorrhoea literally means white discharge (*leucos* = white, *orrhoea*= discharge), usually implied from the vagina in females. In Sanskrit it is called as Shwetapradar (etymology : *Shweta*= white + *pradar*= vaginal discharge in women). The earliest Ayurvedic scripts ie the Atharvaveda or Brihat-trayi as they are known to be described by the ancient rishis , Charak, Sushrut and Vag-Bhatt , have not used the word *Shwetapradar* but have used the word *pradar* or *yonisrava* for vaginal discharge

and raktapradar or asrugdar for blood stained discharge or irregular bleeding. The meaning of the word Shwetapradar is “*shweta* = white and *pradar* = discharge.

श्वेतप्रदर

पाण्डुरे प्रदरे इति श्वेतप्रदरे । पाण्डुरे ऽ असृग्दरे पिबेत् ।

च. चि. ३०।११६। एवं अ. सं. उ. ३१। ७३

मिथ्याचारेण ताः स्त्रीणां प्रदुष्टेनार्तवेन च।

जायन्ते बीजदोषाच्च दैवाच्च शृणु ताः पृथक् ॥

च. चि. ३०/८

विंशतिर्व्यापदोयोनेर्जायन्ते दुष्टभोजनात् ।

विषमस्याङ्गशयनभृशामैथुनसेवनैः॥

दुष्टार्तवद्रव्यैर्बीज दोषेण दैवतः॥

अ.ह.उ. ३३/२७,२८

There are 4 main causes of women’s diseases which are classified into twenty types, *vitiated doshas* and *lakshanas* differ in these. The management is described in various ancient texts and more in details in the modern Ayurvedic text books for undergraduates and postgraduates (Charak, 1983; Sharangdhara, 1931; Kashyap, 2005; Premvati Tewari, 1990; Nirmala Joshi, 1999). A few students’ dissertations on leucorrhoea or cervical erosions are reviewed from libraries. However there is lack of objective records for case descriptions and investigations. Usually oral as well as local therapy has been used in combination and it is difficult to evaluate the need and efficacy of a single therapy, such as Panchvalkal, for the treatment of leucorrhoea.

योन्यामधावनात् कण्डू जाताः कुर्वन्ति जन्तवः।

स स्यादचरणा कण्डवा तयातिनरकाक्षिणी ॥

पवनो ऽ तिव्यवायेन शोफसुप्तिरुजः स्त्रियाः।

करोति कुपितो योनौ सा अतिचरणा मता ॥

च. चि. ३०/१८

उपप्लुता

वातरलेष्मामयव्याप्ता श्वेतपिच्छिलवाहिनी

उपप्लुता स्मृता योनिः॥ अ.सं.उ. ३८/४०

#### 4. Genesis of the study:

Dr Ranjan Bhatt had demonstrated in the laboratory that the Panchavalkal extracts have antibacterial activity against organisms commonly causing cervicovaginal infections (Bhatt RM, Patel Saraswati, 1991). The PVK combination with Shirisha instead of Pakar was effective in the laboratory cultures against fluconazole resistant candida also. Some Ayurvedic postgraduate students' theses have concluded that Panchavalkal kwath is effective in the healing of cervical erosions and leucorrhoea, however the results were evaluated subjectively and there was no published data on clinical activity in women with objective criteria for evaluation.

It is only when a treatment is given alone that the efficacy of the treatment can be properly evaluated hence we envisaged the first study in the series. Subsequently the scope was expanded.

The series of clinical and basic studies which were undertaken and which have been described in subsequent chapters is as follows:

- i) **Objective evaluation of vaginal douche with Panchavalkal kwath alone in leucorrhoea:** This involved the assessment of clinical activity and objective or semiquantitative assessment of leucorrhoea as well as objective documentation with cervical cytology ie Papanicolaou (Pap) smears, vaginal pH and colposcopy. Fresh kwath was prepared daily for women with specific inclusion and exclusion criteria. The modified Panchavalkal with substitution of Pakar with Shirisha was used based on Dr Ranjan Bhatt's laboratory findings showing antifungal activity of Shirisha (N=42).
- ii) **Comparison of PVK Kwath combined with routine systemic (oral Ayurvedic treatment (N=20) with a group of control cases treated with standard allopathic treatment (N=16) with similar selection criteria of inclusion/ exclusion and similar evaluation.**
- iii) **Assessment of Prakriti in women with leucorrhoea (N=75)**

- iv) **Development and Standardisation of a vaginal formulation (cream) from Panchavalkal for greater acceptance and ease of administration**
- v) **Preclinical studies for toxicity and dermal safety evaluation of PVK vaginal formulation (cream) in rats and rabbits**
- vi) **Clinical activity and safety evaluation of PVK vaginal formulation (cream) in women with leucorrhoea with specific inclusion/ exclusion criteria (N=30)**
- vii) **Comparison of clinical activity and safety evaluation of PVK vaginal cream (above group) with syndromic treatment of leucorrhoea in control group of standard care (N= 34).**

The first 3 studies (i, ii, iii) were part of the initial project in 1999 and have resulted in the following publications:

1. *Joshi JV, Bhat RM, Vaidya RA et al. Cervical cytology, vaginal pH and colposcopy as adjuncts to clinical evaluation of Panchavalkal, an Ayurvedic preparation, in leucorrhoea. J Cytology 2004; 21:33-38.*
2. *Nabar SS, Joshi JV, Rege VV, Bhatt RM, Nadkarni DS, Nagral KSD, Joshi BA, Vaidya RA, Vaidya AB. Prakritiparikshan and effects of modified Panchavalkal kwath (douche) in relation to Prakriti and Doshaprakopa in Shwetapradar (Leucorrhoea). J NIMA 2002; Part 1:Vol 9 & Part 11:Vol 10 (2002)*
3. *Joshi JV, Vaidya RA, Rege VV, Nabar NS, Lulla M, Sunder S. Treatment of Leucorrhoea with Traditional Ayurvedic or Allopathic Therapy. J NIMA 2005;47:9-12.*
4. *Joshi JV, Vaidya R, Shah R, Vaidya A. The Phytochemical & Pharmacological Basis of Ayurvedic Plants Used in Treatment of Leucorrhoea and Menorrhagia. J of Res & Ed in Indian Med 2008; 14:1-16.*
5. *Joshi JV, Vaidya RA, Affandi MZ. Cytology in the Diagnosis of Gardnerella Vaginalis infection. (Letter). J Cytology 2007; 23:214.*

6. Joshi JV, Vaidya RA, Rege VV, Joshi BA, Nabar NS, Pandey SN, Jagtap S, Nagral KI, Shah RH, Vaidya ADB. *Objective Evaluation of Ayurvedic Medicinal Plants in Treatment of Leucorrhoea. Proceedings of 2nd International Congress on Ayurveda, Pune, 2006 . pp 456-466.*

These are therefore included as commentaries and previously unpublished images have been used for logical descriptions.

The subsequent studies (iv, v,vi, vii) on vaginal formulation development, preclinical testing and comparison of PVK vaginal formulation with control group treated with standard allopathic antimicrobials have been part of the new CCRAS project and are being published for the first time in this monograph.

All these studies were initiated after permission from human and preclinical ethics committees by Kasturba Health Society's Medical Research Centre in collaboration with Viridis BioPharma Ltd, Ayurved Mahavidyalaya, Sion, and National Institute for Research in Reproductive Health, ICMR.

## Chapter 2. Literature review

*Joshi JV, Jagtap S, Amonkar AJ, Vaidya RA, Paradkar PH, Vaidya AB*

1. **Ayurvedic concepts -Leucorrhoea- shwetapradara- PVK dravyagunavidnyana**
2. **RTIs/ STDs - Therapies/ Lacunae in current therapy**
3. **Phytochemistry & Pharmacology of Barks - Panchavalkal (PVK) plants**

**1. Shwetapradar and the place of Panchavalkal in therapy-** As mentioned in **Chapter 1**, *Hetu-* pathogenesis- *samprapti-* *Shwetapradar* , or white discharge is described as *pradar* or *soma roga* in various texts. The shat-kriya-kaal for this symptom as such is not described but it is recognized that there are 20 types of women's diseases and that these arise from 4 basic causes. Defective behavior or lifestyle, affections of the artava (menses and discharges), genetic causes and unknown causes like fate, wrath of the Gods are the causes of genital diseases in women as per Charak Samhita. In Ashtang Hridaya later Vagbhatt specified additionally abnormal diet, having coitus in abnormal postures, excessive coitus (sexual promiscuity) and use of toxic substances etc. for sexual pleasure as causes of female genital disease (yoni vyapad or yoni-roga).

The word *shweta-pradara* has not appeared in great trios or Brihatrayi i.e. Charaka, Sushruta and Vagbhat samhitas. Comment (*teeka*) by Chakrapani and books like Sharangadhara samhita , Bhavaprakash and Yogratnakara have used the word *shweta-pradara* for white vaginal discharges.

The dominant vitiated dosha can lead to different symptoms associated with gynecological diseases.

## Vataja

## वातज

वातलाहाचेष्टया वातलायाः समीरणः।

विवृद्धो योनिमाश्रित्य योनेस्तोदं सवेदनम् ॥ ९॥

स्तम्भं पिपीलिका सृष्टिमिन कर्करातां तथा।

करोति सुप्तिमायासं वातजांरचापरान गदान् ॥ १०॥

सा स्यात् सशब्दरुक्फेनतनुरुक्षार्तवाऽनिलात् च.चि. ३०-९,१०

## Pittaja

## पित्तज

व्यापत्कट्वम्ललवणक्षाराद्यैःपित्तजा भवेत् ।

दाहपाकज्वरोष्णार्ता नीलपीतासितार्तवा ॥

भृशोष्णकुणपस्रावा योनिः स्यात्पित्तदूषिता । च.चि. ३०-११,१२

## Kaphaja

## कफज

कफो ऽ भिष्यन्दिभिर्वृद्धो योनिं चेद् दूषयेत् स्त्रियाः।

स कुर्यात् पिच्छिलां शीतां कण्डुग्रस्ता-ल्पवेदनाम्॥

पाण्डुवर्णा तथा पाण्डुपिच्छिलार्तववाहिनीम् । च.चि. ३०-१३,१४

**Symptoms (Lakshanas):** The primary symptom or Leucorrhoea or *shweta-pradara* is described as a vaginal discharge and hence the type of discharge can offer a clue to the Ayurvedic etiology: *Pittaja* (dominated by *pittadushti* when there is burning, yellowish discharge). *Kaphaja shwetavrava* is said to occur when there is bad smell, itching (*kandu*) and thick mucoid discharge. *Vataja shwetavrava* occurs when there is yellow mucopurulent or bubbly (*budbuda*) discharge and pain (*ruja*) . When vitiation of all 3 *doshas* occurs *Sannipatik shwetapradara* occurs. This may cause all symptoms like burning, itching, pain, swelling (*shopha, shotha*), and may indicate multiple causes. In severe cases it may be associated with fever (*jwara*), dyspareunia (*maithuna-asahanta*) etc corresponding clinically to Pelvic Inflammatory Disease (PID). Infections including worm infestations may be indicated by *jantu*.

## Sannipatik

सान्निपातिक्

समश्नन्त्या रसान्सर्वानदुषयित्वा त्रयो मलाः।

योनिगर्भाशयस्थाः स्वैर्योनिं युञ्जन्ति लक्षणैः।

सा भवेद्दाहरूलार्ता श्वेतपिच्छिलवाहिनी॥ च.चि. ३०-१४,१५

Sannipatik Yonivyapad can also indicate cancer or arbuda. In recent times it is known from the virological and bacteriological studies as well as long term follow-ups of HPV, HSV and othe STD infections that these infections are strongly associated in a causative manner with Pelvic Inflammatory Disease and also in cervical cancer.

योन्यामधावनात् कण्डूं जाताः कुर्वन्ति जन्तवः।

स स्यादचरणा कण्डवा तयातिनरकाक्षिणी ॥

पवनो ऽ तिव्यवायेन शोफसुप्तिरुजः स्त्रियाः।

करोति कुपितो योनौ सा अतिचरणा मता ॥

च.चि. ३०/१८

उपप्लुता

वातश्लेष्मामयव्याप्ता श्वेतपिच्छिलवाहिनी

उपप्लुता स्मृता योनिः॥ अ.सं.उ. ३८/४०

पाण्डुं सतोदं आस्रावं श्वेतं स्रवति वा कफम् ।

कफ वातायम व्याप्ता सा स्यात् योनिः उपप्लुता ।

The main vitiated dosha is kapha in majority of cases and on the basis of clinical features leucorrhoea appears to be disease of vitiation of kapha.

Kapha aggravated due to its own vitiating factor , influences or vitiates *rasadhatu* of reproductive system , which already influenced by excessive coitus, miscarriage, improper mode of life & dietetics during menstruation & *rutu kala* along with poor hygiene produces white and painless vaginal discharges.

However according to Charaka all gynecological diseases have vitiated Vata (Vata prakopa) as a causative factor:

न हि वातादृते योनिः नारीणां संप्रदुष्यति । च.चि. ३०-११५

*Shweta-pradara* or white discharge is a symptom of all gynecological disorders arising due to vitiation of *kapha* and *vata* – *kapha* (*atyandana, karnini, acharana, aticharana, sheshmala, upapluta, and prasarvini*). In all these diseases besides white discharge per vaginum, specific clinical features described under each disease as given below.

*Kapha* vitiated due to excessive use of *abhisyandi* substances (food or substances which block the *srotas* or passages) reaches reproductive system and causes unctuousness, coldness, itching, and dull pain in vagina. This may result from swelling and obstruction to the normal flow of blood and lymph. The woman looks anemic and discharges yellowish coloured unctuous menstrual blood. This is the opinion of Charaka. Chakrapani has equated this with *kaphaja asrugdara* on the basis that yellowish discharges per vaginum are present during intermenstrual period also.

What is important is to note that excessive sex (implicating multiple sexual partners) and *jantu* (implicating infections were recognized as a major cause of gynecological symptoms and disease.

Other causes of white or watery discharge per vagina include *somaroga* which also causes urinary symptoms.

योन्यामधावनात् कण्डू जाताः कुर्वन्ति जन्तवः।  
 स स्यादचरणा कण्डवा तयातिनरकाक्षिणी ॥  
 पवनो ऽ तिव्यवायेन शोफसुप्तिरुजः स्त्रियाः।  
 करोति कुपितो योनौ सा अतिचरणा मता ॥

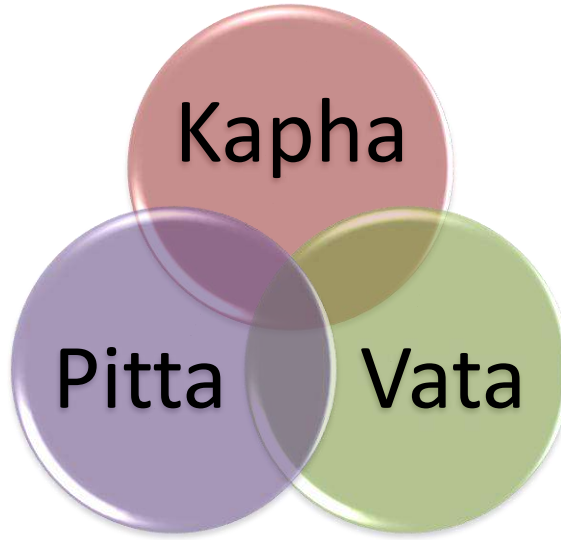
च.चि. ३०/१८

सोम रोग  
 स्त्रीणामतिप्रसंगेन शोकाच्चापि श्रमादपि।  
 अतिसारकयोगाद् वा गरयोगात्तथैव च॥  
 आपः सर्वशरीरस्थः क्षुभ्यान्ति प्रस्रवन्ति च।  
 तस्मास्ताः प्रच्युतः स्थानान्मूत्रमार्गं व्रजन्ति हि। यो.र. - ५६८

The treatment of different types of gynecological differs as per the dominant dosha in Ayurveda as is indicated below:

वाते तैलाम्लसंयुया : (बस्तयो हिताः)	च.चि. ३०-८५
पंचवल्कस्य पित्तार्ता (कल्कं धारयेत्)	
श्यामादीनां कफातुराः (कल्कं धारयेत्)	च.चि. ३०-६२

It is also to be remembered that the patient's *mool- prakruti* or *rogaprakruti* is not *ekadoshik* ie with single *dosha* and usually 2 or more doshas are involved. This may be represented as in the following diagram:

**Figure 2.1: Prakriti nidaan and doshaprakopa in disease as per Ayurveda**

Most diseases are with 1 or 2 prominent doshas and can be cured in this stage – saadhya. When all 3 doshas are affected as in Sannipatik the disease is very severe eg PID, Cancer and becomes incurable or cured with difficulty- asaadhya or kashtasaadhya.

In the present study only uncomplicated cases of leucorrhoea were included and Sannipatik stage was excluded.

Apart from these several other medicinal plants are also mentioned in Ayurveda. However since Panchavalkal is one of the commonest therapies for leucorrhoea and cervical erosions and is used extensively in current gynecological practice and also has been documented in recent times by researchers of biological sciences to have remarkable antibacterial and antifungal and anti-inflammatory activity we undertook a systematic and objective evaluation of Panchavalkal in Shwetapradara. Moreover the vranaropak or wound healing property is clearly mentioned in Ayurveda.

It is also to be noted that we wanted to confirm the clinical usefulness of PVK hence all cases were given uniform treatment irrespective of the prakriti or doshaprakopa. And the prakriti was analysed retrospectively in the different groups.

**Dravyagunavidnyana of Modified Panchavalkal with special reference to the use in leucorrhoea:**

PVK has been described in all classical as well as modern texts for treatment of women's diseases , apart from nonhealing ulcers et.

Combination of 2 or more medicinal plants or dravyas for the benefit of composite action of the dravyas for improving health or treatment of diseases is described under the section of Mishrakagana or Sankhyikiyoga in Ayurveda ( Deshpande, J, Ranade pg 227). The combination of barks of 5 trees is known as Panchavalkal.

पञ्चैते क्षीरिणो वृक्षास्तेषां त्वक् पंचवल्कलम् ॥ भा. नि.

Panchavalkal belongs to the group of Kheerivriksha ie the parts of the tree produce the milky (ksheer) fluid or latex. Because of this the plant extracts have astringent activity. The ayurvedic properties of PVK are described in terms of rasa, veerya, guna and vipaka. These are described in all text books of medicine and dravyagunavidnyana and are briefly given below.

Ayurveda classifies various medicinal products into different groups called as “varga” or “gana”. Panchavalkal is classified under “Ksheerivrikhsas” or latex producing large trees. They are also subclassified into families or “kula”.

Classification as per the most important tree is “Vatadi” group or gana. The following is a summary of the main Ayurvedic texts supported by Phytopharmacology texts such as VM Gogte's Dravyagunavidnyana. The information below is applicable mainly to the use of barks (valkal) and the uses and properties of other parts have not been included in this monograph.

All are latex producing trees and the barks have astringent properties which are essentially responsible for the therapeutic used described. The latex or milky white sticky semiliquid obtained from the barks or leave stems has been described as Ksheera or milk in Ayurveda , and the plants belonging to this category as ksheerivriksha.

The main Ayurvedic indications are for nonhealing ulcers, wound healing ( vranaropana) and women's diseases like white discharge (Shwetasrava or Shwetapradara) or irregular or excessive bleeding (Raktasrava). The mixture (Panchavalkal) can be administered orally in the form of a decoction (Kadha, kwatha or Kashaya) or locally in the form of a paste (kalka) or vaginal douche (Uttarbasti or yonidhawan). The Ayurvedic properties and indications of individual 5 plants are briefly described below.

**Vata (*Ficus bengalensis* Linn) :**

Vata is described as being cool, heavy, astringent and as reducing kapha and pitta.

वटः शीतागुरुर्ग्राही कफपित्तब्रणोपहः ।

वण्यो विसर्पदाहघ्नः कषायो योनीदोषहृत् । भा. प्र.

तथा तृष्णाच्छर्दि मूर्च्छा स्तूपित्तविनाशनः । ध. नि.

It is a large tree , commonly known as the banyan tree and spreads over a large area with its aerial roots. The roots are fibrous hence the branches reach to the ground to support the tree. It has religious and mythological importance . The trunk is greyish white. The leaves are thick, ovoid or elongated and large, about 10 – 15 cms long, and have 3 to 5 veins. Fruits are red, in pairs, and actually contain the floescence. It grows all over India and in tropical countries like Malaysia and Burma.

**Used parts:** The stem bark is mainly used for medicinal purpose. However the latex, leaves, fruit and tender leaves (vatankur) are also used.

**Rasa-** Kashaya; Vipaka- Katu, Veerya- Sheeta, Guna- Guru, and ruksha

**Pharmacological action:** It alleviates kapha and pitta ie It is Kaphaghna and Pittaghna

**Local uses:** It is used in wound healing and nonhealing ulcers. It is also used for joint swellings and arthritis. It is used for various indications including diseases of the eye and teeth. The decoction can be used for vaginal douches for vaginal infections and cervical erosions, and pelvic inflammation.

**Oral uses:** Same indications.

Since it is astringent and stops bleeding (Stambhaka) it is used in the treatment of dysentery, diarrhea, ulcerative colitis. Its anti-inflammatory (Shothahara) properties are used in uterine swelling, leucorrhoea, pelvic inflammation. Its antidiuretic property is used in the treatment of Diabetes (Meha). Recent studies have shown hypoglycemic effect and anticancer activity. It is also used in skin diseases and to improve complexion (Varnya).

Bhava – Prakasha describes its use in the treatment of Visarpa, probably synonymous to Herpetic eruptions, due to its ulcerhealing and its property of relieving the severe burning sensation (Pitta-hara).

### Udumbara (*Ficus racemosa* Linn)

उदुम्बरो जन्तुफलो यज्ञाडगो हेमदुग्धकः ।

उदुम्बरो हिमो रुक्षो गुरुः पित्तकफास्रजित् ।

मधुरस्तुवरो वण्यो ब्रणशोधनरोपणः ।

भा. प्र.

This a tall tree, about 10 to 14 meters high and has a reddish smoky bark. The leaves are about 7 to 10 cms and have 3 veins. The ripe fruits are red in colour. The fruits carries

many small worms and is really an inflorescence. It grows all over India and in some other tropical countries.

**Used parts:** Bark, fruit, latex, roots .

**Rasa** (bark) - Kashaya ; Vipaka- Katu, Veerya- Sheeta, Guna- Guru, and ruksha

Pharmacological action: It alleviates kapha and pitta ie It is Kaphaghna and Pittaghna. It has vishaghna properties in some poisonings.

**Uses:**

**Local-** It is anti-inflammatory, analgesic and wound healing hence used as a paste in nonhealing ulcers. It, particularly the latex, is also used in the treatment of local swellings due to lymph nodes (granthi), cancer (arbuda) , testicular inflammation or cancer. The decoction can be used for gargling in the oral ulcers.

Since it is astringent the oral decoction is also used in the treatment of dysentery, diarrhea, ulcerative colitis. Due to its Pittaghna quality it is specially used for burning and thirst. It is useful for the treatment of epistaxis and bleeding piles. The kwath is used in Prameha or Diabetes mellitus and as an antidiuretic.

**Orally and locally** (as local douches or uttarbasti) it is used in the treatment of uterine diseases, inflammation, cervical erosion and leucorrhoea and menorrhagia.

**Ashwattha or Peepal** (*Ficus religiosa Linn*)

अश्वत्थपिप्पलो दुर्जरः शीतः पित्तश्लेष्मत्रणास्रजित् ।  
गुरुः तुवरको रुक्षो वण्यो योनिविशोधनः । भा. प्र.

It is also a large tall tree , commonly known as the Peepal tree and has religious and mythological importance . The leaves are smooth, shiny, large, with 5 to 7 veins and

heart shaped, with long stalks. Fruits are red and contain fluorescence. It grows all over India and in tropical countries like Malaysia and Burma and Shri Lanka.

The stem bark is mainly used for medicinal purpose. However the latex, leaves, fruit are also used.

**Rasa-** Kashaya, Madhur; **Vipaka-** Katu, **Veerya-** Sheeta, **Guna-** Guru, and **ruksha**

Main Pharmacological action: It alleviates kapha and pitta ie It is Kaphaghna and Pittaghna

**Plaksha** (*Ficus infectora* or *Ficus lacor* **Buch-Ham**)

Large tree uncommon but found in most regions in India.

प्लक्षः कषायः शिशिरो व्रणयोनिगदापहः ।  
दाहपित्तकफास्रघ्नं शोथहाः रक्तपित्तहृत् । भा. प्र.

This is a huge tree with greenish grey bark. The leaves are large with 4 to 10 pairs of veins. The fruits are whitish, small and round. It grows all over India but is not found very commonly. Has to be cultivated.

Rasa: Kashaya, Veerya : Sheeta, Vipaka: Katu, Kaphapittaghna

**Shirisha** (*Albezzia lebbeck* **Benth**)

This is a large tree grown all over India and with bipinnate leaves.

क्वचित् कपितनस्थाने शिरीषो वेतसः क्वचित् ।  
कैयदेवनिघण्टुः ओषधिवर्गः ॥४३८॥  
केचित्तु पारिषस्थाने शिरीषं वेतसं परे वा वदन्तीति विशेषः ।  
भा. नि. वटादिवर्गः ॥ १५ ॥

It is written in Kaiyadeo Nighantu and Bhava-Prakash that Shirish or Vetas can be used as an alternative plant in Panchavalkal (PVK). Dr Ranjan Bhatt's microbiology

experiments showed that combination with shirish gave superior antimicrobial activity, particularly against the Candida species, which commonly infect the female lower genital tract. Hence we used Shirisha instead of Pakar. The Ayurvedic properties describe the plant as sweet, light, and cooling and alleviating asthma, ulcers and also acting against poisons. Useful in allergic skin diseases.

शिरिषो मधुरोऽनुष्णस्तिक्तश्च तुवरो लघुः ।

दोष शोष विसर्गघ्नः कासत्रण विषापहः। भा.प्र.

**Rasa-** Kashaya / Madhura, Tikta; Veerya- Slightly ushna, Vipaka- katu, Guna- Laghu, Tridosahara

Part used: Stem bark mainly, leaves

Dr Ranjan Bhatt clearly showed that the combined bark extracts of 5 plants were superior to individual bark extracts, and that modified Panchavalkal with substitution with Shirisha was superior to Classical Panchavalkal.

Subsequent to Dr Ranjan Bhatt's work (Saraswati Patel 1991), others have also shown clinical use of PVK in prevention of gynecological sepsis and in cervical erosions and it is suggested that it may be useful even in PID (Palep et al 2003, 2004, Girja, 2006).

However none have used objective criteria for evaluation like Pap smears and colposcopy, Vaginal pH and compared it with allopathic treatment using same criteria for subject inclusion and exclusion in the clinical studies as we have reported in Chapter 3 and Chapter 6.

**Table 2.1. Summary of Ayurvedic properties of Modified Panchavalkal\***

Name of plant	Properties			
	Rasa	Veerya	Vipaka	
<i>Vata</i>	Kashaya	Sheeta	Katu	Dosha – Kaphaghna, Pittaghna Dhatu – Rakta (Menorrhagia, complexion enhancer, bleeding disorders)
<i>Ashvattha</i>	Kashaya	Sheeta	Katu	Dosha – Kphapittashamak Dhatu – Rakta chaemostatic, complexion enhancer (Astringent)
<i>Udumbar</i>	Kashaya Madhur	Sheeta	Katu	Dosha – Alleviates pitta and kapha Dhatu Acts on raktadhatu, enhances complexion, helps in wound healing, cures haemorrhagic disorders, varnya, vranaropan, rakta pittaghna
<i>Plaksha</i>	Kashaya	Sheeta	Katu	Dosha – Kaphaghna, pittaghna Dhatu – Rakta
<i>Shirish</i>	Kashaya Tikta	Mild Ushna	Katu	Dosha – Tridosahara (Shaman) Dhatu – Rasa (Skin) rakta (herpes) Vishaghna

\*Adapted from Joshi JV et al , JREIM, 2008

## 2. RTIs/ STDs – Therapies / Allopathic options & lacunae

Leucorrhoea as a symptom has been described in Chapter 1.2 and the prevalence and problem of resistance has been highlighted. The list of organisms which can cause RTIs/ STDs is given below ( <http://www.popcouncil.org/pdfs/RTIFacsheetsRev.pdf>).

“RTIs are widespread. The World Health Organization estimates that each year, there are over 333 million new cases of curable STIs. In addition, UNAIDS calculates that in 2000 alone, 5.3 million people became infected with HIV. RTIs that are not sexually transmitted are even more common” (WHO, 1994,1998, 2009; Hawkes 2002; Paul et al 1999, Patel V et al 2006).

From etiologic point of view RTIs can be 1) Endogenous 2) Iatrogenic 3) Sexually Transmitted- the last being most common. Common organisms causing RTI/STIs are listed below:

### Sexually Transmitted Infections (STIs):

#### Common bacterial infections

*Neisseria gonorrhoeae* (causes gonorrhoea or gonococcal infection)

*Chlamydia trachomatis* (causes chlamydial infections)

*Treponema pallidum* (causes syphilis)

*Haemophilus ducreyi* (causes chancroid)

*Klebsiella granulomatis* (previously known as *Calymmatobacterium granulomatis* causes granuloma inguinale or donovanosis).

*Mycoplasma hominis*

*Ureaplasma urealyticum*

*Actinomyces spp*

#### Common viral infections

*Human Immunodeficiency Virus* or HIV (causes AIDS)

*Herpes simplex Virus* type 2 or HSV 2 (causes genital herpes)

*Herpes simplex Virus* type 1 or HSV 1 (Less common, causes orogenital syndrome)

*Human Papilloma Virus* or HPV (causes genital warts and certain subtypes lead to cervical cancer in women)

*Hepatitis B Virus* (causes hepatitis, may lead to cirrhosis or cancer of the liver)

*Hepatitis C Virus* (causes hepatitis, may lead to cirrhosis or cancer of the liver)

**Parasites**

*Trichomonas vaginalis* (causes vaginal and cervical trichomoniasis)

**Fungi**

*Candida albicans* (causes vulvovaginitis in women; inflammation of the glans penis and foreskin [balano-posthitis] in men).

*Candida frusei*

**Asymptomatic STIs can be sexually transmitted.** Many STIs occur without symptoms, in the incubation period or if incompletely treated. For example, up to 70% of women and a significant proportion of men with gonococcal and/or chlamydial infections experience no symptoms at all. Both symptomatic and asymptomatic infections can later lead to the development of serious complications, discussed below. STIs adversely affect the health of women

Untreated STIs can have critical implications for reproductive, maternal and newborn health. STIs are the main preventable cause of infertility, particularly in women.

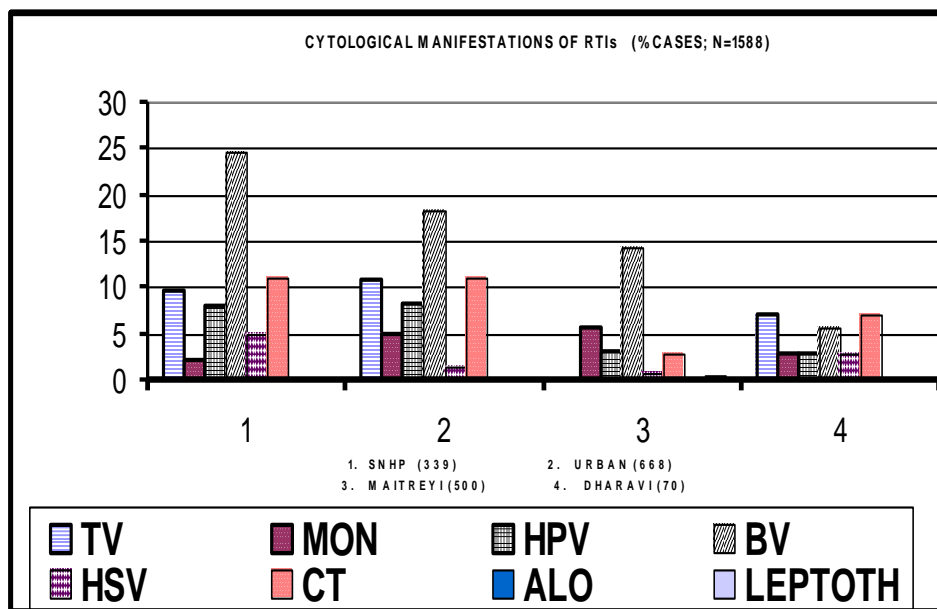
For example, 10 - 40% of women with untreated chlamydial infection develop symptomatic pelvic inflammatory disease. Post-infection tubal damage is responsible for 30 - 40% of cases of female infertility. Furthermore, women who have had pelvic inflammatory disease are 6 - 10 times more likely to develop an ectopic (tubal) pregnancy than those who have not, and 40 - 50% of ectopic pregnancies can be attributed to previous pelvic inflammatory disease.

**Diagnosis of RTIs/ STIs:** The various methods used for diagnosis are listed below:

1. Clinical
2. Vaginal pH
3. Colposcopy
4. Cytology- Pap smears, Wet vaginal smear, Gram smear, Geimsa St smear
5. FITC
6. ELISA
7. PCR
8. Culture
9. Serology

Out of these Pap smear is the only method which can give a clue to as many as 7 RTIs (*Trichomoniasis, Chlamydiasis, Bacterial vaginitis, Leptothrix, Actinomyces, HPV infection, HSV infection, Fungal hyphae or spores*) with a single test simultaneously with the screening for cervical cancer and precancer (Bethesda system, Joshi JV et al, Mali BN et al, Kiviat et al). It also indicates the severity of infection because of the changes in cell morphology and leucocytic infiltration and distribution. Hence it was used in the present clinical studies one of the measures of response to treatment. As already indicated in Chapter 1 the sensitivity may be low but the specificity is high. The graph below gives the prevalence of Cytological manifestations of RTIs in Pap smears from different groups of women evaluated by us. These were presented at the cytology conference (Unpublished data, 2001). Several other reports have been published as given earlier.

**Figure 2.2. Cytological manifestations of RTIs in different groups of women as shown in Pap smears and Wet smears**



Key : SNHP- SPARC NANDIGRAM project in semitribal area, Urban Clinics of Bhanvan's SPARC, Maitreyi Women's health checkup (Forty +), Dharavi slum women (unpublished data)  
 TV= Trichomonas vaginalis, Mon=Monilia, HPV= Human Papilloma Virus Infection, BV= Bacterial vaginitis, HSV= Herpes Simplex Virus infection, CT=Chlamydia trachomatis infection, ALO=Actinomyces Like Organisms, Lepto=Leptothrix

**For all other organisms eg viruses, individual different tests can be used but these are very expensive, or not available (eg Virus culture, Chlamydia culture, IgM etc) and this was beyond the purview of the projects.**

**Management: The general management is indicated in Chapter 1 with a focus on partner therapy.**

The following drugs are commonly used for multiple infections:

Metronidazole, Secnidazole, Satronidazole, Azithromycin, Doxycycline, Cephalosporins, Penicillin, Myconazole, Fluconazole, Clindamycin

### **Advantages and Disadvantages of antimicrobials:**

If taken appropriately and in adequate doses most individuals are cured of the RTI/ STI. However with changing lifestyles the RTI/STIs have assumed epidemic proportions. Hence mass use as well as inadequate dosing due to side effects or due to poor compliance has led to drug resistance in large proportions of populations. Drug resistance is a major problem in the management of RTIs. In spite of the possible serious complications listed in Chapter 1 many persons, particularly the youth indulge in high risk behavior along with poor compliance. Thus there is scope for novel therapies with less side effects and perhaps less drug resistance. (White TC, 1998; Sobel, 1999; Somani, 2000; Ferris et al, 2004; Bradshaw 2006, Cannon RD, 2007, Secor, 2012). Intolerance to allopathic drugs is another indication for use of alternative therapies like Ayurved (Zargooshi, 2012).

The demonstration of PVK extract efficacy in fluconazole resistant *Candida* species by Bhatt RM et al is indeed one of the reasons why Ayurvedic therapies can be explored for treatment of leucorrhoea.

### **3. Phytochemistry and pharmacology of Panchavalkal:**

The classical Panchavalkal plants as well as the 3 alternative or substitute plants have several medicinal properties and are used for diverse medical indications. . The stem bark

is the most effective and biologically active, however the fruits, leaves and flowers are also used in some medical conditions. In this monograph we have restricted review to the phytochemistry and pharmacology of the barks alone as these are used in “Panchavalkal kwath” and for the indication of uncomplicated leucorrhoea or cervical erosion only.

### Phytochemistry:

The Phytochemistry of the barks of the Panchavalkal plants is summarized below in Table 4.1. Part of this was reviewed by us earlier (Joshi et al , 2008) however it has been researched and updated as more phytochemistry studies of the bark or whole plant have been recently carried out by various scientists (CCRAS 1990; CCRAS 1994; CCRAS 2001; Rastogi 1989; Rastogi 1994; Jain 1994; Swami, 1996; Khan & Javed, 1998; Gogte VM , 2000; Gupta AK,2004; Kumar, 2007;Joshi U, 2008, Patil 2010). A summary of these is given in Table 4.3.

**Table 2.2. The phytochemicals in barks of classical and modified anchavalkal**

Sanskrit name	Phytoconstituents
<i>Vata</i> <i>Ficus bengalensis</i> Linn	Glycosides, Tannins, Triterpenes, $\beta$ -sitosterol, Mesoinositol, other sterols, Quercetin-3-galactoside, Rutin, Friedelin, Leucoanthocyanin, rhamnosides, begolenoside, leucocyanidin
<i>Ashwattha</i> <i>Ficus religiosa</i> Linn	Sterols, $\beta$ -sitosterols. Coumarins, Tannins, Aminoacds, Vit K1, Aliphatic alcohols
<i>Udumbar</i> <i>Ficus racemosa</i> Linn <i>Ficus glomerata</i>	$\beta$ -sitosterol, Stigmasterol, $\alpha$ -amyrin acetate, Lupeol, Lupeol acetate, Taraxasterol ester, Glucose, Friedelin, Tannins, Tetracyclic triterpenes, glauanol, Hentriacontane, Leucoanthocyanin, Leucoanthocyanidin, , Psoralens,
<i>Plaksha</i> <i>Ficus infectora</i> <i>Ficus lacor</i>	$\alpha$ -amyrin, $\beta$ -amyrin, lupeol, stigmasterol, and compesterol, infectorin, scutellarein, scutellarein glucoside, sorbifolin, and bergapten, bergaptol
<i>Shirisha</i> <i>Albizzia lebbeck</i> Benth	D- catechin, melacacidin, leucoanthracyanidin, lebbecacidin, H-sitosterol, betulinic acid, caffeic acid, kaempferol, quercetin, lupeol and Albizziahexoside A

**Phytopharmacology:**

The aqueous and alcoholic extracts contain different compounds and all these compounds have been shown to have antiinflammatory, antibacterial, antiparasitic, antifungal or woundhealing properties. Hence in the kwath the hydrophilic compounds are concentrated whereas the hydroalcoholic extracts hydrophobic compounds are also concentrated hence whilst we used the kwath in the first study for formulation development we were able to incorporate hydroalcoholic extracts and could expect more activity.

Antibacterial properties of Panchavalkal plants have been shown by several researchers. Dr RM Bhatt was one of the first to demonstrate the antibacterial and antifungal activity of hydroalcoholic extracts in in vitro studies against organisms commonly infecting the female genital tract (Bhatt et al, 1984; 1985, 1995, Patel S, 1991). Organisms studied included *Pseudomonas* spp and *Klebsiella* spp. She also showed that the combination had superior activity as compared with individual plant extracts and that the combination with Shirish as an alternative to Pakar was superior and could act against Fluconazole resistant *Candida albicans*. Others who have confirmed antibacterial or antifungal activity are Swami & Bisht, 1996; Bindu, 1997; Mandal, 1997; Srinivasan et al, 2001; Mahato et al, 2005; Kumar et al 2007; Nair & Chanda, 2007; Faysal, 2008, Aswar et al, 2008; Hemaiswarya 2009, Vyas et al, 2011; Palep et al, Ashar et al 2012; Khan MS et al, 1998, 2012;

The wound healing activity and antimicrobial properties have been reported by Bhatt et al and Mehta D et al (Unpublished data). Antioxidant activity has been evaluated by others (Anandjiwala, 2008;) and is likely to be useful for antiinflammatory action and healing of wounds. Astringent activity which leads to reduced vaginal discharge and reduced wetness is also reported.

In conclusion the plants used in modified panchavalkal have phytoconstituents which have antimicrobial, antifungal and anti inflammatory activities justifying the biological plausibility of the therapeutic use of Panchavalkal in leucorrhoea.

**Chapter 3.****Objective clinical evaluation of Use of PVK kwath in Leucorrhoea**

*Joshi JV, Rege VS, Nabar NS, Nadkarni DS, Affandi MZ, Vaidya RA*

- 1. Clinical study on Traditional Kwath**
- 2. Pilot study on comparison of Ayurvedic PVK kwath with Allopathic therapy**
- 3. Evaluation of Prakriti in leucorrhoea**

**1. Clinical study on Traditional Kwath**

Leucorrhoea is the commonest symptom in gynecology and more than 75% of women suffer from it during their lifetime. The text books and dissertations from Ayurvedic medical colleges are replete with statements on use of Panchavalkal in leucorrhoea or cervical erosions. Dr Ranjan Bhatt's laboratory work *in vitro* supported the Ayurvedic concept however she demonstrated that the use of Shirisha instead of Parisha gave better antifungal (anticandida) activity. It is well known that *in vitro* or *in vivo* and *in human* results may sometimes be widely disparate. There were also some postgraduate theses on PVK but these were unpublished and used only subjective criteria for evaluation. All these cannot be considered as evidence for efficacy as there is no published data in peer reviewed medical journals on the clinical efficacy. The following literature, laboratory or clinical based studies were reviewed:

- i) Ayurvedic texts as described in Chapter 2.1 (Brihatttrayi, Laghutrayi, Other texts)
- ii) Phytochemistry and phytopharmacology of extracts and components of Classical and modified PVK as in 2.3.

iii) Studies carried out by Dr RM Bhatt and colleagues and students (Bhatt et al 1985, 1986, 1999; Patel, 1991) and by Dr HS Palep and colleagues (Mehta & Palep, 1994; Bindu et al, 1997)

iv) Postgraduate students' dissertations in Ayurvedic medical colleges, Ayurvedic journals

It was observed that no objective criteria were used in the clinical studies on leucorrhoea and documentation was subjective.

Hence this clinical study was planned to document the effects of use of Panchavalkal kwath alone in women with leucorrhoea. The study was supported by CCRAS.

The aim of this clinical study was to determine whether there was any objectively demonstrable effect of use of the traditional kwath alone in cases of leucorrhoea or cervical erosions. Hence it was decided to prepare the PVK kwath in the traditional way as described in the Ayurvedic text and use it in women with chronic leucorrhoea. The modified PVK was used because in laboratory studies the combination with substituted Shirisha showed better antifungal activity than the classical PVK (Chapter 2).

This study was supported by the CCRAS research grant and carried out in collaboration with Late Dr Vanita Rege, Head of the Department of Obstetrics & Gynecology of the Ayurvedya Prasarak Mandal's Ayurved Mahavidyalaya, Sion. A weekly joint leucorrhoea clinic has been set up to investigate and follow up women with leucorrhoea and for screening for cervical precancer and cancer. A binocular colposcope was installed in this clinic and patients were referred to the pathology laboratory of the Sion Ayurved hospital for blood collection. The sera were separated and transferred in cold thermos to the Bhavan's SPARC laboratory for initial routine clinical biochemistry and organ function tests to exclude any women with abnormal biochemistry including HIV and VDRL tests. All investigations including Pap smears were carried out at the Bhavan's SPARC which

had a laboratory Quality control program in place under the guidance of Dr SJ Talwalkar. All Pap smears were evaluated using the Bethesda System and the criteria as reported by Joshi et al in previous publications ( Bethesda System,1993; Joshi et al 1991, 1993, 1994, Mali & Joshi 1986, 1993 ). Colposcopy was used as per the International criteria for classification as negative, inflammation, Acetowhite areas, CIN 1,2,3 or Cancer and Unsatisfactory.

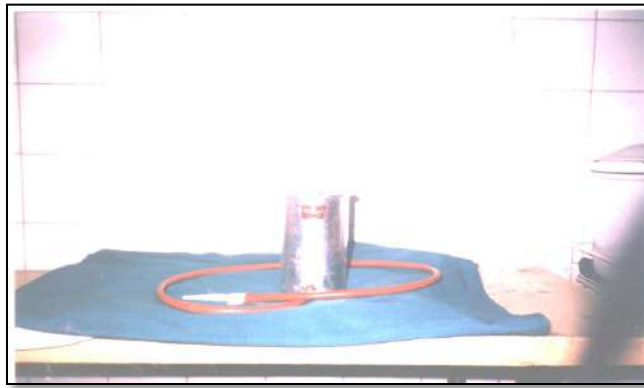
The criteria for patient inclusion and exclusion were defined so as to exclude the bias and to exclude cases of PID as these cases require urgent multipronged therapy and the effect of PVK kwath alone cannot be assessed. The study was undertaken after permission of the “Institutional Ethics Committee”, an intersystem Ethics committee with experts from Allopathy, Ayurved and Basic Scientists. The results of the study have been presented in various conferences including Indian Association of Cytology, at Nagpur, and published in Indian Journal of Cytology (Joshi et al, 2004) with data analysis in 42 cases who underwent treatment with kwath alone for first 2 weeks and were assessed for clinical symptoms, signs, as well as objective evaluation by symptom scoring, colposcopy, vaginal pH, and Papanicolaou smears initially and at 2 weeks. The post therapy effect was observed by repeating the investigations again at 1 month ie 2 weeks after discontinuation of the kwath. Daily douches under sterile conditions were given in the gynecological department to ambulatory cases of leucorrhoea with definite criteria of inclusion and exclusion. Women underwent examinations pretherapy, at 2 weeks and at 1 month. A specially developed Case Record Form was used.

The salient features of the study and previously unpublished figures, graphs and microphotographs are given below.

The kwath was prepared from a mixture of the barks of 5 PVK plants, which was purchased from a standard Ayurvedic firm. The preparation of the kwath has been described in details earlier (Joshi et al 2004) as per Bharat Bhashaya Ratnakar (1938).

A measured 25 gms of standard powder (5 gms of each PVK plant) from a registered Ayurvedic Pharmacy was used for preparation of fresh kwath everyday for the woman. The daily douches were given in the gynecological ward of the hospital using the douche can and a disposable plastic nozzle for each day for the women so that cross infections were prevented.

**Figure 3.1. Douche can and plastic disposable nozzle used for Daily douching with PVK kwath**



25 gms of PVK bark powder  
used for kwath preparation



The vaginal pH was recorded using the Qualigen pH paper and testing the pH from the anterior vaginal wall.

The pH of the kwath was 5.5. The anterior vaginal pH in women with leucorrhoea varied from 3.5 to >7 and the effect of PVK kwath has been described in details earlier.

Pap smears were carried out using a disposable spatula and endocervical brush. The prelabelled slides were kept ready in the trolley as shown in the Fig. 3.1.2.

**Fig. 3.2. Trolley for Pap smears and colposcope at the collaborative leucorrhoea clinic at Sion Ayurved Hospital**

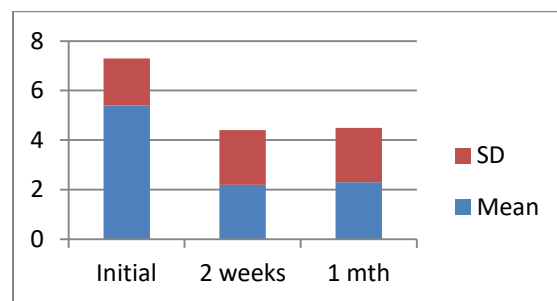


*Legend: Figure shows the i) gynec table, ii) colposcope,iii) sterile trays with autoclaved specula, iv) disposable spatula and brush v) spray fixative vi) sterilium vii) sterile gloves,viii) slide box, ix) apron*

It was observed that 85.7% of women had clinical relief or cure from the use of PVK kwath alone ( $p < 0.001$ ; Chi square test). The symptom score was reduced as shown graphically below.

**Figure 3.3. Mean and SD semiquantitative symptom**

Score before treatment, at 2 week and at 1 month



Initial vs 2 weeks and 1 month-  $p < 0.05$  ; paired t test.

**Figure 3.4. Colposcopy as a method of assessment: Cases excluded from study**

a) Early cancer of the cervix



b) Endocervical polyp



**Figure 3.5. Colposcopic picture before and after PVK kwath treatment**

Before treatment

Large erosion with oedematous cervix and vagina

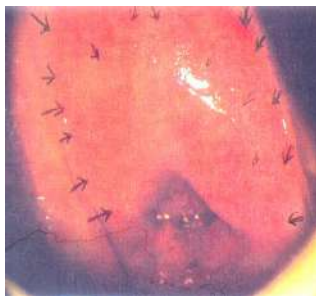


After treatment- Reduced edema, healing clean erosion

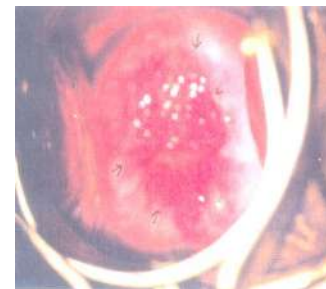


**Figure 1.6. Severe edema and vascularity and size of erosion reduced after PVK douche treatment**

Very severe edema- erosion covering almost the whole of cervix



Post -therapy- clean healing smaller erosion



The changes in Papanicolaou smears also demonstrated a reversal to normal pattern with reduction in inflammatory exudate as seen in Figures 3.7 and 3.8.

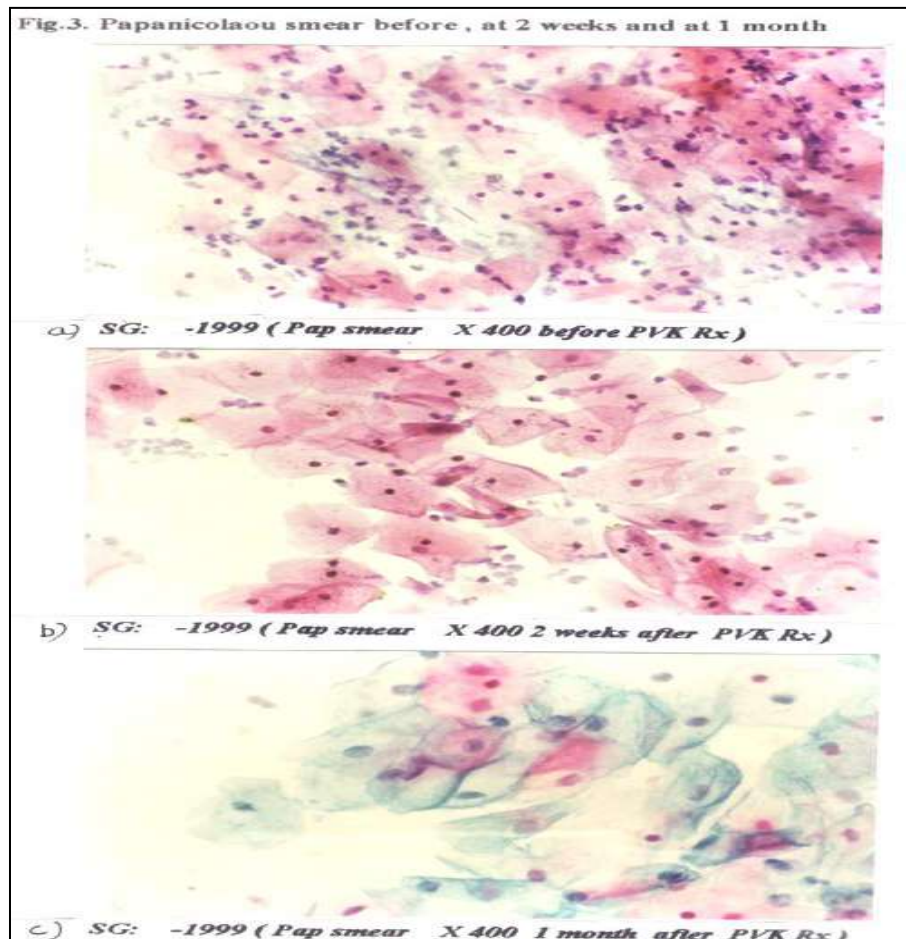
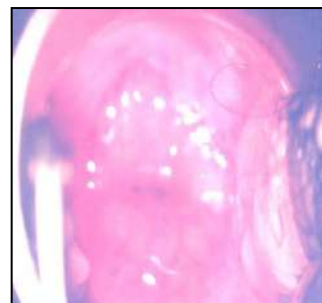


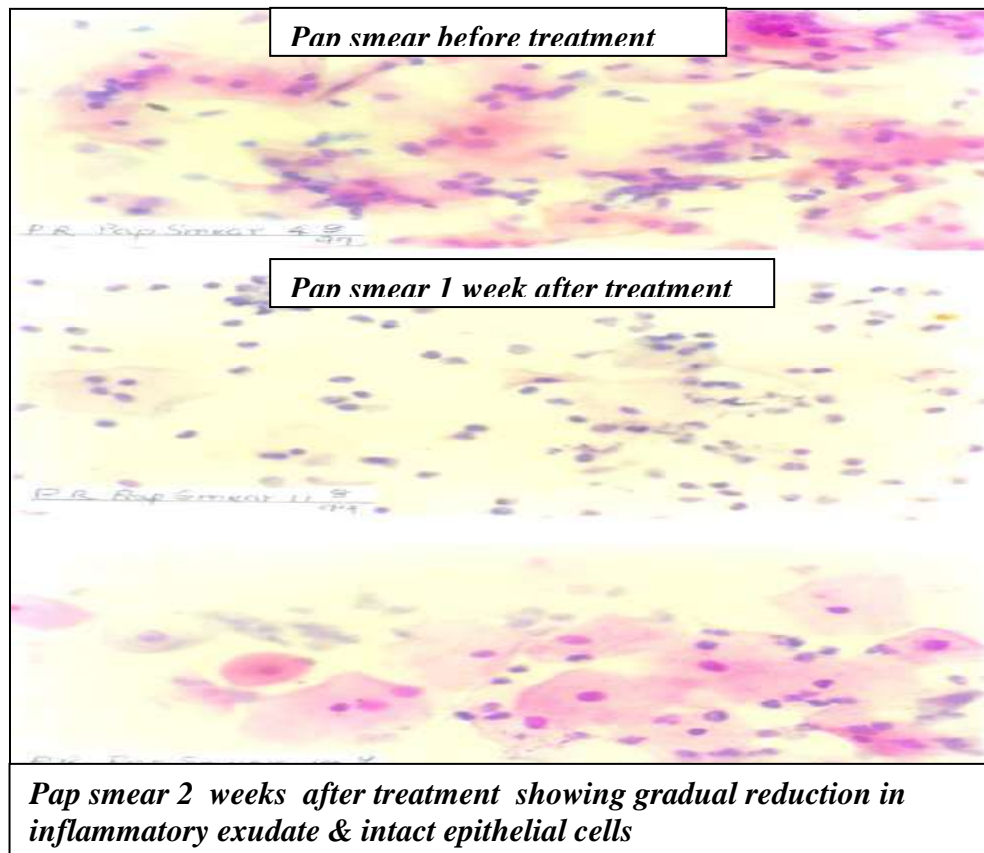
Figure 3.8. Severe edema and vascularity reduced after PVK douche treatment in

Before PVK treatment

After PVK treatment



**Figure 3.9. Cytology as a method of assessment in the same case.**



Thus a marked improvement in the leucorrhoea score was seen. This was corroborated by the colposcopic demonstration of improvement in the healing of cervicitis and cervical erosions as shown in the figure below. Colposcopy demonstrated reduced congestion, inflammation, discharge and showed epithelialisation of eroded areas in 19/ 21 cases.

**Microbiology:** Culture was carried out in 28 cases before treatment and aerobic and anaerobic bacteria and fungal infections were identified as shown below:



#### **Summary & Discussion:**

Panchavalkal is recommended for gynecological diseases in Ayurveda. However no published data is available except for students dissertations or few reports with subjective analysis (TAKalkar, 1994; Khataavkar, 1998; Jain, 1999). The present study was based on Reverse Pharmacology principle of Ayurvedic literature review, current practices and experimental evidence in microbiology showing superior action of PVK combination with Shirisha over classical Panchavalkal. There was objective evaluation using additional investigations like cytology, wet smear, colposcopy and vaginal pH for demonstration of activity of the daily kwath, comparable to allopathic treatment. The study also documented some failures and hence a follow up speculum examination rather than subjective symptom relief should be taken as the end point in such clinical studies.

Treatment of 42 cases of leucorrhoea with modified freshly prepared PVK kwath alone given on an outpatient basis for 2 weeks resulted in significant subjective improvement in tandem with objective markers like colposcopy and Pap smears as well as vaginal pH in 85% of cases.

## 2. Pilot study on comparison of Ayurvedic PVK kwath with Allopathic therapy

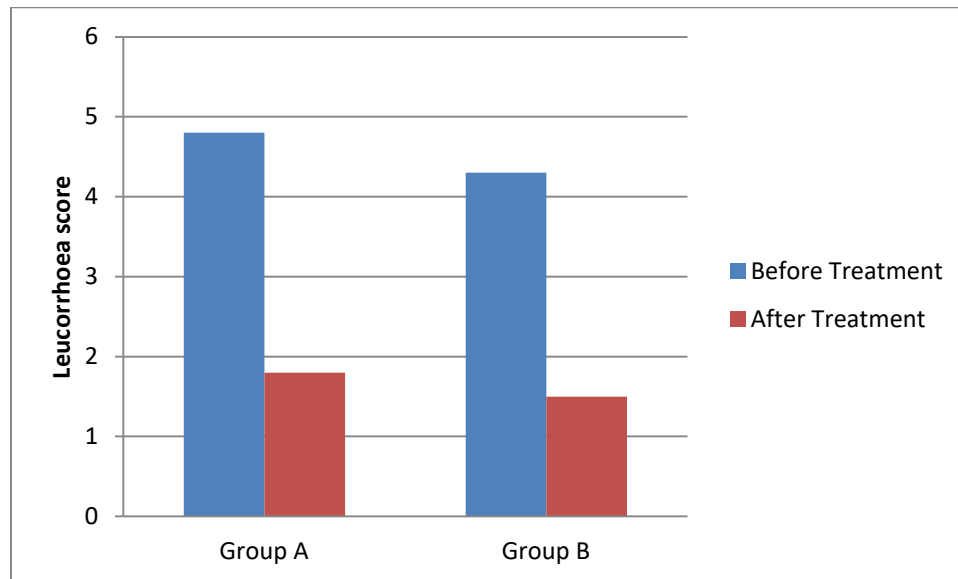
### Comparative Allopathic

**Rationale:** Having seen that the PVK kwath alone is effective in giving subjective relief in the primary symptom of leucorrhoea it was decided to undertake a pilot study for comparison of this treatment with standard allopathic therapy. Parallel case enrollment was carried out with identical inclusion and exclusion criteria in both groups as given earlier. The allopathic treatment was based on national and international CDC based guidelines. Twenty cases were enrolled in the kwath group and 16 were enrolled in the allopathy group. However in this study systemic or oral ayurvedic therapy as being practiced by the gynecologists at the Ayurved Mahavidyalaya was started from Day1 and continued till 1 month. The results of this pilot study also have been published (Joshi et al, 2005) and the summary as well as the previously unpublished figures are given below.

Subject selection criteria for inclusion and exclusion were similar to the previous study. A total of 200 women were screened to enroll 36 women with specific inclusion/exclusion criteria as in 3.1.

Women in Group A, Ayurvedic treatment Group A (N=20) received the oral systemic therapy as per the standard of care in Ayurvedic teaching hospital for 4 weeks and local kwath douche for 10 days as described earlier. Pap smears and colposcopy and Vaginal pH were carried out. Ayurvedic medicines with multiple ingredients which varied according to the patient's constitution, history and ayurvedic pathogenesis were given orally two or three times a day for upto 4 weeks. Allopathic group B received standard of care treatment with FAS-3 kits for husband and wife. Women in both groups responded similarly. In both groups the scores were similarly and significantly reduced ( $p < 0.01$ ), 90% in Group A and 88% in Group B with partial or complete relief of symptoms.

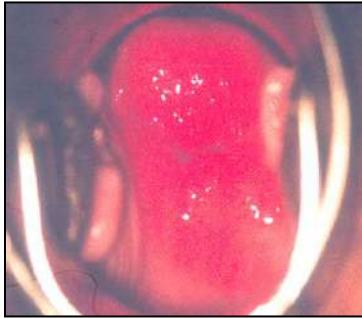
**Figure 3.10. The score for specific symptom of amount of leucorrhoea before and after treatment**



The score for specific symptom of amount of leucorrhoea as assessed by visual grading at each visit was reduced significantly from  $4.8 \pm 0.5$  (SEM) to  $1.8 \pm 0.4$  (SEM) in group A (  $p < 0.01$ ) and in group B from  $4.3 \pm 0.54$  (SEM) to  $1.5 \pm 0.6$  (SEM) ( $P < 0.01$ ). Overall 18/20 (90%) in group A and 13/16 (88%) in group B reported partial or complete relief from leucorrhoea.

**Figure 3.11. Colposcopy after treatment with allopathic treatment shows a large persistent erosion**

Before allopathic treatment



After allopathic treatment

persistent large erosion



**Figure 3.12. Colposcopy after treatment with modified PVK douche treatment and systemic oral Ayurvedic therapy shows healing in a large erosion & cervicitis**

PVKD +S

Before treatment



2 weeks after treatment



Thus it was concluded that Modified PVK vaginal douche with systemic oral Ayurvedic therapy has similar efficacy to allopathic treatment in uncomplicated leucorrhoea (Joshi et al, 2005).

In this pilot study however the systemic therapy was not standardized nor uniform but as per the outpatient practice in the Ayurvedic college. Usually Pushyanug churna, Lodhra churna, Nagkesar churna, Krumikuthar tablets, Triphala guggulu tablets, chandraprabhavati tablets, and sometimes sariva, trivanga and kamadudha rasa were also given depending on the patient's prakriti, doshaprakopa (symptoms and signs) and duration of disease. In view of the smaller number that could be studied in this project it was decided to assess a larger group for such a comparative study in future. This was possible in the second CCRAS study when the usefulness of PVK vaginal formulation cream along with standardized uniform Ayurvedic systemic oral tridoshahara formulation therapy was compared with standard of care allopathic therapy as described in Chapter 6.

### **3. Response to treatment in relation to prakriti and doshaprakopa**

We studied Prakriti in 75 cases of leucorrhoea and the response to PVK douche was evaluated in 42 cases which were selected for the CCRAS study. This has been reported earlier (Nabar et al, 2003). A specially designed case record form for leucorrhoea and prakritinidan was used.

The distribution in leucorrhoea cases was vata pitta (30%), pitta kapha (29%), kaphapitta (19%), pittavata (16%), vata kapha (3%), and kaphavata (3%). Pitta as a component was observed in 94% of cases. Kapha component was observed in 70% cases. We did not observe ekadoshaja or sama prakriti in this group.

The response to Modified PVK kwath was analysed in treated cases and was best in the pitta dominant and kapha dominant cases justifying the sutra:

**पंचवल्कस्तु पित्तार्ता ।**

**Conclusions:**

This chapter summarises the findings of the earlier CCRAS project which primarily was aimed at determining the activity and clinical safety of Modified PVK kwath alone in the treatment of uncomplicated leucorrhoea . Daily vaginal douche with freshly prepared kwath relieved the symptoms, fully or significantly in 87% of cases. Additionally there was objective evaluation with semiquantitative symptom records, Pap smears, colposcopy and vaginal pH which showed significant improvement or cure in many cases.

The combined used of kwath with oral Ayurvedic treatment was compared with a parallel group of allopathic standard of care therapy and showed similar results. In both groups partner treatment was given with single dose kits for syndromic treatment of leucorrhoea.

Prakriti analysis in cases of leucorrhoea showed pitta as the dominant prakriti in these cases with vata vitiation also in majority. In women treated with PVK the best response was observed in those with pitta and kapha dominance.

Most of these results have been published earlier as mentioned but have been summarized here alongwith previously unpublished photographs and figures.

**Chapter 4.****Panchavalkal (Modified) Vaginal Formulation development***Mehta D, de'Souza A, Shah A, Paradkar PH, Amonkar AJ*

- 1. Pharmacognosy- Passport Data – selection of source of plant material**
- 2. Tests on bark**
- 3. Vaginal drug delivery system**
- 4. Development of the vaginal gel**
- 5. TLC & standardization of extracts**
- 6. Formulation- quality control**
  - Heavy metals
  - Pesticides
  - Aflatoxin content
  - Microbial load

**Rationale for vaginal drug delivery system for treatment of leucorrhoea:**

The vaginal douche used and described in Chapter 3 had to be prepared fresh every day and was a tedious as well as time consuming process for the health assistant who prepared it everyday, and also for the physician who administered it every day. Additionally it was also time consuming, uncomfortable and costly for the patient consumer who was required to visit the hospital daily for 14 days. Hence there was a great need to develop a vaginal formulation which could be self administered at home by the patient. Vaginal formulations, eg vaginal creams or pessaries are available as user friendly local applications for other drugs like miconazole, clotrimazole, clindamycin, and mycostatin as also lactate gel preparations. Most of them are available as OTC (Over The Counter) medicines. However only one Ayurvedic marketed formulation is available as vaginal cream and that is V-gel® which is widely used by Ayurvedic physicians for the treatment of leucorrhoea. Pentaphyte® is a formulation from Panchavalkal (2% extract) but is essentially developed for cutaneous application and not for vaginal delivery. Preclinical testing by vaginal application in rabbits has not been carried out with this cream.

The second major advantage of a local formulation is that a higher concentration can be applied to the inflamed site and this can result in greater efficacy. The ancient rishis had thought about this and the use of kalka (application of paste) and varti (form of vaginal pessary) as local preparations apart from yoni-dhawan (douching) or pichu (application of medicated oils with silk or cotton balls) has been described in Atharva Veda, BhavaPrakasha and Charaka. Kalka (fresh paste) and varti (elongated dried formulation tabloid) similar to a pessary have been described as early as 3000 to 5000 BC in Charaka- Samhita. However the preparation requires daily formulation from fresh leaves or kalka. Vartis are finger like formulations in which the paste is dried on a silk cloth which may be partly dried and could be used per vaginum, and these may be stable for a week or so. However there are no hygienically prepared stable formulations. Hence we initiated a programme to develop a vaginal formulation for the treatment of leucorrhoea so that the therapy is i) self administered ii) convenient and user friendly iii) cost effective & iv) saves the provider time as well as the user time (in terms of hospital visits) v) has more stability than the kwath.

Vaginal pessaries have also been attempted by us and by others (Mehta , Palep et al) but these were not clinically useful because of side effects like burning of the vagina (Unpublished Data), probably due to the acidic pH.

Due to the recent epidemiologic spread of diseases like Human Immunodeficiency Syndrome and other sexually transmitted infections (STIs/ STDs) diseases the development of safe and effective vaginal / topical microbicides has attracted the attention of researchers all over the world. The use of a vaginal microbicide allows the “female controlled chemical barrier method” to be used as a preventive method against STIs.

Panchavalkal has shown unique effectiveness against multiple organisms as discussed in Chapter 2 hence it can have a broad spectrum of activity. Viridis BioPharma Pvt Ltd have the previous experience of developing a cream from 5 Ayurvedic medicinal plants for burns and for skin application for episiotomy wounds. Panchavalkal ointment was studied in patients of Tata Department of Plastic Surgery, Sir J. J. Group of Hospitals, Mumbai (Unpublished Data, Mehta D , Bhatt RM et al). It was clinically tested in 15

burn patients & 5 plastic surgery patients for healing + infection control. Superficial burns were totally cleared of infections and healed within 18 days. Deep wounds were cleared of infections. Periodical bacteriological tests were made before, during and after the treatment of all the patients. Hundred percent recovery was observed. All wounds treated with Panchavalkal ointment achieved rapid healing. A cooling effect was experienced by the patients and a rapid epithelisation of the wound was also observed.

Thus based on Reverse Pharmacology path with Ayurvedic experience and microbiological experiments by Late Dr RM Bhatt and Dr Dilip Mehta the Modified Panchavalkal vaginal cream was developed as a vaginal formulation as given below.

### **1. Pharmacognosy- Passport Data – selection of source of plant material:**

#### **a) Collection of Plant Materials :**

i) Selection of Appropriate Trees: The trees which have acquired desired growth and maturity were selected for collection of bark. 'Bark' is the peripheral layer of protective tissues, which develops on the stem or on roots of the plants, after attaining certain period of maturity or growth by the concerned plant species.

ii) Techniques for collection of bark: Barks were removed from the selected trees by making incisions about 1'' deep on the stem, at different angles and at various heights of the stem, taking care that no injury occurs to the internal vascular tissue system of the stem. With the help of a sharp iron knife, bark was removed in flakes from the demarcated position and angle of the stem. Numbers of trees of the same species were chosen for collecting the bark.

A collected bark of each species was numbered and a tag bearing, the correct name of the plant, date of collection, locality etc. were maintained and the bark was stored in cotton bags.

#### **b) Processing of the collected Materials:**

The collected materials from various localities were brought to BLAT (BLATTER HERBARIUM ST. XAVIER'S COLLEGE). They were spread either on the floor on

news papers or on large tables with its respective tag, for drying under room temperature. The materials were stirred periodically to avoid attack by any fungi or micro-organisms. Scrutinies of the drying specimens were done periodically to ensure their safety. It took about more than two and half months time for the proper drying of the materials.

**c) Storing of the Materials:**

The completely dried materials were packed in neat dry cotton bags individually along with its respective Scientific name, Vernacular name, locality of collection, period of collection and accession number. The packed dry materials were finally stored in a steel cupboard for the supply to the concerned institution.

**d) Preparation of Data on Bark Samples:**

The collected fresh samples of the Bark from the concerned plant species were studied in detail for their external or morphological characters and their internal structures or Anatomical peculiarities.

**e) Chemical tests**

Various chemical tests were also conducted to detect the presence or absence of various chemical components and their action with the respective Chemicals.

**f) Standardisation of Test:**

Standardisation of the test was made based on the final results of the various tests with the materials.

**g) Study on Macroscopical peculiarities of the bark samples:**

The following parameters were considered for the macroscopical peculiarities of the bark samples:

1. Colour of the bark
2. Odor of the bark
3. Flexibility of the bark
4. Appendages on the bark
5. Ornamentation/Designs on the Bark
6. Thickness of the Bark.

Photographs of the particular tree bark leaves and roots etc of each species have been taken. Morphological peculiarities of the outer surfaces of the bark of each species

and inner surface of the particular species etc. were critically studied and illustrations were made for the characteristics of the concerned species.

Macroscopic studies of the following species were done.

Microscopic structures also were prepared and they are incorporated in the text with details of concerned species.

#### **h) Microscopic Studies :**

Transverse Longitudinal section (T. L. S.) of the bark of the concerned species, and T.S. of aerial root of Banyan were taken separately with sharp blade. Thin sections of the concerned bark/root was selected and they were stained with 50% alcoholic safranin in petridish for about 5-10 minutes, and then Washed 50% alcohol and finally mounted separately in glycerin on clean slide with the help of coverslip taking care that no air bubbles enter into the tissues. Each section was observed and studies critically under a compound microscope. Peculiarities of the cell, cell inclusions, tissues etc. was sketched separately for reference purpose.

The following plants were collected:

**Table 4.1. List of plants for pharmacognosy and bark collection**

1.	<i>Ficus beghalensis</i> Linn	'Wad'	Family - Moraceae
2.	<i>Ficus religiosa</i> Linn	'Pipal'	Family - Moraceae
3.	<i>Ficus racemosa</i> Linn	'Umber'	Family - Moraceae
4.	<i>Ficus infectoria</i> Roxb.	'Pipli'	Family - Moraceae
5.	<i>Albizia lebbek</i> (Linn)	'Siris'	Family - Mimosaceae

**Taxonomic details:****Sanskrit name: Vata; Local Name: Wad****Scientific Name – Ficus benghalensis Linn.****Family – Moraceae.****Description of the Plant:**

Large tree, with many aerial roots from the branches. Leaves coriaceous, ovate, or orbicular – ovate, entire, glabrous. Receptacles sessile, in pairs, axillary, globose, red when ripe, Ostiole prominent. White latex oozes out when the fruit is removed. Fruit with 3 persistent, board perianth lobes. Receptacle grooved within, the Periphery.

Fresh Bark about 2m thick, green with white dots, ash coloured with persistent circular leaf bases. Inner portion of the bark on both sides light orange coloured with loose dust like structures which extends up to 1 cm on both the sides of the central fibery elongate uniform portion which is about 1.8 cm broad, whitish. Fibers rough, comparatively tightly organized, off- white in colour.

Peel of the material from the dorsal surface and the ventral surface were taken by blade. They were mounted in water and focused under a compound microscope for detailed structure under the low power and high power objectives of the compound microspore. Diagrams were made pertaining to the structures observed under the microscopes.

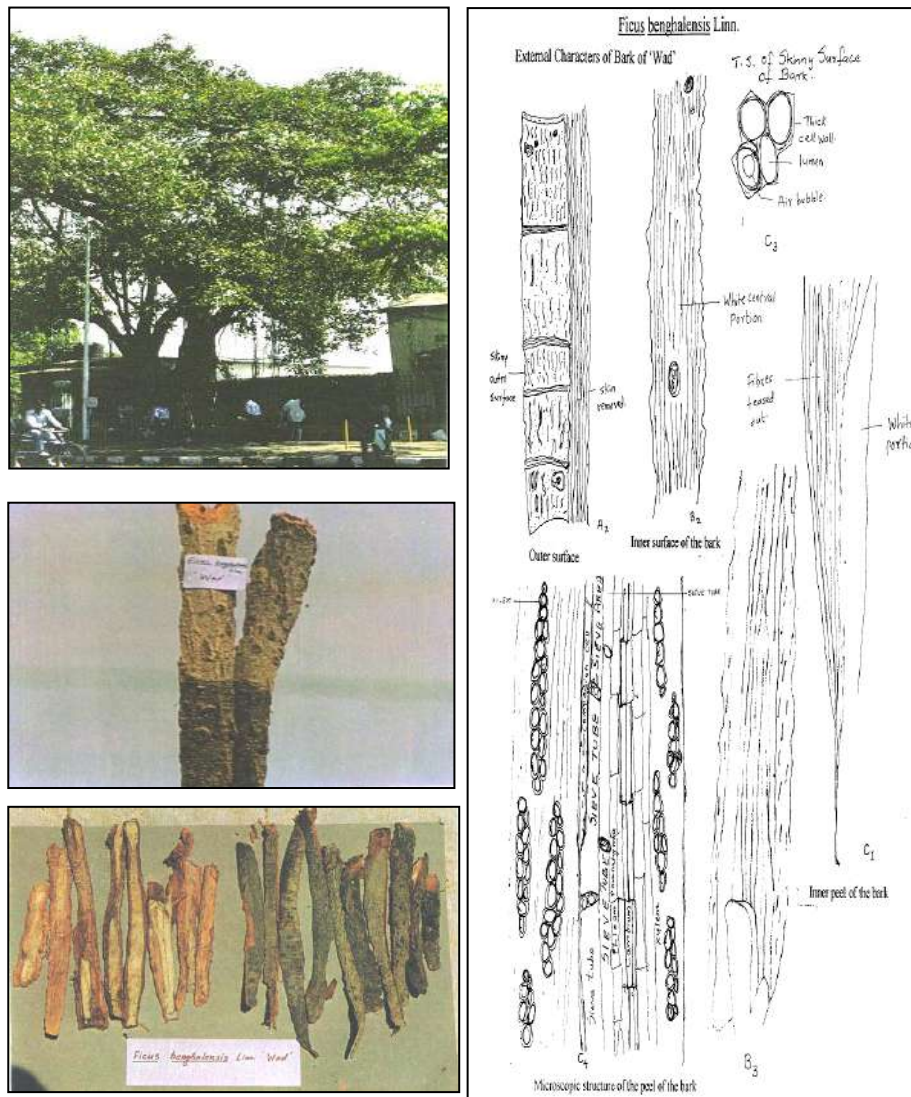
Microscopic structures also were prepared and they are incorporated in the text with details of concerned species.

**Microscopic Studies :**

Transverse Longitudinal section (T. L. S.) of the bark of the concerned species, and T.S. of aerial root of Banyan were taken separately with sharp blade. Thin sections of the concerned bark/root was selected and they were stained with 50% alcoholic safranin in petridish for about 5-10 minutes, and then Washed 50% alcohol and finally mounted separately in glycerin on clean slide with the help of coverslip taking care that no air

bubbles enter into the tissues. Each section was observed and studies critically under a compound microscope. Peculiarities of the cell, cell inclusions, tissues etc. was sketched separately for reference purpose.

**Figure 4.1. *Ficus benghalensis* L. “Wad” – Banyan Tree**



Macroscopic and microscopic features are depicted in the Figure above.

Another set of peeling from the same material was used for staining 50% alcoholic safranin was used for staining. The peeling was stained in a petridish for about five

minutes. The stained materials were then mounted on a clean slide in a drop of glycerine and focused under the C. M. for the detail study.

**Sanskrit name: Ashwattha; Local name: Pipal**

**Scientific Name – *Ficus religiosa* Linn.**

**Family – Moraceae.**

**Description of the plant:**

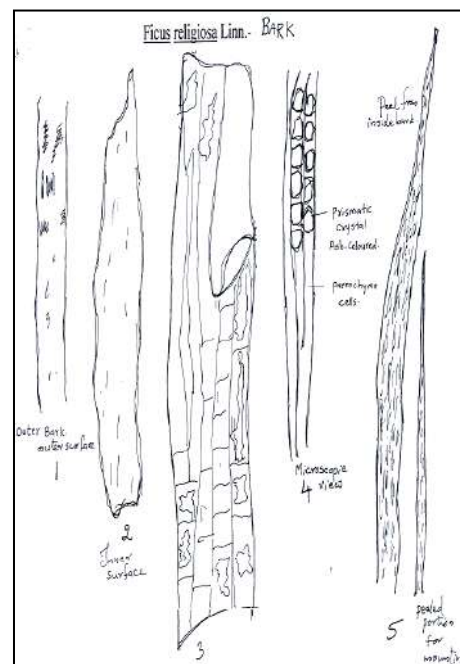
Large tree, usually epiphytic. Leaves ovate – rotund, narrowed at the apex into a linear – lanceolate tail, entire, glabrous, coriaceous, 5 – 7 nerved. Tender leaves yellowish – pink. Receptacle sessile, rounded, green, small, axillary, in pairs.

**Figure 4.2. *Ficus religiosa* l. – Pipal**

**A fruiting branch**



**Inner & Outer surface/peel**



Bark heavy, thick, off-white or yellowish with very thin greenish dry, skinny peels, with slight folding as thick as human hair. Inside the bark, there is a thin covering and inner to the outer skin there is an orange- yellowish area.

Thickness of the bark about 0.8 cm. peeling takes from inside bark when wetted with water becomes darker brownish or chocolate coloured.

**Microscopic Characters / Internal structure of the bark:**

Section of the bark was taken; they were mounted in water and observed under Compound Microscope.

The bark under the compound microscope shows thin walled parenchymatous cells with prismatic crystals arranged within the cells one below the other. The powdered sample of the bark looked dark purplish and rough to touch. Under microscope, bacteria of various types such as Cocci, Bacillus, Spiral etc were noticed. Red ants were seen in the stored sample.

T. L. S. of the bark under C. M. – No special structures were seen under C. M. TLS, does not show any lenticels or fissures. Inner bark is lamellate.

**Sanskrit name: Oudumbar; Local Name 'Rumad'**

**Scientific Name – *Ficus racemosa* Linn.**

**Family – Moraceae.**

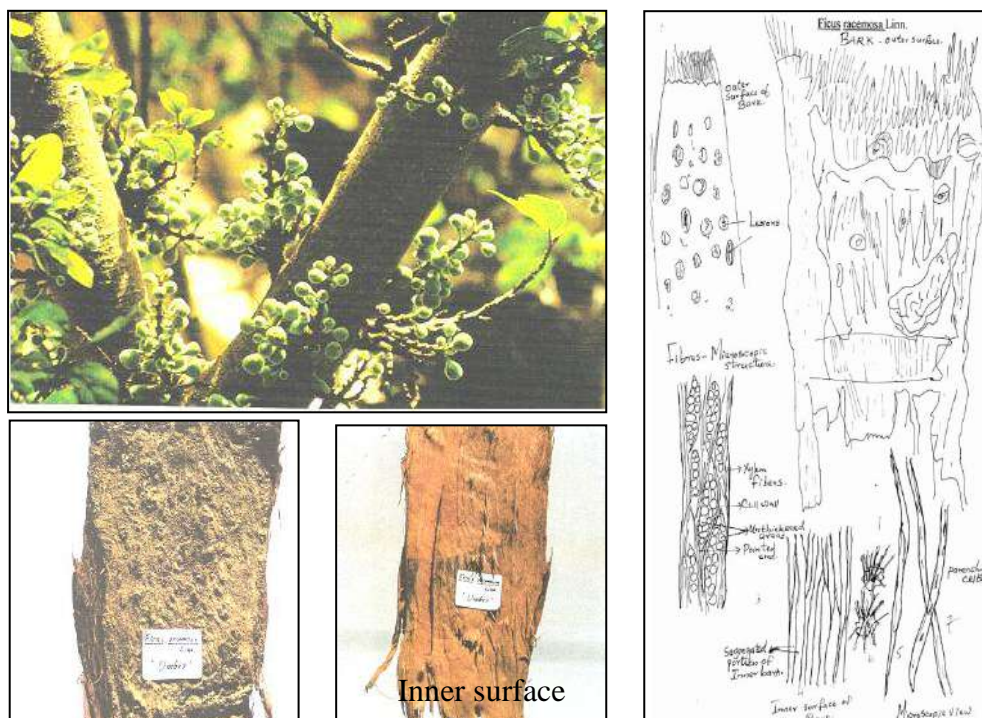
**Description of plant:**

Evergreen tree. Leaves simple, alternate, ovate – oblong or ellipticlanceolate, entire, glabrous, 3–nerved from base. Petiole pinkish. Stipule pinkish, opposite, sagittate, tapering to apex. Receptacle shortly pedunculate, on short leafless branches, sub – globose, green coloured with white hairs when young, red when ripe, smooth or pubescent.

**Microscopic Characters / Microscopic Characters:**

Bark soft, outer colour light brownish, inner surface dirty pink coloured. Bark into pieces when cut, surface of the bark thick, soil or mud coloured, more or less 0.1 – 0.2 mm thick with raised circular or oblong lesions, tightly fixed with the inner portion of the bark, not peeling off. Inner part of the bark loose, tearing into longitudinal sheets or clusters of fibers. Inner side pink or purple coloured when freshly removed from the tree. Colour comes out while touching with other objects; Bark holds water, and feels cool while touching. Layers of sheets can be removed from the inner surface of the bark.

**Figure 4.3. *Ficus infectoria* l. – Udumbar**



Microscopic Characters / Internal structure of the bark:

Fibers colored, light pinkish – soft, easily segregating from outside skin. Under microscope, fiber show two – three layered, separating walls, open areas broader, more in number. fibers in two to three rows, no regular arrangements.

Powder of the bark in small pieces of short fiber, very fine, powder comparatively in less quantity. Microscopic structure shows only parenchymatous cells with tannin coating.

Receptacle shortly pedunculate, on leafless part of the branch, subglobose green coloured, white hairs on surface when young, smooth or pubescent, red when ripe.

**Sanskrit name: Plaksha; Local Name : 'Pipli'**

**Scientific Name – *Ficus infectoria* Roxb.**

**Family – Moraceae.**

**Description of the plant:**

Large deciduous tree with yellowish white, thin latex, Leaves arranged on the branch; ovate – oblong, abruptly acuminate, margin wavy, entire, 3 – nerved. Bud scale stipule broad, narrowing to the apex. Receptacle axillary, sessile, globose, in pairs, whitish. The whole tree gets flooded with white fruit which is a very fascinating sight. The tree is comparatively rare in wild habitats also in cultivation.

Bark about 1.5-cm thick, ash coloured or slightly blackish in colour on outer surface with thin lines running longitudinally in between. Bark breaks easily. No odour. Inner surface coloured off-white with a tinge of pink, edges turning slightly inwards after two days of drying, grooved within, colour depends to pink within, on drying.

**Microscopic Characters / Internal Characters:**

Cells parenchymatous with cross walls containing rounded, colorless leucoplasts within the cells. Fibers from inside peels off easily. Tannin contents seen in the cells.

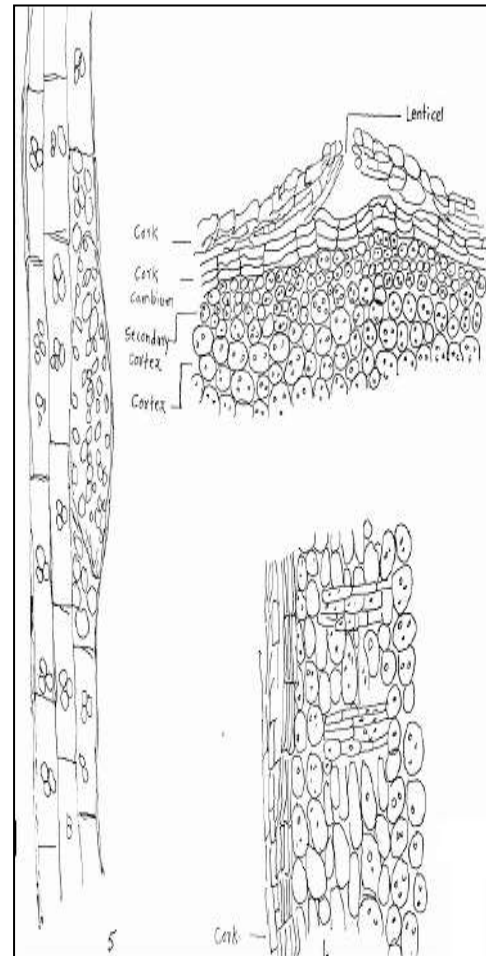
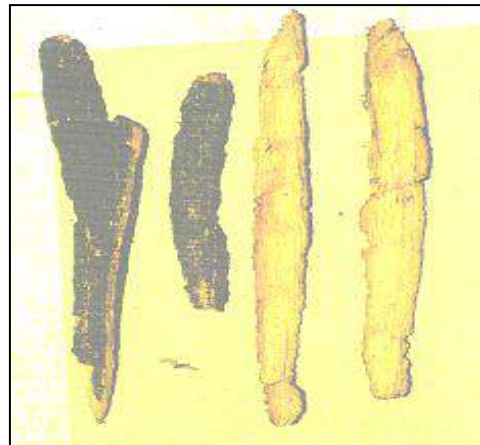
**T.L.S. of Bark:**

T.L.S. of bark shows distinct lenticels; bark consisting of Phellum, Phellogen and Phelloderm consist of about 10 layers of rounded parenchymatous cells, of which 4-5 layers of outer cells just below the cork-cambium consists of very compactly arranged rounded parenchymatous cells without any intercellular space, the cells contain plenty of chloroplast within it. This forms the secondary cortex.

The inner cortex is the primary cortex which consists of comparatively bigger, rounded, thin walled parenchymatous cells with intercellular spaces and few chloroplast in each cell. The presence of lenticel is distinctive feature of this species.

**Figure 4.4. *Ficus infectoria* Roxb. –Plaksha- Parish Pipali**

Tree in fruiting condition



**Sanskrit name: Shirish; Local Name: Siris**

**Scientific Name - Albizzia lebbbeck (Linn)**

**Family - Mimosaceae**

A medium to large tree, of multi-stemmed widely spreading habit (to 30 m diameter) when grown in the open, but capable of good log form in plantation. Height to 20 m. Bark rough, grey; inner bark reddish. Leaves bipinnate, rachis 70-90 mm, rachillae 1-5 pairs, 50-70 mm. Leaflets 3-11 pairs, oblong to elliptic-oblong, asymmetrical, 15-65 mm x 5-35 mm, glabrous, entire, initially bright green and folding at night, maturing to a duller glaucous green and fixed rachis. Fully but briefly deciduous in the dry season. Inflorescence an axillary cluster of 15-40 pedicellate flowers. Peduncle to 100 mm, pedicel 1.5-5 mm, corolla inconspicuous, free filaments numerous, 15-30 mm. Entire inflorescence, fluffy, 60 mm diameter, yellow-green with distinctive pleasant fragrance. Pod flat oblong 120-350 mm x 30-60 mm, stiff-papery when ripe, swollen over seeds, dehiscent. Seeds 3-12 per pod, brown, flattened, 7 x 1.5 mm.

**Macroscopic Characters / External Structure:**

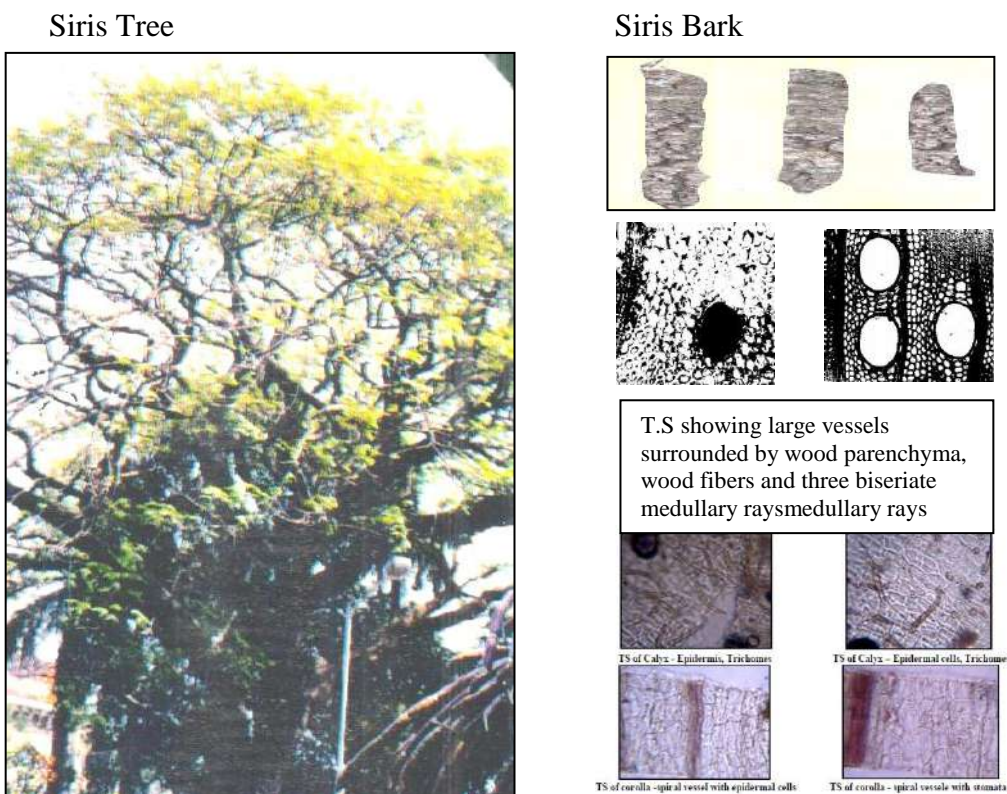
The heartwood of *Shirisha* appeared dark brown in colour streaked with dark and brown shades. On organoleptic examination, very mild characteristic odour with slightly astringent taste was perceived. The surface characteristic of heartwood was appeared to be hard and course in touch, rough texture and splintery in fracture characteristic.

The diagnostic characters of the powder are plenty of broken fragments of isolated and groups of thin walled and occasional thick walled fibers with blunt or pointed end associated with ideoblasts embedded with oxalate crystals; longitudinally cut fragments of vessels and tracheids with bordered pitted thickening and beaded walls. Lenticular masses of tangentially cut medullary rays associated with fibres and fragments of radially cut medullary rays crossing the vessels, parenchyma or fibers, Prismatic crystals of calcium oxalate scattered as such throughout the powder and parenchymatous cells embedded with minute starch grains. Occasional small groups of transversally cut fibers were also found.

**Microscopic Characters/ Internal Characters:**

Transverse sections shows isolated big vessels scattered throughout the section or rarely in radial groups. At places they are often blocked with tyloses impregnated with tannin. In longitudinal section, they exhibit numerous closely arranged minute bordered pits and slit like pores. The fibers are plenty occupying the major area of the section. They are thin walled usually arranged in tangential and radial rows and at places embedded within broad zone of radially running groups of metatrachial parenchyma embedded with prismatic crystals of calcium oxalate. Vesicentric parenchyma encircling the vessel and paratracheal parenchyma with broad radially running bands are also seen at places. Medullary rays are uni to tri seriate. Their cells are pitted and rectangular in shape. In tangential longitudinal section uniseriate medullary rays are seen as a vertically running linear bands while the multiseriate medullary rays as a lenticular areas, embedded with rows of prismatic crystals of calcium oxalate and dark brown colouring matter. In radial longitudinal section, the medullary rays appear as narrow horizontally running bands crossing the vessels and fibres. Parenchymatous cells are pitted and are embedded with starch grains and calcium oxalate crystals. Starch grains being very minute in the vesicentric parenchymatous cells.

**Figure 4.5. Albizzia lebbeck (Linn)- Shirish**



## 4.2 Tests on Bark Powder

	Chemical	<u>Ficus religiosa</u> L. 'Pipal'	<u>Ficus benghalensis</u> L. 'Vad'	<u>Ficus infectoria</u> Roxb. 'Pipali'	<u>Ficus racemosa</u> L. 'Umber'	<u>Albizia lebbek</u> (Linn) – "Siris"
1.	50% Sulphuric Acid	Black	Black residue, suspension pale brown.	Black	Black	Black heavy solution
2.	50% Nitric Acid	Dark Yellow	Golden solution in between, residue down and top suspension	Yellow solution with material on top	Pale brown solution, dark brown residue down.	Dark yellow solution less suspended material on top
3.	50% Acetic Acid	Brown Solution, dark brown residue.	Pale yellow solutions in less quantity, brown residue more.	Light brown solution, particle down.	Peach colour solution, dark brown residue.	Light brown solution with little residue down
4.	50% Hydrochloric Acid	Clear Solution, black residue.	Light yellow solution, dark brown residue down.	Pale brown solution, dark brown residue down with suspended material on top.	Peach colour solution, dark brown residue	–
5.	Xylene	Clear Solution, brown residue.	Clear Solution, residue down.	Clear Solution, brown residue.	Clear Solution, with very little residue down.	Light yellow solution brown residue down
6.	Alcohol	Pale brown solution.	–	Pale brown solution, brown residue.	Peach colour solution, dark brown residue	Light brown solution top, dark brown middle portion and pale brown residue down
7.	Aqueous (Distilled Water)	Yellow solution, dark brown residue.	Solution yellow with tinge of brown, residue dark brown, diffusing.	Pale brown solution, dark brown residue down top suspended floating powder particles.	Clear Solution, brown particles settle down.	Peach coloured solution and dark brown residue, brown small few particles floating on the top of the solution

**STANDARDIZATION OF CHEMICAL TESTS**

**‘Umber’:** **Ficus racemosa** Linn.

Bark powder treating with 50% Nitric acid gives Brown residue

**‘Pipal’:** **Ficus religiosa** Linn

Bark powder treating with Nitric acid gives dark yellow solution.

**‘Wad’:** **Ficus benghalensis** Linn.

1. Bark powder treating with acetic acid gives pale yellow solution, dark brown residue down.
2. Bark powder treating with 50% Hydrochloric acid gives pale yellow solution top, dark brown residue down.

**‘Pipli’:** **Ficus infectoria** Roxb

Bark powder treating with Alcohol gives clear solution with dark brown residue down.

**‘Siris’:** **Albizzia lebbeck** Linn

1. Bark powder treating with Sulphuric Acid gives Black heavy solution.
2. Bark powder treating with nitric Acid gives dark yellow solution less suspended material on top.
3. Bark powder treating with Acetic acid gives light brown solution with little residue down.
4. Bark powder treating with xylene light yellow solution brown residue down.

5. Bark powder treating with Alcohol gives light brown solution top, dark brown middle portion and pale brown residue down.
6. Bark powder added in distilled water shows peach coloured solution and dark brown residue, brown small few particles floating on the top of the solution.

**Preformulation studies:**

Preliminary studies were carried out to determine the best solvent to extract the active principles with maximum antibacterial activity. It was observed that butanol and ethanol were superior to other solvents and ethanol was the best solvent. Butanol itself shows some bacterial inhibitory activity. Panchavalkal with Shirish was used as in it showed best antibacterial and antifungal activity against common pathogenic organisms in leucorrhoea (Patel & Bhatt , 1991).

**Mutagenicity Test:** There was no mutagenicity with the Modified PVK extract as tested by the method of Ames et al.

The bark powders were submitted to gamma-radiation prior to extraction to get rid of any residual bacterial load. The antibacterial activities were reported with the microbe free extracts.

**2. Ideal Vaginal drug delivery system:**

Vaginal formulations have acquired major significance since the onset of the HIV epidemic world over. This mode of prevention empowers women for prevention of the Sexually Transmitted Diseases like HIV. Many virucidal agents have been studied recently and workshops and conferences are being held every year throughout the world (Obeiro et al, 2012). One of the editors (JV Joshi) has the experience of participating as a Trial monitor in an international multicentred WHO Phase I Clinical trial of Chondroitin sulphate (CS) as a Vaginal Microbicide studied in NIRRH, ICMR.

A vaginal drug delivery system requires special considerations and the qualities of an ideal vaginal formulation have been described by Rencher(2001) as given below:

1. Safe when self-administered and non-irritating
2. Is effective against many vaginal pathogens including STIs
3. Effective within a wide range of pH
4. Stable
5. Acceptable consistency for both partners
6. Acceptable colour, should not stain clothes
7. Acceptable odour, for both partners
8. Easy to dispense in repeated doses
9. Compatible with the use of various contraceptives- systemic or local

**3. Development of the vaginal cream with carbopolymer base gel vs petroleum jelly:** The earlier vaginal creams in India and abroad were prepared with an aqueous base, usually petroleum jelly. Our literature search revealed that petroleum jelly, though otherwise safe, was associated with following disadvantages:

- i) It was not supposed to be used with condoms as it can cause weakening and rupture of the condoms which are very commonly used by male sexual partners for prevention of pregnancy and / or STIs (Rosen & Rosen, 1999; European Working Group on HIV Infection, 1993, Voeller et al, 1989).
- ii) It was not being used in the preparation of the modern vaginal microbicides which were being developed by international agencies (Rencher, 20001).

Hence in the present study the vaginally compatible gel, carbopolymer 940 (IP) was used as the gelling agent for the preparation of the cream from plant extracts.

The pH of the various bark extracts was observed to be as follows:

**Table 4.2. pH of Panchavalkal Plants used in the study**

PVK Plant Bark	pH
<i>F bengalensis</i>	5.75
<i>F infectoria</i>	7.02
<i>F religiosa</i>	5.20

<i>F racemosa</i>	7.00
<i>A lebbeck</i>	3.55

Carbopol 940 (IP) was used as a gelling agent in preparation of the cream.

### **Panchavalkal Cream Formulation**

#### Formula

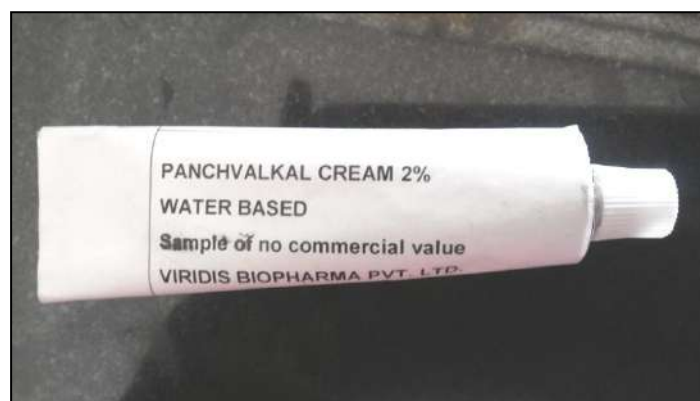
- Ficus religiosa – 0.4% w/w
- Ficus infectoria – 0.4% w/w
- Ficus bengalensis – 0.4% w/w
- Ficus racemosa – 0.4% w/w
- Albizzia lebbeck – 0.4% w/w
- Carbopol 940 – 0.58%
- Propylene Glycol – 7.0%
- Purified water – q.s.

#### **Process:**

1. The extracts were mixed together and ground to fines so as to pass through ASTM 100#.
2. Purified water was heated to 60 to 65<sup>0</sup> C and propylene glycol was added to it.
3. Carbopol 940 was added to this solution slowly under stirring till it completely dissolved.
4. The finely divided mix of the extracts was added to the solution from step 2 and the mixture was stirred vigorously. The temperature was maintained between 60 and 65<sup>0</sup>C.
5. Finally the pH was adjusted between 4.5 and 7 with the help of Triethanolamine..

6. The cream thus formed was allowed to cool to room temperature under continued stirring.
7. It was then filled into tubes and the tubes were sealed.

**Figure 4.6. Modified Panchavalkal Vaginal Cream tube**



The individual bark extracts as well as the cream (final formulation) were subjected to TLC, HPTLC and purity studies (microbial load etc)

#### **4. High performance thin layer chromatography (HPTLC) of Panchavalkal extracts:**

The extracts and formulation were analysed by HPTLC (high performance thin layer chromatography) method on silica gel plates. Earlier individual samples were analysed for suitable solvent system and concentration on TLC. Optimum concentration of each extract which gave better separation was selected and applied on HPTLC plates and developed in one solvent system. Quantitative HPTLC was not possible due to the variation of plant species. Samples were labeled as follows:

P1. *Ficus recemosa*

P2. *Ficus infectoria*

P3. *Ficus benghalensis*

P4. *Ficus religiosa*

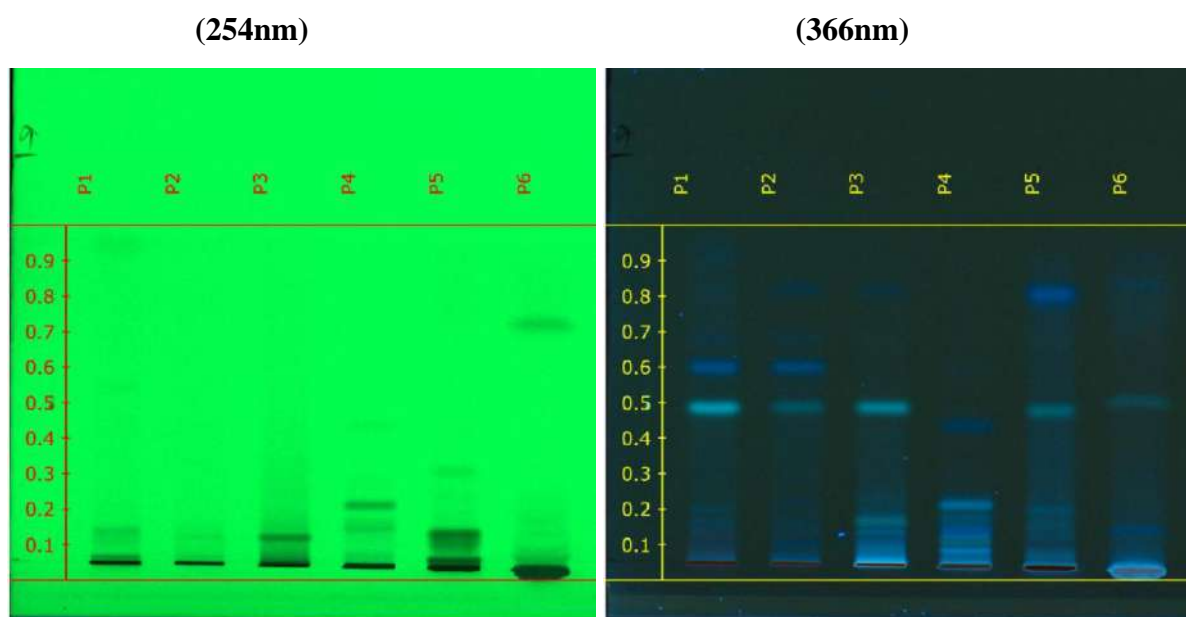
P5. *Albizia lebbek*

P6. Formulation

**HPTLC procedure:**

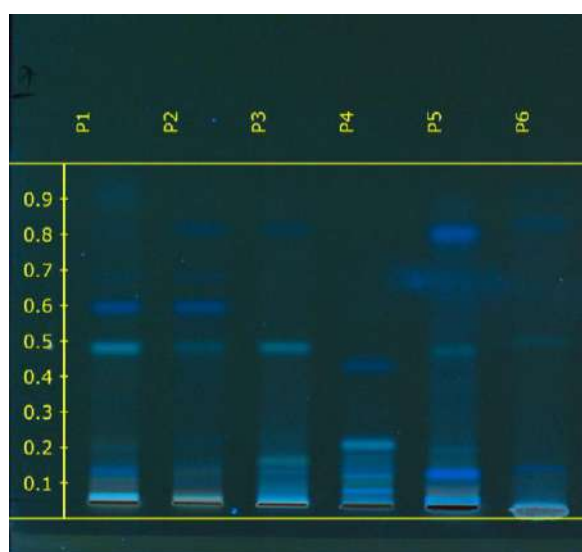
1. Silica gel plates were cut to the desired size using scissors. A line 1.5 cm parallel to the bottom of the plate was drawn using a very light pencil and being careful not to scratch the silica gel.
2. Samples were applied on the plate using Camag (Wilmington, NC, USA) Linomat V, an automated applicator equipped with a 100- $\mu$ L syringe
3. Following settings were used for the sample application: band length 6 mm, application rate 4 s  $\mu$ L<sup>-1</sup>, table speed 10 mm s<sup>-1</sup>, distance between bands 4 mm, distance from the plate side edge 6.5 mm, and distance from the bottom of the plate 1.5 cm
4. Sample was taken in a glass microsyringe and sprayed along a 5 mm path on the pencil line.
5. Plates were run in solvent system Toluene:ethyl acetate:formic acid (90:10:1). Using a pair of long forceps, the top of the plate was grasped and placed in the tank oriented with the spotted sample just above the level of the solvent.
6. The tank was covered tightly and the plate was allowed to remain undisturbed until the ascending solvent line reaches the top of the plate (approx. 15-20 minutes).
7. The TLC plate was removed by grasping the top edge with forceps and air dried under chemical fume hood.
8. Plates were observed under 254 nm and 360 nm wavelengths.
9. The plate was heated at 100°C for 3 minutes and derivatized with Natural Product (NP) reagent (1g of 2-aminoethyl diphenylborinate in 200ml of ethyl acetate). It was further sprayed with anisaldehyde sulphuric acid reagent and again heated for 3 minutes.

**Figure 4.7.a. HPTLC plate developed in toluene:ethyl acetate:formic acid (90:10:1) system and observed under UV (254nm and 366nm).**



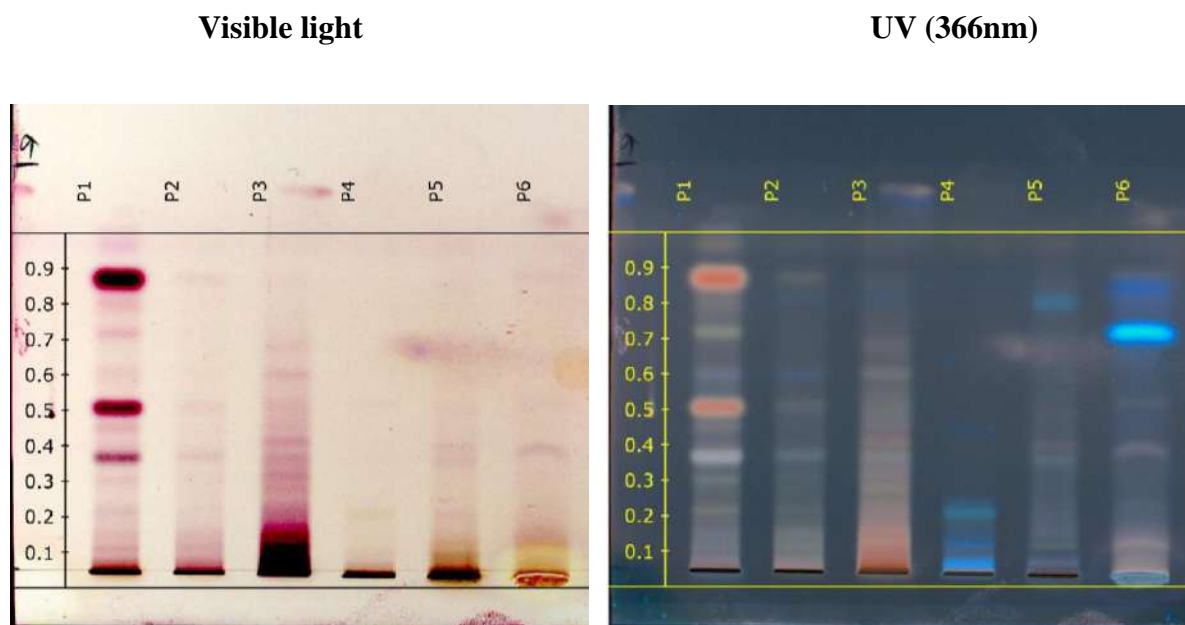
4 spots at the same RF were observed in P1, P2, P3 and P5 samples and formulation at 366nm. The plate was further derivatized with natural products reagent.

**Figure 4.8. HPTLC plate after derivatization with NP reagent and observed under UV (366nm)**



Derivatization with NP reagent increased the intensity and visibility of the flavonoid compounds in P1-P6 extracts.

**Figure 4.9.a. HPTLC plate after subsequent derivatization with anisaldehyde sulphuric acid reagent and observed in visible light and under UV (366nm)**



All the samples showed accentuation of respective compounds (P1-P6) in visible and UV (366nm) lights.

The extra spot seen in formulation (P6) lane after development at 254nm and after derivatization with ASR at 366nm can also be seen in the graph and can be an ingredient in the formulation.

**Conclusion:** HPTLC confirmed the presence of flavonoids in hydroalcoholic extracts as well as in the formulation.

**5. Formulation- quality control- Heavy metals**

- Pesticides
- Aflatoxin content
- Microbial load

4 tubes (35gms X 4) of formulation from medicinal plants (KHS/CCRASPVK/09) for pesticide and aflatoxin analysis as follows:

**Pesticides:** Analysis and detection limits for the following pesticides was carried out at Reliable Analytical Laboratories laboratories ( Govt certified)

**Table 4.3: Pesticide Residue –(AOAC 2007)**

Sr. No.	Tests	Units	Results	Quantification Limit	Test method
1	Aldrin	mg/kg (ppm)	BLQ	0.01	GC-MS
2	Dieldrin	mg/kg (ppm)	BLQ	0.01	GC-MS
3	Alpha-HCH	mg/kg (ppm)	BLQ	0.01	GC-MS
4	Beta –HCH	mg/kg (ppm)	BLQ	0.01	GC-MS
5	Gama-HCH	mg/kg (ppm)	BLQ	0.01	GC-MS
6	DDT	mg/kg (ppm)	BLQ	0.01	GC-MS
7	DDD	mg/kg (ppm)	BLQ	0.01	GC-MS
8	DDE	mg/kg (ppm)	BLQ	0.01	GC-MS
9	Quinolphos	mg/kg (ppm)	BLQ	0.01	LC-MS/MS

**Aflatoxins:** Analysis and detection limits for the following types were carried out at the certified laboratories (AOAC methods):

**Table 4.4: Aflatoxin- TLC (AOAC 2005)**

Sr. No.	Tests	Results
1	Aflatoxin B1, ppb	Not detected
2	Aflatoxin B2, ppb	Not detected
3	Aflatoxin G1, ppb	Not detected
4	Aflatoxin G2, ppb	Not detected

(Detection Limit, ppb :2.5)

**Heavy metals:** 105 gms (35gmsX 3) of formulation from medicinal plants for Heavy metal (Ahmed et al, WHO 1998a) and microbial load analysis as follows:

**Table 4.5. Heavy metals**

Heavy metal	Estimated amount/gm	Method	Permissible limit as per (FDA & WHO)
Lead	2.1 ppm	AAS (Kojuncu et al, 2004)	10 ppm
Cadmium	Nil	AAS (Kojuncu et al, 2004)	0.3 ppm
Arsenic	Less than 1 ppm	Stain method (WHO, 1998)	10 ppm
Mercury	Nil	Colorimetric with Dithizone reagent (Jamaluddin et al, 2003)	1 ppm

Bee Pharmo Labs Pvt Limited (Govt certified)

**Table 4.8. Microbial load (WHO, 1998)**

<b>1.Total aerobic count (By plate count method)</b>	80 CFU/GM
<b>a. Total bacterial count</b>	70 CFU/GM
<b>b. Total fungal count</b>	10 CFU/GM
<b>2.Pathogens</b>	
<b>a. <i>Salmonella</i></b>	Absent
<b>b. <i>Enterobacteria</i></b>	Absent

**Conclusions:**

Due to side effects of allopathic drugs and possibility of drug resistance there is a great need to develop effective and tolerable herbal vaginal cream for treatment of leucorrhoea in women. Such creams have been developed and studied by Talwar et al, Salhan et al, and Chopra et al for prevention of sexual transmission of HIV. However Palep et al and Ashar et al have studied Pentaphyte cream for the prevention of anaerobic infections and it is a marketed preparation. This cream is extensively used by Ayurvedic physicians for the prevention of postoperative wounds after hysterectomies or episiotomies. In the present study the vaginally compatible gel was used to develop a vaginal cream from modified Panchavalkal for the treatment of uncomplicated leucorrhoea like bacterial vaginitis. KHS/CCRASPVK/09, 2 % cream was developed from bark extracts of Panchavalkal with bioactive extracts as shown by microbiological culture studies. The cream had acceptable colour and odour and consistency. It had acceptable levels of heavy metals, pesticides, aflatoxin and microbes. The extracts as well as the formulation showed bioactive component of stigmasterol in TLC and HPTLC. Hence it was decided to take up the cream for preclinical testing.

This was ready for preclinical testing as described in the next chapter. Small sample sizes of 0% and 4% creams were also made available for preclinical testing.

**Chapter 5.****Preclinical study of Panchavalkal Vaginal cream  
KHS/CCRASPVK/09 for vaginal tolerability in rabbits  
and dermal tolerability in rats**

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# Kasturba Health Society's Medical Research Centre & ICMR Advanced Centre for Reverse Pharmacology in Traditional Medicine

**1. Introduction****2. Animal Laboratory Studies- Prerequisites****3. Vaginal tolerability in rabbits****4. Dermal toxicity in rats****5. Conclusion****1. Introduction:**

In view of the RTI global epidemic the development of an effective and safe vaginal microbicide has become a research priority. The KHS/CCRASPVK/09 cream was developed from Ayurvedic Medicinal Plants, to be used as a vaginal formulation for the short term (10 days) treatment of leucorrhoea. The preclinical toxicity study of various extracts was already carried out in rats and rabbit vagina (Bhatt RM et al; Mehta D et al Unpublished Data). Moreover the clinical study with the Modified PVK kwath as reported in Chapter 3 did not show any clinical toxicity and had shown comparable safety and activity as compared to the control group with allopathic therapy.

However the new regulations for global acceptance required additional dermal safety test in rats and rabbit as per CCRAS guidelines. Although it will be mandatory to advise

abstinence or condoms during use, the male penis may be accidentally exposed to it. It is therefore necessary to ensure that there will be no cutaneous toxicity of the cream. Systemic absorption in women, though expected to be minimal, is still possible as exemplified by Nanoxynol-9 (Chvapil et al, 1980) and if it occurs, it should not lead to any organ toxicity. The present study included evaluation of local as well as systemic tolerability, and toxicity if any, of PVK cream (Hematology, Serum Biochemistry and Organ histology).

Preclinical studies were designed as per the CCRAS guidelines in collaboration with the National Centre for Preclinical Reproductive and Genetic Toxicology, at National Institute for Research in Reproductive Health, Indian Council of Medical Research (NIRRH, ICMR) and were carried out at the animal house & laboratories of NIRRH as per Schedule Y (1945) (CCRAS, 1990,1996). The present monograph describes the safety evaluation of PVK vaginal formulation based on CCRAS guidelines and Reverse Pharmacology principles (Lavekar GS,2010; Vaidya AB, 2010).

## **2. Animal Laboratory Studies- Prerequisites**

The animal study was designed to establish the preclinical safety of Panchavalkal Vaginal cream KHS/CCRASPVK/09. Preclinical study of was performed in two species viz., Holtzman rats and New Zealand white rabbits. Clearance for the use of animals in the study was obtained from the Institutional Animal Ethics Committee (IAEC) prior to the initiation of the study with an approval number NIRRH/IAEC/ 03-09. These experiments were performed in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), India.

## **3. Vaginal tolerability in rabbits**

### **Objective:**

To determine Sub-Acute Dermal toxicity of KHS/CCRASPVK/09 in rats and Vaginal toxicity in rabbits including target organs involved for establishing its safety in humans.

### **Guideline:**

The study was carried out as per Schedule Y.

**Materials and Methods:****Test Article Details:**

Test substance	: KHS/CCRASPVK/09
Batch/lot number	: July 09
Characteristics	: Gel form
Sponsor	: Kasturba Health Society, Med. Res. Centre

KHS/CCRASPVK/09 is an Ayurvedic preparation in gel form. The intended route of administration is Intra vaginal application.

The formulation was stable at room temperature and it was kept in plastic tubes at room temperature.

**RABBIT STUDY:****Test Article Administration:**

Route: Intra Vaginal Application

The dose was directly applied daily intra-vaginally.

**Test Animals:**

New Zealand white rabbits were procured from Sainath agencies (CPCSEA Registration no: 282/CPCSEA) were used in the study. Total 24 female rabbits of 24-28 weeks of age were randomly selected and assigned to the control and treatment group after the acclimatization to the housing for two weeks prior to the start of the study. The weight variation of animals used did not exceed  $\pm 20\%$  of the mean weight.

**Animal Husbandry:**

Animals were fed a diet of lucern grass and in-house-prepared concentrates. *Ad-Lib*, fresh and filtered (purified by UV and reverse osmosis) drinking water was provided to all the animals throughout the study. The animals were kept in steel cages maintained under

controlled temperature ( $23 \pm 1^{\circ}\text{C}$ ) and humidity ( $55 \pm 5\%$ ), and in a 14hr light/10h dark cycle. One animal was housed in a single cage.

**Study Design:**

Animals were randomized before acclimatization and divided in three test groups and a control group. The therapeutic dose (TD) was taken as mid dose, low dose was  $\frac{1}{2}$  TD and high dose was 2TD. All the animals were handled in an identical manner.

All the animals belonging to control and treatment groups were applied intra-vaginally with vehicle and test substance (~volume) respectively over a period of 28 days including holidays. Animals were sacrificed 24 hrs after the last dose of administration. The grouping of the animals was as per Table 5.1.

**Table 5.1. The Grouping was as follows:**

Gr. No.	Groups	Dose	Number of Females
1	Control	0%	6
2	Treatment (Low)	1%	6
3	Treatment (Mid)	2%	6
4	Treatment (High)	4%	6

During the treatment period, the animals were daily examined for avert clinical signs, morbidity, and mortality, if any. The weight of each rabbit was recorded on the day of commencement of treatment, weekly thereafter and at necropsy. Application site i.e. vagina was observed daily for signs of toxicity viz., Erythema, edema, swelling and closure of introitus.

**Figure 5.1.** Method of vaginal application of the test substance**Hematological and Biochemical Analysis:**

At the end of the treatment, the animals were bled from the orbital sinus for clinical pathology assessment which included analysis of various hematology parameters. Hematological analysis was performed using an automatic hematological analyzer Abacus (Diatron). The parameters included: Red Blood Cell (RBC) Count, White Blood Cell (WBC) Count, Hemoglobin (Hb), Hematocrit (Hct), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Platelets count. The differential leukocyte counting was performed with an optical microscopy after staining with Leishman's stain (Sigma-Aldrich) and, in each case, 100 cells were counted.

For biochemical analysis, blood was centrifuged to obtain serum, which was stored at  $-20^{\circ}\text{C}$  until determination of the following parameters viz. Total Protein, Albumin, Globulin, Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT), Cholesterol, Alkaline Phosphatase, Glucose, Creatinine, Urea, Uric Acid, Triglycerides, Bilirubin (Total And Direct), Calcium and Phosphorous. The serum biochemistry was performed by using fully automated serum biochemistry analyzer- EM 200 (ERBA) with biochemical kits from Spinreact, S. A. Ctra Santa Coloma, Spain.

**Gross Pathology, Organ weight, and Histopathology:**

After completion of dosing period, on 29th day the animals were euthanized using intravenous over dose of anesthetic (Sodium Thiopentone) and necropsied for the gross evaluation of the various organs. The necropsy also included careful dissection of various target organs like heart, liver, spleen, kidneys, intestine and stomach, determination of absolute organ weight, and calculation of organ weight to body weight ratios (Percent Relative Organ Weight). Finally, the dissected tissues were fixed in 10% neutral buffered formalin, processed (Tissue processor Leica ASP300), and embedded (Paraffin Embedder Leica EG1150 H) in paraffin wax. Sections (5-7 $\mu$ m) (Fully Automated Rotary Microtome Leica RM2255) of these tissues taken on glass-slides were stained using a combination of hematoxylin-eosin before observing under a microscope for histopathological evaluations.

**Statistical Analysis:**

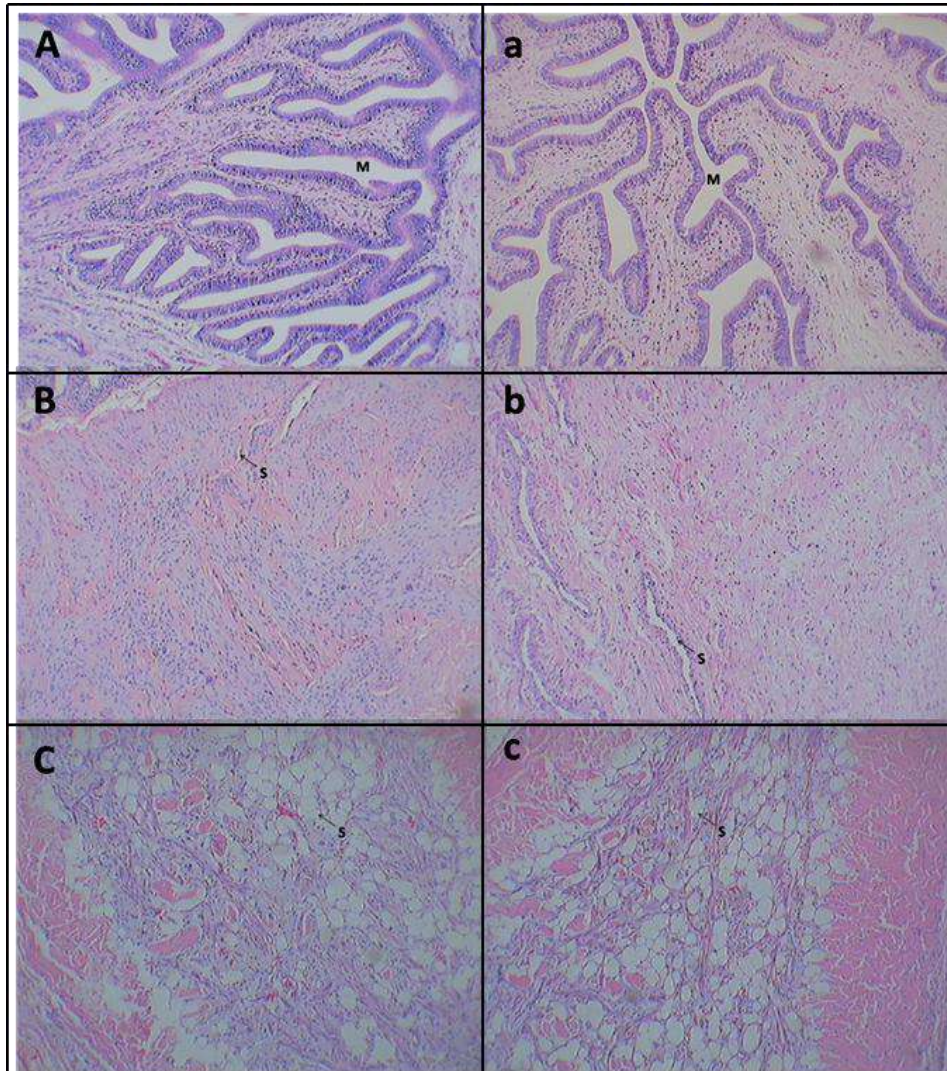
For all the toxicological evaluations, the results of the treatment groups were compared with those of the control group. Data was expressed as mean  $\pm$  S.D. and was analyzed by two-tailed Student's t-test. Paired t-test was applied for hematological and biochemical values observed before and after treatment. Differences were considered significant at  $P < 0.05$ .

**Results:**

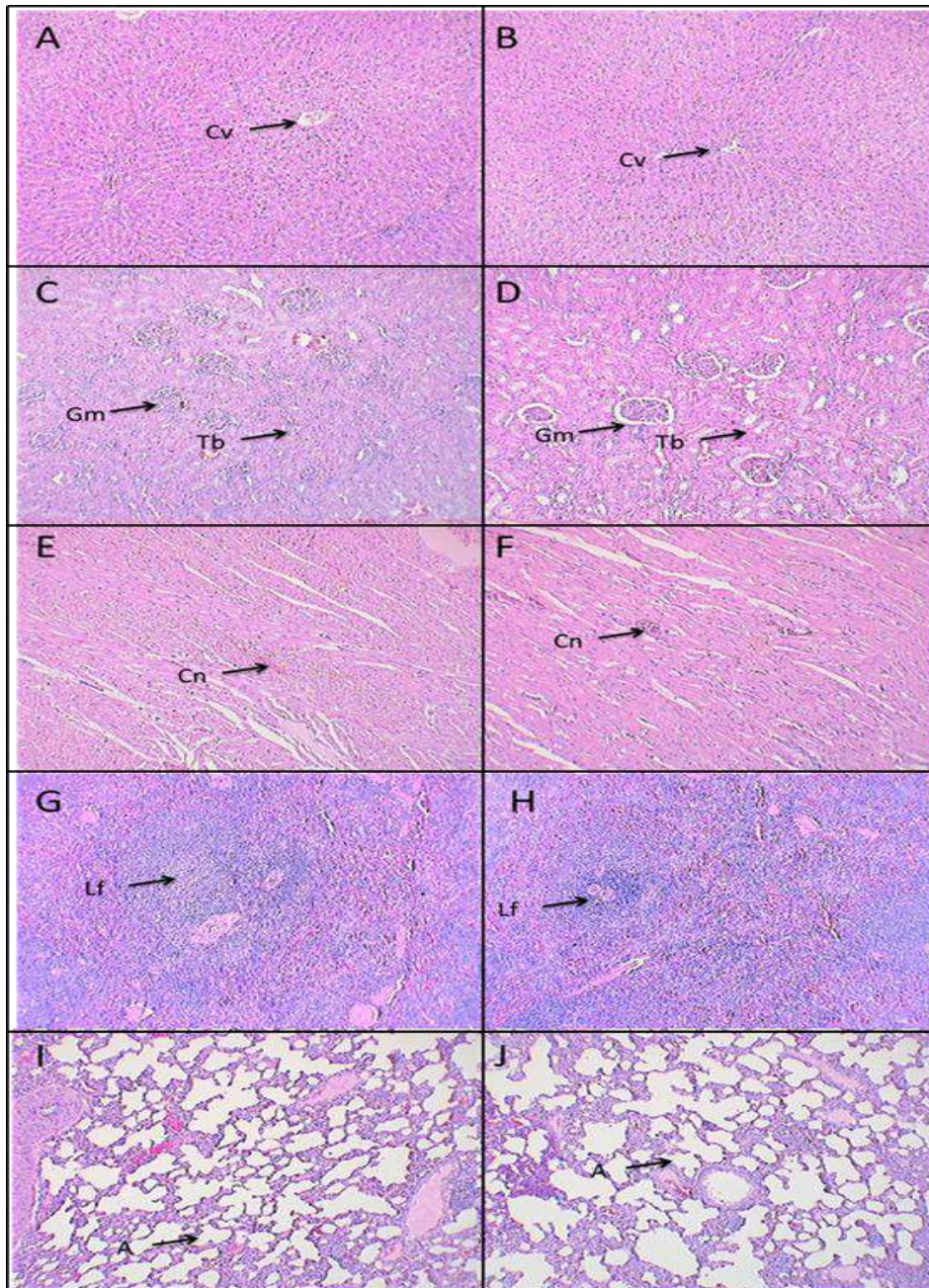
All the animals showed normal behavior throughout the study. No mortality was observed in control as well as treated groups during the period of 28 day. There was no significant difference in the weekly body weight between three treatment groups and that of control. The mean weekly body weight is given in Table 5.2. There was no significant difference in various hematological parameters in treatment group as compared to control. Average hematological values are given in Table 5.3. There was no significant difference in various clinical chemistry parameters between control and treatment group. Average clinical chemistry values are given in Table 5.4.

There were no significant difference in absolute organ weights and relative organ weights of treated group and control group. Absolute organ weight and relative organ weights are given in Table 5.5 and 5.6.

Terminally sacrificed animals did not show any gross pathological findings (Figure 5.3). Histopathology was carried out to identify various lesions like; liver: Periportal mononuclear cell (MNC) infiltration, Focal Hepatocyte degeneration, Kidney: focal tubular regeneration; Thymus: Atrophy. The application site i.e. different parts of vagina did not show any signs of toxicity (Figure 5.2). All the lesions are summarized in Table 5.7. **All the lesions observed were incidental / spontaneous findings and these were not related to the test substance as similar observations were noted in control group also.**

**Figure 5.2.** Histology of different parts of Vagina application site

(A: Upper region Control 0% at 100x, a: Upper region Treated 3% at 100x,  
B: Middle region Control 0% at 100x, b: Middle region Treated 3% at 100x,  
C: Lower region Control 0% at 100x, c: Lower region Treated 3% at 100x)  
**Key:** S: sinusoidal structures, M;Mucosa)

**Figure 5.3:** Histology of **rabbit** vital organs

(A: Liver control 0%, B: Liver treated 3%, C: Kidney control 0%, D: Kidney treated 3%, E: Heart control 0%, F: Heart treated 3%, G: Spleen control 0%, H: Spleen treated 3%, I: Lung control 0%, J: Lung treated 3%)

**Key:** Cv: Central vein, Gm: Glomeruli, Tb: Tubule, Cn: Congestion, Lf: Lymphoid Follicle, A: Alveoli.

**Table 5.2. Mean weekly Body weight (Kgs), Values,unit (Mean± SD n=6).**

Week	Group I	Group II	Group III	Group IV
0 th day	2.121±0.175	2.212±0.183	2.125±0.116	2.213±0.231
1st week	2.157±0.161	2.172±0.121	2.090±0.139	2.203±0.223
2nd week	2.163±0.161	2.225±0.142	2.135±0.122	2.238±0.242
3rd week	2.265±0.122	2.352±0.133	2.292±0.114	2.378±0.221
4th week	2.272±0.181	2.330±0.127	2.250±0.120	2.408±0.227

**Table 5.3 Average of Hematological Parameters, Values (Mean± SD).**

Parameter(units)	Group I	Group II	Group III	Group IV
Hamoglobin (gms%)	11.67±0.516	12.30±3.503	9.77±2.699	10.90±0.751
RBC(per cmm)	6.56±0.458	6.87±1.987	5.59±0.861	5.82±0.700
PCV(% ml)	38.65±1.750	36.43±4.674	35.18±3.644	35.90±3.005
MCV (Micro)	61.52±2.173	60.50±2.168	64.83±9.908	60.83±2.137
MCH(MeanHb/per red cell)	18.50±0.672	17.95±0.952	18.58±0.966	18.38±0.488
MCHC(Hb/RBC)	30.30±0.253	29.72±1.026	30.22±0.847	30.23±0.476
WBC(per cmm)	10.25±1.962	9.88±1.995	7.80±2.268	10.77±3.676
Lymphocytes(%)	39.00±5.404	44.67±5.538	43.17±2.563	45.67±7.202
Neutrophils (%)	57.00±4.290	50.33±5.574	51.67±2.422	50.50±7.287
Monocytes(%)	2.00±1.673	3.00±1.549	3.17±1.602	2.17±1.169
Platelets(%)	180.17±78.070	154.00±63.198	146.50±45.558	175.67±47.546

**Table 5.4 Average of Biochemical Parameters, Values (Mean± SD)**

Parameters,(unit)	Group I	Group II	Group III	Group IV
Total protein (Gms%)	6.03±0.253	6.41±0.356	6.22±0.296	6.55±0.739
Albumin(Gms%)	3.85±0.525	4.00±0.634	3.70±0.131	3.94±0.508
Globulin (Gms%)	2.18±0.457	2.41±0.599	2.52±0.272	2.61±0.710
SGPT (Units/ml)	65.12±18.739	75.76±11.854	60.22±11.478	65.03±27.662
SGOT (Units/ml)	12.19±3.835	14.77±8.283	12.14±3.432	16.80±4.887
ALP (Units/ml)	127.22±45.498	124.85±64.019	126.29±47.140	121.14±23.685
Bilirubin (mg %)	0.09±0.036	0.13±0.026	0.09±0.015	0.08±0.049
Direct Bilirubin (mg%)	0.04±0.020	0.06±0.033	0.05±0.026	0.05±0.015
Cholesterol (mg%)	32.31±5.978	45.01±21.138	38.82±16.679	42.49±21.627
Glucose (mg%)	144.58±14.929	169.45±50.581	124.78±15.075	110.60±36.966
Urea (mg%)	71.85±12.390	79.15±12.267	53.76±10.508	59.00±19.668
Uric Acid (mg%)	0.67±0.120	0.63±0.177	0.64±0.103	0.74±0.305
Creatinine (mg%)	0.82±0.064	0.84±0.144	1.19±0.149	1.16±0.153
Calcium (mg%)	16.71±1.677	16.69±0.717	14.56±1.065	15.43±1.517
Phosphorus (mg%)	3.76±0.267	3.89±0.642	4.68±0.869	4.56±0.677
Triglyceride (mg#)	93.14±60.453	119.21±28.418	93.29±26.359	101.33±27.849

**Table 5.5 Average Body Weight (gms) and Absolute organ weight (gms) Values,units (Mean± SD).**

Organs	Group I	Group II	Group III	Group IV
Body Weight	2301.67±177.473	2330.00±127.436	2250.00±120.333	2407.50±226.578
Heart	7.140±2.109	7.222±0.614	8.542±2.262	7.538±0.882
Liver	69.297±12.363	76.825±10.310	74.948±6.612	80.410±9.908
Kidney	12.508±2.106	15.383±2.482	12.947±1.245	13.592±1.795
Adrenal	0.362±0.144	0.343±0.109	0.313±0.079	0.313±0.049
Spleen	1.088±0.204	1.182±0.318	1.505±0.571	1.502±0.568
Uterus	6.983±3.211	6.698±0.844	5.095±3.794	6.443±3.990
Ovaries	0.283±0.078	0.330±0.139	0.377±0.171	0.288±0.057

**Table 5.6 Average Relative Organ Weight Values (Mean± SD).**

Organs	Group I	Group II	Group III	Group IV
Heart	0.31±0.074	0.31±0.035	0.38±0.095	0.32±0.059
Liver	3.01±0.490	3.31±0.525	3.34±0.359	3.35±0.381
Kidney	0.54±0.070	0.66±0.113	0.58±0.066	0.57±0.082
Adrenal	0.02±0.007	0.01±0.005	0.01±0.004	0.01±0.002
Spleen	0.05±0.008	0.05±0.016	0.07±0.023	0.06±0.019
Uterus	0.30±0.127	0.29±0.040	0.22±0.160	0.26±0.144
Ovaries	0.01±0.004	0.01±0.006	0.02±0.007	0.01±0.002

**Table 5.7 Summary of Gross and Histopathological Findings**

Observations	Group I	Group II	Group III	Group IV
	0%	1%	2%	4%
	F	F	F	F
	6	6	6	6
<b>Liver:</b>				
Peri-portal MNC infiltration	2	X	X	2
Focal Hepatocyte Degeneration	1	X	X	1
<b>Thymus:</b>				
Atrophy	1	X	X	0
<b>Kidney:</b>				
Focal Tubular Regeneration	2	X	X	2

Key: MNC = Mononuclear cell infiltration,

**Summary and Discussion**

In the present study, all the animals belonging to control group and treated groups did not show any abnormal clinical signs.

There was no mortality throughout the study. There was no significant decrease in weekly body weight, hematological parameters, clinical chemistry values, terminal body weight, absolute and relative organ weight between control and treatment group.

The lesions observed in the histopathology were spontaneous/ incidental findings and not due to treatment as these lesions were also present in control and well comparable between control and high dose groups. After summarizing all the observations, the test substance did not produce any treatment related adverse effect during 28 days of the study.

**4. Dermal toxicity in rats****Study Objective:**

To determine Sub-acute dermal toxicity of KHS/CCPRASPVK/09 in rats and identify target organs involved for establishing its safety in humans.

**Guideline:**

The study was carried out as per Schedule Y

**Summary**

Two groups of six males and six females each of Holtzman rats were applied with 500 mg of 0 % and 2 % of test substance by dermal application for 28 days and were sacrificed 24 hrs. after the application of last dose. The rats were examined daily for signs of toxicity. Body weight and food consumption were recorded during the experimental period along with the incidence of mortality and signs of ill health. Laboratory investigations were performed on blood at termination of the study. All animals, sacrificed at termination of the study were subjected to complete necropsy and weights of organs were recorded. The results were evaluated statistically using Students 't' test.

Histopathological evaluation was performed on the tissues listed in the protocol in all rats belonging to control and high dose group.

No clinical signs and symptoms of toxicity were observed in animals receiving test substance. Test substance did not induce any adverse effect on food intake and weekly body weight in control and treatment groups. Data on hematological parameters and biochemical parameters revealed no adverse effect upon treatment and all values were within normal range as compared to control. Necropsy and histopathological analysis along with absolute and relative organ weight showed no toxicity related changes.

The result of the present study demonstrated that KHS/CCPRASPVK/09 does not cause any observable toxicity at doses used in the study when administered for the period of 28 days.

### **Materials and Methods:**

#### **Test Article Details:**

Test substance	: KHS/CCPRASPVK/09
Batch/lot number	: July 09
characteristics	: Gel form
Sponsor	: Kasturaba Health Society

KHS/CCPRASPVK/09 is an ayurvedic preparation in gel form. The intended route of administration is Intra vaginal application.

The formulation was stable at room temperature and it was kept in plastic container at room temperature.

#### **Test Article Administration:**

1. Route: Dermal Application
2. Dose was directly applied daily on shaved skin. The test substance was applied as thin as possible to form a uniform film covering approximately 10 per cent of the total body surface area.

**Test Animals:**

Holtzman strain rats bred in the animal house facility of NIRRH were used in the study. Total 24 animals together with 12 males and 12 females of 10-12 weeks of age were randomly selected and assigned to the control and treatment group after the acclimatization of five days prior to the start of the study. The weight variation of animals used did not exceed  $\pm 20\%$  of the mean weight of each sex.

**Animal Husbandry:**

The rats were housed in polypropylene cage containing autoclaved corn cobb as bedding material that was replaced on a weekly basis. Throughout the study, rats were provided with soy-free, in-house-prepared rat pellets (consisting of crude protein, fiber and nitrogen free extract) prepared at the institute and filtered drinking water (purified by UV and reverse osmosis), ad libitum. All the animals were maintained at the controlled temperature of  $23 \pm 10^\circ\text{C}$ , humidity of  $55 \pm 5\%$ , in a 14 h light/10 h dark cycle.

**Study Design:**

Animals were randomized before acclimatization and divided in a test group and a control group. The therapeutic dose (TD) was taken as high dose. All the animals were handled in an identical manner.

All the animals belonging to control and treatment group were gavaged with vehicle and test substance respectively over a period of 28 days including holidays. The Grouping was as Table 5.8.

**Table5.8.** Grouping of the animals:

<b>Gr. No.</b>	<b>Groups</b>	<b>Dose</b>	<b>Number of Males</b>	<b>Number of Females</b>
1	Control	0%	6	6
2	Treatment	2%	6	6

During the treatment period, the animals were daily examined for avert clinical signs, morbidity, and mortality, if any. The body weight and food consumption were recorded weekly throughout the dosing period.

**Figure 5.4** Dermal Application sites in rats



**Hematological and Biochemical Analysis:**

At the end of the treatment, the animals were bled from the orbital sinus for clinical pathology assessment which included analysis of various hematology parameters. Hematological analysis was performed using an automatic hematological analyzer Abacus (Diatron). The parameters included: Red Blood Cell (RBC) Count, White Blood Cell (WBC) Count, Hemoglobin (Hb), Hematocrit (Hct), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Platelets count. The differential leukocyte counting was performed with an optical microscopy after staining with Leishman's stain (Sigma-Aldrich) and, in each case, 100 cells were counted. For biochemical analysis, blood was centrifuged to obtain serum, which was stored at  $-20^{\circ}\text{C}$  until determination of the following parameters viz. Total Protein, Albumin, Globulin, Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT), Cholesterol, Alkaline Phosphatase, Glucose, Creatinine, Urea, Uric Acid, Triglycerides, Bilirubin (Total And Direct), Calcium and Phosphorous. The serum biochemistry was performed by using fully automated serum biochemistry analyzer- EM 200 (ERBA) with biochemical kits from Spinreact, S. A. Ctra Santa Coloma, Spain.

**Gross Pathology, Organ weight, and Histopathology:**

After completion of dosing period, the animals were euthanized using  $\text{CO}_2$  chamber and necropsied for the gross evaluation of the various organs. The necropsy also included careful and consistent dissection of various target organs like heart, liver, spleen, kidneys, intestine and stomach, determination of absolute organ weight, and calculation of organ weight to body weight ratios (Percent Relative Organ Weight). Finally, the dissected tissues were fixed in 10% neutral buffered formalin, processed (Tissue processor Leica ASP300), and embedded (Paraffin Embedder Leica EG1150 H) in paraffin wax. Sections ( $5\ \mu\text{m}$ ) (Fully Automated Rotary Microtome Leica RM2255) of these tissues taken on glass-slides were stained using a combination of hematoxylin-eosin before observing under a microscope for histopathological evaluations.

**Statistical Analysis:**

For all the toxicological evaluations, the results of the treatment groups were compared with those of the control group. Data was expressed as mean  $\pm$  S.D. and was analyzed by two-tailed Student's t-test. Differences were considered significant at  $P < 0.05$ .

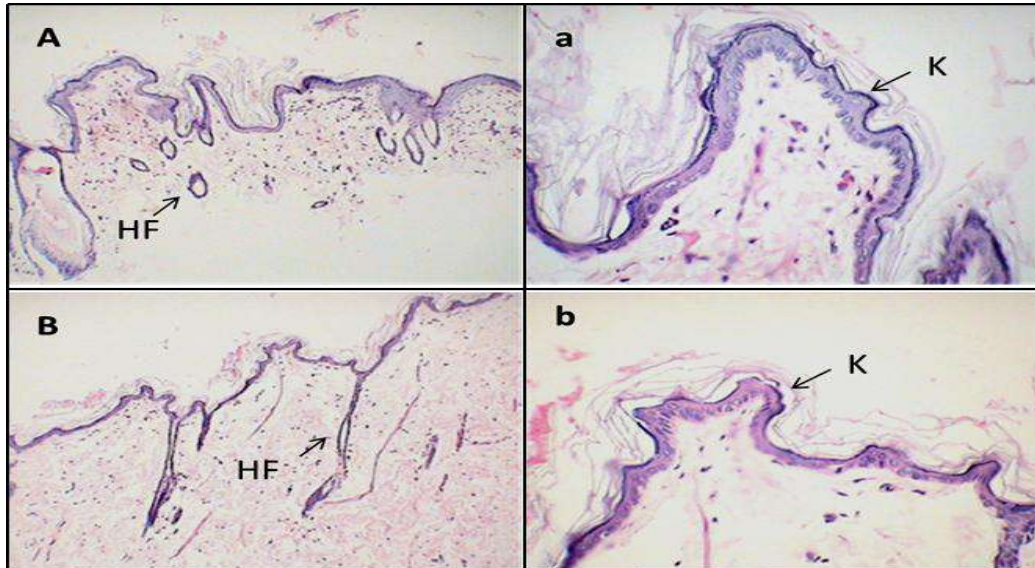
**Results:**

All the animals showed normal behavior throughout the study. No mortality was observed in control as well as treated groups during the period of 28 day. There was no significant difference in the weekly feed consumption between treatment group and that of control in either sex. The mean weekly feed consumption is given in Table 5.9. No significant difference was observed in the weekly body weight between three treatment groups and that of control. The mean weekly body weight is given in Table 5.10.

In males and females there was no significant difference in various hematological parameters and clinical chemistry as compared to control and treatment group. Average hematological values and biochemical values were given in Table 5.11 and 5.12 respectively.

There were no significant difference in terminal body weight in males as well as females of treated groups and control group. Also there was no difference in absolute organ weight and relative organ weight in either sex; the values are given in table 5.13 and 5.14. Terminally sacrificed animals did not show any gross pathological findings. Histopathological findings revealed various lesions like; liver: Periportal MNC infiltration, Focal Hepatocyte degeneration, kidney: protein casts in tubules, focal tubular regeneration; Lung: hyperplasia of BALT, Bronchopneumonia. Application site did not show any toxicity related changes in either sex figure 5.5 and 5.6. All the lesions are summarized in Table 5.15. All the lesions observed were incidental / spontaneous findings and these were not related to the test substance and well comparable to historical control.

**Figure 5.5** Histology of skin from application site (A: Control 0% at 100x, a: Treated 2% at 400x, B: Control 0% at 100x, b: Treated 2% at 400x) Key: HF: Hair follicle, K: keratin.



**Table 5.9** Mean of weekly Feed Intake Values (Mean± SD).

Sex: Male

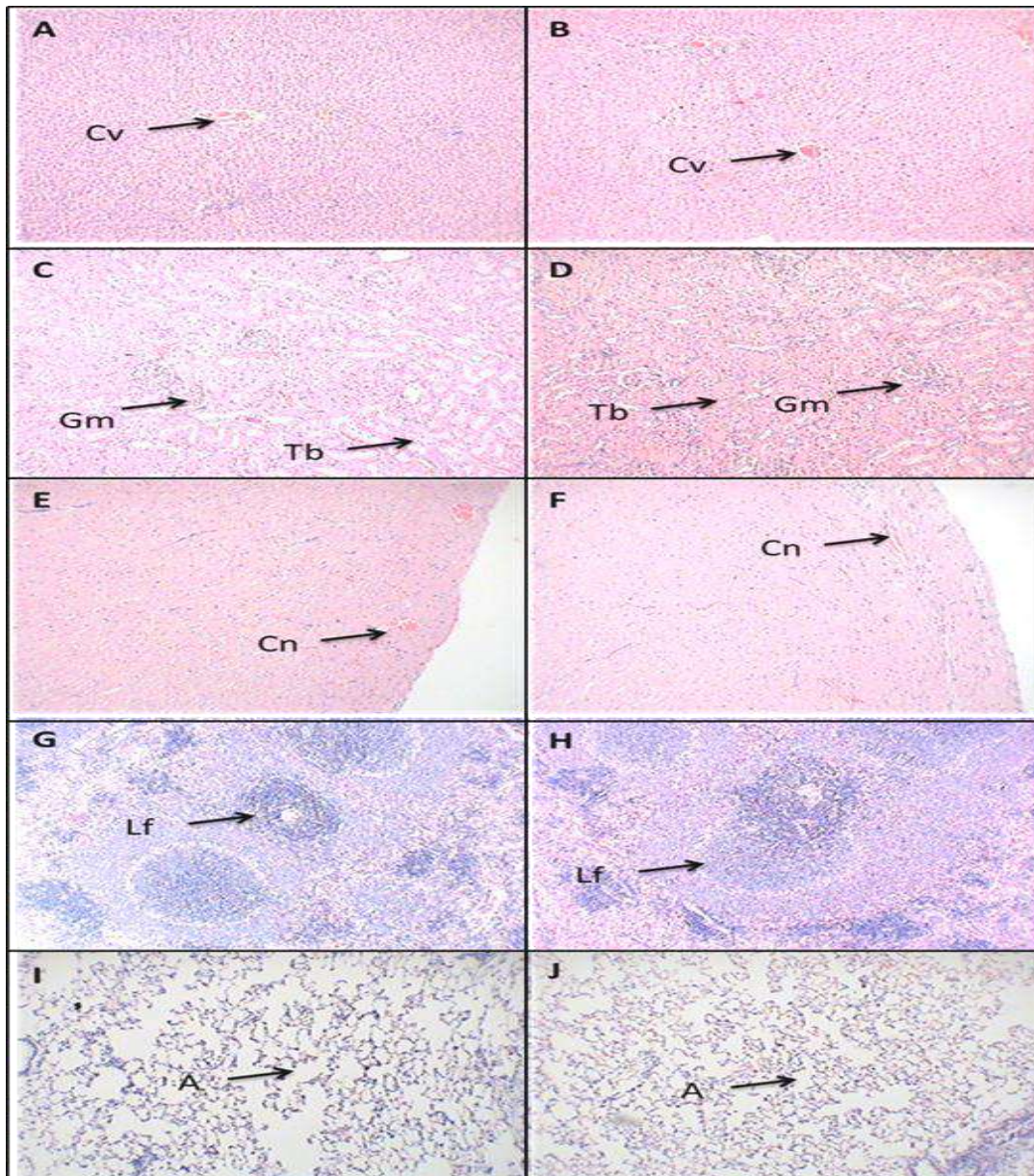
Week	Group I	Group II
1st week	111.417±6.346	110.700±7.558
2nd week	118.033±6.136	117.367±10.701
3rd week	126.400±7.891	112.733±13.771
4th week	124.500±7.517	107.667±26.080

Sex: Female

Week	Group I	Group II
1st week	76.100±1.937	73.067±1.626
2nd week	85.600±4.831	81.267±0.896
3rd week	87.067±4.772	84.333±3.246
4th week	86.100±4.802	85.200±2.943

Values (Mean± SD).

**Figure 5.6 :** Vital organ histology from rat dermal study (A: Liver control 0%, B: Liver treated 2%, C: Kidney control 0%, D: Kidney treated 2%, E: Heart control 0%, F: Heart treated 2%, G:Spleen control 0%, H: Spleen treated 2%, I:Lung control 0%, J: Lung treated 2%) key: Cv: Central vein, Gm: Glomeruli, Tb: Tubule, Cn: Congestion, Lf: Lymphoid Follicle, A: Alveoli



**Table 5.10 Mean weekly Body weights Values (Mean± SD).**

Sex: Male

<b>Week</b>	<b>Group I</b>	<b>Group II</b>
<b>0 th day</b>	400.667±19.541	387.000±32.044
<b>1st week</b>	395.000±20.425	382.667±41.941
<b>2nd week</b>	415.000±21.643	398.667±51.531
<b>3rd week</b>	423.333±22.651	401.667±63.162
<b>4th week</b>	429.000±24.158	425.500±32.995

Sex: Female

<b>Week</b>	<b>Group I</b>	<b>Group II</b>
<b>0 th day</b>	260.667±28.835	243.333±16.379
<b>1st week</b>	257.667±28.821	242.167±18.830
<b>2nd week</b>	276.000±26.952	262.333±13.125
<b>3rd week</b>	277.000±25.853	265.333±12.565
<b>4th week</b>	279.000±27.590	265.333±12.817

**Table 5.11 Average of Hematological Parameters Values (Mean± SD).**

Sex: Male

Parameter	Group I	Group II
Hamoglobin	16.57±0.937	15.27±0.787
RBC	11.60±0.769	10.85±0.584
PCV	53.03±3.102	49.03±3.422
MCV	45.67±1.211	44.00±2.098
MCH	14.23±0.301	14.05±0.432
MCHC	31.20±0.727	31.10±0.841
WBC	8.89±2.085	12.585±4.310
Lymphocytes	77.17±3.869	75.33±2.875
Neutrophils	19.67±3.983	21.00±2.366
Monocytes	1.83±0.753	2.00±0.894
Platelets	640.50±223.463	636.17±122.196

Sex: Female

Parameter	Group I	Group II
Hamoglobin	14.92±0.508	15.18±0.643
RBC	9.73±0.232	10.06±0.409
PCV	47.81±1.488	49.00±2.270
MCV	49.00±1.414	48.50±0.837
MCH	15.30±0.297	15.03±0.398
MCHC	31.15±0.596	30.92±0.757
WBC	6.58±3.041	7.62±1.987
Lymphocytes	77.00±2.828	76.67±3.204
Neutrophils	19.50±3.619	20.33±3.204
Monocytes	2.17±0.753	1.67±0.816
Platelets	698.17±196.496	556.00±284.998

**Table 5.12 Average of Biochemical Parameters Values (Mean± SD) ,** Sex: Male

Parameters	Group I	Group II
Total protein	7.783±0.248	7.400±0.759
Albumin	3.883±0.313	3.083±0.454
Globulin	3.900±0.456	4.317±0.492
SGPT	133.150±35.324	114.433±61.027
SGOT	259.667±31.420	248.467±59.260
ALP	456.917±61.582	430.700±72.814
Bilirubin	0.350±0.105	0.317±0.172
Direct Bilirubin	0.233±0.052	0.233±0.186
Cholesterol	125.717±8.431	111.283±28.610
Glucose	108.317±14.547	94.767±12.238
Urea	37.100±2.037	39.683±3.293
Uric Acid	1.817±0.512	1.367±0.821
Creatinine	0.833±0.103	0.833±0.137
Calcium	10.650±0.838	9.517±1.169
Phosphorus	0.833±0.103	0.833±0.137
Triglyceride	196.517±48.283	192.567±49.432

Sex: Female

Parameters	Group I	Group II
Total protein	7.517±0.440	7.183±1.036
Albumin	3.417±0.147	3.700±0.490
Globulin	4.100±0.494	3.483±0.655
SGPT	74.333±6.519	69.683±13.009
SGOT	201.567±33.026	191.850±41.046
ALP	373.333±110.741	319.033±71.179
Bilirubin	0.267±0.103	0.367±0.137
Direct Bilirubin	0.200±0.110	0.300±0.089
Cholesterol	127.017±18.182	131.550±11.339
Glucose	82.033±26.142	79.817±12.975
Urea	35.767±3.993	40.083±4.366
Uric Acid	1.900±0.537	2.267±0.797
Creatinine	0.917±0.075	0.783±0.133
Calcium	12.183±3.129	11.083±1.238
Phosphorus	7.300±4.512	6.050±3.831
Triglyceride	120.850±40.122	129.500±39.775

**Table 5.13 Average Body Weight and Absolute organ weight Values (Mean± SD).**

Sex: Male

<b>Organs</b>	<b>Group I</b>	<b>Group II</b>
<b>Body Weight</b>	429.000±24.158	425.500±32.995
<b>Heart</b>	1.582±0.209	1.535±0.170
<b>Liver</b>	14.533±1.619	13.922±1.703
<b>Kidney</b>	2.957±0.207	2.950±0.443
<b>Adrenal</b>	0.154±0.018	0.163±0.003
<b>Spleen</b>	0.942±0.136	0.948±0.085
<b>Brain</b>	1.990±0.237	1.950±0.233
<b>Testis</b>	3.863±0.329	3.560±0.615
<b>Epididymis</b>	1.707±0.210	1.729±0.212

Sex: Female

<b>Organs</b>	<b>Group I</b>	<b>Group II</b>
<b>Body Weight</b>	279.000±27.590	265.333±12.817
<b>Heart</b>	1.108±0.155	1.263±0.210
<b>Liver</b>	8.066±1.150	8.393±0.671
<b>Kidney</b>	1.997±0.186	2.018±0.104
<b>Adrenal</b>	0.136±0.022	0.134±0.020
<b>Spleen</b>	0.764±0.132	0.662±0.081
<b>Brain</b>	2.142±0.288	1.907±0.084
<b>Ovaries</b>	0.315±0.076	0.248±0.062
<b>Uterus</b>	1.550±0.227	1.480±0.256

**Table 5.14 Average Relative Organ Weight Values (Mean± SD).**

Sex: Male

<b>Organ</b>	<b>Group I</b>	<b>Group II</b>
<b>Heart</b>	0.370±0.055	0.360±0.025
<b>Liver</b>	3.381±0.221	3.264±0.185
<b>Kidney</b>	0.689±0.031	0.693±0.088
<b>Adrenal</b>	0.036±0.005	0.039±0.003
<b>Spleen</b>	0.219±0.028	0.223±0.016
<b>Brain</b>	0.465±0.057	0.462±0.071
<b>Testis</b>	0.901±0.057	0.832±0.090
<b>Epididymis</b>	0.398±0.043	0.407±0.046

Sex: Female

<b>Organ</b>	<b>Group I</b>	<b>Group II</b>
<b>Heart</b>	0.397±0.030	0.477±0.080
<b>Liver</b>	2.896±0.347	3.174±0.344
<b>Kidney</b>	0.716±0.026	0.762±0.053
<b>Adrenal</b>	0.049±0.009	0.050±0.008
<b>Spleen</b>	0.273±0.027	0.250±0.033
<b>Brain</b>	0.769±0.076	0.720±0.038
<b>Ovaries</b>	0.113±0.025	0.094±0.023
<b>Uterus</b>	0.561±0.102	0.562±0.116

**Table 5.15 Summary of Gross Histopathological Findings**

Observations	Group I		Group II	
	0%		2%	
	M	F	M	F
	6	6	6	6
<b>Liver:</b>				
Focal Hepatocyte degeneration	1	0	0	0
Periportal MNC infiltration	0	0	1	1
<b>Kidney:</b>				
Focal Tubular degeneration	1	0	0	0
Protein cast in tubules	1	0	0	0
<b>Lung:</b>				
Hyperplasia of BALT	2	4	1	2
Bronchopneumonia	0	2	0	2

Key: MNC = Mononuclear cell infiltration, BALT = Bronchiole associative Lymphoid tissue

### 5.Summary and Discussion

In the present study, all the animals belonging to control group and treated groups did not show any abnormal clinical signs. Weekly feed did not vary throughout the 28 day.

There was no mortality throughout the study. There was no significant decrease in feed consumption, weekly body weight, hematological parameters, clinical chemistry values, terminal body weight, absolute and relative organ weight between control and treatment group.

The lesions observed in the histopathology were spontaneous/ incidental findings and not due to treatment as these lesions were well comparable between control and high dose

groups. After summarizing all the observations, the test substance did not produce any treatment related adverse effect during 28 days of the study.

### **5. Summary and Conclusions:**

Four groups each consisting of six females of New Zealand white rabbits were applied 500 mg of 0 %, 1%, 2 % and 4% of test substance by intra vaginal application for 28 days and were sacrificed 24 hrs after the application of last dose. The rabbits were examined daily for signs of toxicity. Body weight was recorded during the experimental period along with the incidence of mortality and signs of ill health. Laboratory investigations were performed on blood at termination of the study. All animals, sacrificed at termination of the study were subjected to complete necropsy and weights of organs were recorded. The results were evaluated statistically using Students 't' test. Histopathological evaluation was performed on the tissues listed in the protocol in all rabbits belonging to control and high dose group.

No clinical signs and symptoms of toxicity were observed in animals receiving test substance. Test substance did not induce any adverse effect on weekly body weight in control and treatment groups. Data on hematological parameter and biochemical parameters revealed no adverse effect upon treatment and all values were within normal range as compared to control. Necropsy and histopathological analysis along with absolute and relative organ weight showed no toxicity related changes.

The result of the present study demonstrated that KHS/CCRASPVK/09 does not cause any observable toxicity at doses used in the study when administered for the period of 28 days.

The results of the present study demonstrated that KHS/CCPRASPVK/09 does not cause any observable dermal toxicity in rats at doses used in the study when administered for the period of 28 days. It is known that vaginal application of Nonoxynol -9, a common spermicide and microbicide, and transdermal and vaginal application of contraceptive steroids can get absorbed but are nontoxic at the marketed doses. It was therefore

reassuring that there was no local as well as systemic toxicity in rats following dermal application of this Ayurvedic vaginal cream.

Lastly, vaginal route of application is used for local diseases so that higher local concentrations are available. However it can also be used for systemic therapy to avoid the first pass effect due to intestinal or liver metabolism as in case of contraceptive steroids. Thus systemic pharmacological effect or toxicity may result sometimes from local application also. The authors have some experience of vaginal drug delivery systems (Shikarey ZK, Joshi JV et al, 1987, Vaidya RA et al) for contraceptive steroids. Recently the vaginal route has also been explored as local and systemic pharmacotherapy of Hormone Replacement Therapy (HRT) (Luengratsameerung et al, 2013).

There was no systemic toxicity from local application or any possible absorption. This was reassuring in view of the reports of absorption in blood of a commonly used vaginal spermicidal drug Nonoxynol -9 (Chvapil et al, 1980). Occasionally allergic rash or reaction is reported in males after sexual intercourse with a female who has applied a vaginal cream (Zargooshi et al, 2012). No dermal toxicity in male or female rats or in rabbits was observed after 28 days local application and the possibility of male partners developing local reaction due to sexual intercourse with females with vaginal PVK cream application was excluded in the rats and rabbits.

The most important part of the study was also demonstration of local, systemic, hematologic and organ safety in all animals in the two species studied over 28 days with the PVK cream. One group of rats was additionally studied 2 weeks post therapy and did not show any delayed effects.

**Chapter 6.****Clinical study of safety and activity of A) PVK vaginal cream (Group 1) and B) Control cases treated with standard allopathic treatment (Group 2) in uncomplicated leucorrhoea**

*Joshi JV, Jagtap S, Paradkar PH, Walwatkar P, Agashe S, Vaidya RA*

- 1. Introduction**
- 2. Treatment schedules :**
  - a) PVK Vaginal cream + Oral Ayurvedic Formulation**
  - b) Treatment schedule of control cases**
- 3. Subject selection**
- 4. Investigations**
- 5. Results & Discussion**
- 6. Future Research**

**1. Introduction**

This second CCRAS supported clinical study was based on the Reverse Pharmacology principle to develop an Ayurved inspired, user friendly formulation for treatment of RTIs in women, and to evaluate its effectiveness and tolerability in uncomplicated leucorrhoea. Apart from information in Ayurvedic texts or students' dissertations on PVK kwath there was no published or documented evidence on the clinical effects of PVK kwath on symptoms, signs and no objective evaluation was available. Postgraduate University dissertations by MD Ayurved students have shown effectiveness in a very subjective manner. Dr Ranjan Bhatt's studies in vitro clearly established that the Modified PVK with *Albizia lebbek* instead of *Calamatus rotang* was more effective against organisms commonly affecting the cervix and vagina in women. However there was no published clinical evidence to support the laboratory findings. Hence the clinical evaluation of the Modified PVK, formulated as a traditional kwath, with 5 plants in equal proportions was first carried out by us (1999-2002) in collaboration with Ayurvedya Prasarak Mandal's Ayurved Mahavidyalaya, Sion (APM's AMS) (Chapter 3 in this monograph).

In this initial CCRAS project objective methods of evaluation like gynecologic cytology, vaginal pH and colposcopy were used for the first time for Ayurvedic treatment of leucorrhoea (Joshi et al, Journal of NIMA, 2003; Indian Journal of Cytology, 2004). More than 85 % of women showed improvement or total relief from symptoms. Correlation with prakriti and comparison with parallel control standard treatment group was also carried out in subsets (Chapter 3).

Subsequently we developed the user friendly Vaginal PVK cream (Chapter 4), and after preclinical evaluation (Chapter 5), this second clinical study on Panchavalkal was initiated in women with leucorrhoea as the presenting symptom. This project was supported by CCRAS apart from the infrastructure support from the Kasturba Health Society's Medical Research Centre and Ayurvedya Prasarak Mandal's Ayurved Mahavidyalaya, Sion which collaborated for the study. In this study not only was the safety and clinical activity of the cream was confirmed but also partial or complete relief of symptoms was seen in more than 90% of cases. Similarly more than 90% of cases were relieved of symptoms also in the control group showing equivalent symptom relief in PVK and control group, and the results are encouraging. We had earlier shown similar results with the PVK kwath and allopathic control group as discussed in Chapter 3.

A vaginal formulation/ cream or pessary has the advantage over the local douche with kwath that a women does not have to visit the clinic for daily application and she can apply it on her own at her residence. The cream also has a better shelf life, can be kept at home and need not be prepared fresh every day. Moreover it remains in contact with the vaginal mucosa for a longer time. The compliance is better and a larger proportion of women can be protected against complications of Reproductive Tract Infections (RTIs). The results of the study show that it is possible to formulate an effective standardised vaginal formulation with Panchavalkal extracts so that user friendly Ayurvedic formulation can be available to the masses. This is therefore a traslational research application as per the needs of the National Research programme including the Indian Council of Medical Research.

**Complementary Oral Formulation:** As per the principles of Ayurveda leucorrhoea also has a systemic component hence in this study oral tridoshahara therapy was added after 2 weeks of local therapy with vaginal cream. Ideally the systemic therapy should have been added from the beginning (first day of treatment) but we wished to know the clinical side effects and tolerance as well as the local effect of the PVK vaginal cream alone hence the oral formulation was added only after 2 weeks and given for a period of 2 weeks.

**Objectives:**

- a) Study of the tolerability, effectiveness and safety of PVK vaginal formulation applied for 10-12 days followed by 2 weeks of systemic oral formulation in cases with leucorrhoea (Group 1)
- b) Compare the same with a group of leucorrhoea cases treated with standard allopathic therapy with forcanazole + azithromycin + secnidazole single dose followed by anticonorrhoeal dose of Cephadroxyl in women with persistent endocervicitis or erosion after 2 weeks (Group 2)

**Type of study:**

It was decided before starting the project that in view of the different systems of medicine, different routes of administration and different dose schedules a randomised blind study was not possible. Women were enrolled from two centres, an Ayurvedic General Hospital and from Kasturba Health Society's Medical Research Centre in Mumbai. The study was open labeled and non-randomised but both Groups 1 and 2 had identical criteria for selection of cases, investigations for efficacy and safety, and outcome measures.

**Consideration of Ethics :** The project was approved by Independent Inter System Biomedica Ethics Committee (ISBEC) (Currently registered with DCGI for BA/BE studies).

## 2. Test treatment schedule : PVK Vaginal cream + Oral Ayurvedic Formulation

**a. PVK Vaginal cream local treatment:** The KHS/CCRASPVK/09 cream (35 gms per tube), standardized and tested as in Chapter 4 and tested preclinically as in Chapter 5 was preserved at room temperature in a cool dry place in the clinic and same instructions were given to the patients. They were advised to keep it out of reach of children's hands.

**Method of application:** 3 gms of the cream were measured and shown to each woman separately on the index finger stall on the first day of application which was demonstrated to them. Thereafter they applied the cream at home twice daily, after bath in the morning and before retiring for sleep in the night. The women were provided with 24 disposable finger stalls (used by doctors for conducting a digital per rectal examination) so that they could apply the cream with ease and hygienically. They were informed about the importance of proper application and washing their hands with soap and water after the application. It has been our experience that many women from the lower socioeconomic groups and sometimes even from high socioeconomic group do not use the vaginal applicators comfortably and are afraid of self injury, however this easy hygienic method of using disposable finger stalls was acceptable to all. Moreover the addition of the applicator increases the cost of production and also increases the biowaste.

They were advised to stop application 2 days before the follow up gynecological examination was due so that the Pap smears, vaginal pH and colposcopy results were not affected by the prior use of cream.

**Oral formulation:** As mentioned earlier a standardized oral formulation, specially prepared in consultation with 3 senior Ayurvedic gynecologists and 3 Clinical pharmacologists, was supplied by Charak Pharmaceuticals Pvt Ltd for use for 2 –4 weeks after the local application of the vaginal cream. The composition of the oral capsules was as follows: Guduchi extract (*Tinospora cordifolia*) 250 mg + Triphala extract (*Terminalia chebula, Terminalia belerica, Emblica officinalis*) 175 mg + Trikatu extract

(*Zingiber officinale*, *Piper longum*, *Piper nigrum*) 75 mg per capsule.

**Dose:** 2 capsules twice daily after meals for 2 to 4 weeks after completion of the vaginal cream application for 12 days and gynecologic examination at 14 days.

The formulation was prepared by Charak Pharmaceuticals which is recognized by the regulatory authorities as per the GMP guidelines of WHO and CCRAS.

#### **b. Treatment schedule of control cases**

Women in Control Group 2 were treated as per the updated National guidelines (NIRRH) and CDC guidelines, 2006. It may be noted that in the comparative pilot study in 2002 reported in Chapter 3 the CDC and National guidelines of 1998 were followed whereas in the recent study the CDC and National guidelines of 2002 and 2006 were followed.

As in the study with the PVK Kwath in Chapter 3 women with positive serum HIV or TPHA test for syphilis and women suspected of having PID were excluded from the project. The reason for this was that the presence of either disease required a totally different and intensive line of treatment and could not have been advised the use of Panchavalkal cream alone or the syndromic treatment of leucorrhoea.

Syndromic treatment of leucorrhoea was given as follows:

Treatment of control group was as follows

- a) Forcanazole 150 mg single dose after dinner
- b) Azithromycin 1 gm single dose prior to lunch
- c) Secnidazole 2 gms single dose after dinner

**Partner treatment:** All drugs were to be taken by both, husband and wife. Drugs were to be stored at room temperature and out of reach of children.

Persons with reduced gastrointestinal tolerance to allopathic drugs were advised to take the medicines in a 2 or 3 day schedule rather than in a single day. This is a standard practice which we follow and may be the reason why gastrointestinal side effects were minimal in the control group also.

d) Women with persistent erosion and discharge at 2 weeks were also treated with Cephadroxyl 500 mg BD for presumptive gonorrhoea, along with their partners.

**Contraception:** Majority of women had undergone tubal ligation (>90%) or their husbands used condoms for contraception. All women were informed of the need to abstain during the study period and all agreed and complied with the same.

**Partner Treatment:** All husbands were contacted either at the first or second clinic visit when they accompanied their wives or telephonically during the clinic visit by the woman volunteer. Majority of husbands also signed the volunteer consent form as witnesses. All husbands were prescribed allopathic syndromic treatment for presumptive STI (except for few cases who were widows or separated). All were asked about history of allergy to allopathic drugs, concurrent medical treatment, drug compliance, and about side effects after treatment.

Husbands of women in Group 1 (Panchavalkal) were also treated with syndromic single dose treatment.

**3. Subjects and methods:** Subject selection criteria were similar to those used in the earlier study with the PVK kwath (Chapter 3) and are detailed below:

**Selection criteria for both groups:****Inclusion criteria :**

- 1) History of chronic leucorrhoea or itching of more than 6 weeks duration
- 2) Willingness to use PVKV vaginal formulation alongwith systemic Ayurvedic treatment as the only method of treatment during the study period of 6 weeks in group 1 and willingness to comply with allopathic treatment in Group 2
- 3) Willingness to come to the hospital once a week for a minimum of 1 month
- 4) History of tubal ligation or consistent condom use for contraception
- 5) Abstinence from sexual intercourse during study period of minimum of 2- 4 weeks (or husband willing to use condoms during study period)
- 6) Age between 20 and 50 years
- 7) Informed written consent

**Exclusion criteria:**

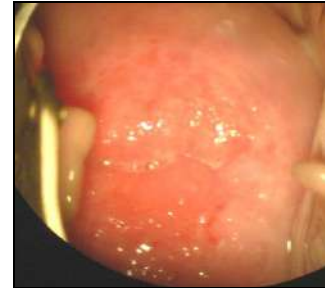
- 1) Pelvic Inflammatory Disease (PID) using standard criteria- low abdominal pain, leucorrhoea, rebound abdominal tenderness, dyspareunia, tenderness of uterus or fornices, fever, leukocytosis (at least 3 criteria positive).
- 2) Abnormal colposcopy (Fig 6.1)
- 3) Abnormal Papanicolaou smear with intraepithelial or invasive cancer (Fig.6.2)
- 4) Unexplained vaginal bleeding
- 5) Abnormal findings on pelvic Sonography
- 6) Pregnancy
- 7) Fibromyoma, polyp, ovarian tumour or cyst, prolapse
- 8) Systemic disease like hypertension, diabetes, heart disease, tuberculosis
- 9) Severe anaemia ie Hemoglobin < 8 gms%
- 10) Use of antibiotics or local vaginal formulation within the previous month
- 11) Endocervical smear positive for gonococci, or positive VDRL or HIV test

**Figure 6.1. Colposcopy in a case with a) endometrial polyp b) Intraepithelial Neoplasia**

a)



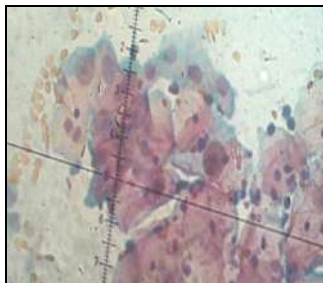
b)



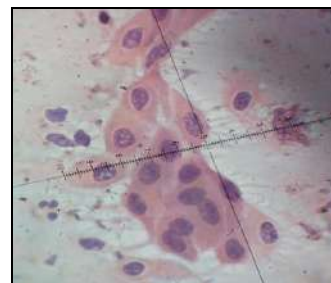
*Both cases were excluded from study*

**Figure 6.2. Pap smears a) Low Grade Squamous Intraepithelial Neoplasia b) High Grade Squamous Intraepithelial Neoplasia**

a)



b)

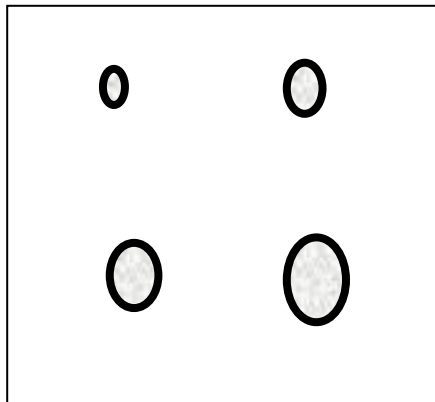


*Both cases were excluded from study*

**4. Investigations:****Clinical Investigations:**

- i) A Case Record form was designed and used after the informed consent at initial examination and women were advised to come at 2 weekly intervals for history and symptomatology.
- ii) The primary symptom of leucorrhoea was scored semiquantitatively as in the previous study.

**Figure 6.3. Leucorrhoea symptom scoring by semiquantitative diagrammatic method**



*( Adapted from Joshi et al, 2004)*

- iii) Other symptoms like itching were scored as absent (Score 0), mild (Score 1), Moderate (Score 2), or Severe (Score 3)
- iv) Weight and Blood Pressure were recorded initially and every 2 weeks
- v) Systemic and gynecological check up initially and every 2 weeks
- vi) Ectocervical and endocervical Pap smears with disposable sterile spatula and brush on 2 slides, initially and every 2 weeks followed by colposcopy

vii) Colposcopy with 5% acetic acid application was carried out when indicated. The size of the erosion was recorded in pictorial diagram indicating the percentage (%) area of ectocervix covered by the erosion. Erosions were classified as small (<25% area), moderate (25 to 50 %) and large (>50%) area covered on ectocervix.

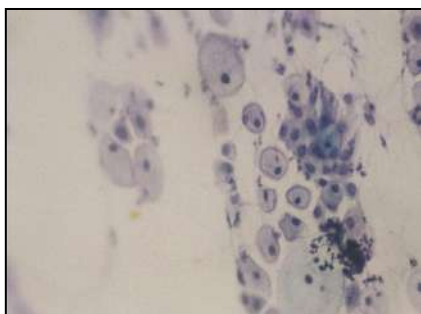
viii) Vaginal pH with Qualigens pH paper applied to the other end of spatula inserted in the anterior vaginal fornix, initially and every 2 weeks (Garcia et al,1999).

#### Laboratory Investigations:

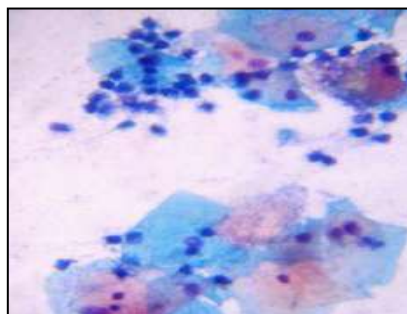
**i) Papanicolaou smear :** Collection with a presterilised spatula and brush on 2 slides, assessed by Bethesda system and as we have reported earlier (Bethesda 2001 , Bethesda 2003a, b, Joshi et al,1991, 1994, 1996, 2004, 2007) with micrometry in selected cases. Whilst Bethesda System in 1993 was followed for earlier analysis of Pap smears (Chapter 3) for this study the New Bethesda System (2001) was followed (Figure 6.4). Cytological manifestations of infections were noted as described earlier and in Bethesda System , 2001.

**Fig 6.4. Cytological manifestations of STIs, a) *HPV* infection and b) *Trichomonas vaginalis***

***HPV* Koilocytosis**



**b) *Trichomonas vaginalis***



**ii) Blood tests (organ function tests):** Complete blood count (CBC), Random blood sugar (RBS), Total Cholesterol, ASAT, ASLT, Total proteins, Creatinine, VDRL, HIV done in at least 20 cases from each group. The laboratory has an autoanalyzer and an external quality control program in place.

**iii) Serum Interleukin-6 (IL-6) levels:** using Enzyme Linked Immunosorbent Assay, ELISA ((Biosource IL-6 EASIA kit; KAP 1261) in a subset of cases

**iv) Routine urine examination-** Proteins, Sugar, Microscopic

**Primary end points:**

**i) Amount of leucorrhoea-** semiquantitative scoring as done and reported earlier by us and shown in the diagram above

**ii) Local Symptom scoring** as mild, moderate or severe for –burning, itching, local swelling, erosions, pain etc- percentage of patients with the symptom as well as semiquantitative scores were be considered.

**iii) Colposcopy-** None of the cases with abnormal colposcopic findings of polyps, or intraepithelial neoplasias were included in the study. The size of erosions as indicated by estimated percentage of surface of portio vaginalis of the cervix by the erosion was recorded before and after treatment.

**iv) Persistence and severity of specific infections and erosions** as shown by wet smear, Pap smear and colposcopy

**v) Anterior Vaginal pH** using semiquantitative pH paper (Qualigens)

**Secondary end points:**

**vi) Systemic symptoms** like body ache, malaise, heaviness, backache etc

**vii) Proinflammatory biomarker** -Serum Interleukin-6 levels

**viii) Side effects of treatment**

**ix) Safety evaluation-** Weight, Blood pressure, symptoms signs, Blood tests- CBC, ESR, SALT, SALP, ASAT, Creatinine, Total cholesterol, Blood urea, Blood sugar, HIV, VDRL, Urinalysis for albumin, sugar and microscopy

**x) Prakriti :** This was assessed in 21 cases of Panchavalkal cream Group 1 and was analysed with respect to outcome.

**5. Results & Discussion**

During the study period a total of 1206 new cases underwent preliminary Pap smear testing and screening. Out of these 63 cases were enrolled for the study; 34 cases were enrolled for the PVK cream group and 31 were enrolled for the control group. Out of 34 cases in Group 1, 3 cases were lost to follow up and 1 withdrew due to a minor side effect within 1 week hence treatment outcome was evaluated in 30 cases. Out of 31 cases enrolled in control treatment Group 2, 2 cases was lost to follow up and 29 cases were evaluated. The mean age, parity and body mass index (BMI) of the two groups was similar as shown in the Table 6.1.

**Table 6.1. Sociodemographic characteristics of women in the study****Mean & SEM of age, weight, height, BMI & parity**

Variable	Group1 N=30	PVK cream	Group 2 N=29	Control
	Mean	SEM	Mean	SEM
Age (Years)	37.0	1.8	33.5	1.1
Weight (Kg)	60.2	4.1	59.3	2.5
Height(Mtrs)	1.5	0.02	1.5	0.01
BMI	25.0	1.8	24.98	0.89
Parity	2.5	0.2	1.68	0.18

*\*Student t test- No significant differences between the groups; BMI= Body Mass Index*

The menstrual history in the two groups was uncharacteristic, however 4 cases in the study group were postmenopausal. Their response was not different from the others in the group. All women in the control group were regularly menstruating.

**Table 6.2. Menstrual history in the participating cases**

<b>Menstrual history</b>	<b>Group1 (N=30)</b>	<b>Group 2 (N=29)</b>
<b>Regular cycles</b>	26	29
<b>Irregular</b>	-	-
<b>Menopausal</b>	4	-
<b>Total</b>	30	29

**Safety evaluation:** Clinical examination, weight, blood pressure did not reveal any adverse effects during treatment in any cases in both the groups. Haematological and other organ function and urine examination did not change after treatment in both groups indicating safety of both modes of treatment.

**Side effects: Group1:** None of the cases in Group 1 complained of any local allergy, discomfort, irritation, burning, swelling or itching during the use of PVK cream. However one case discontinued treatment on Day 4 because she felt that the application of cream was messy.

**Group 2:** 4 women and 3 husbands complained of minor epigastric pain, or 2/3 loose motions after allopathic treatment. However none discontinued treatment.

**Compliance and acceptability:** Drug compliance was good for both treatment groups. Partners of women were compliant in more than 95 % of cases in both groups in sexually active subjects. PVK cream was acceptable and did not produce any side effects. Only 1 case discontinued the treatment within 1 week as she found the use of cream too messy.

Women in both groups were offered alternative (either Allopathic or Ayurvedic ) therapy if they failed to respond to either therapy at the end of 4 to 6 weeks follow up.

### Response of primary symptom of leucorrhoea:

**Table 6.3. Leucorrhoea score (semiquantitative) in Group 1 and Group 2**

	Group1	PVK Cream	Group 2	Control
Status	Mean	SEM	Mean	SEM
<b>Before Treatment</b>	3.43	0.59	4.68	0.53
<b>After Treatment</b>	1.82*	0.45	3.00**	0.43

*Paired t test (before vs after treatment) : \*  $p < 0.05$  \*\*  $p < 0.05$ ; Wilcoxon Rank test (before vs after treatment) : \*  $p < 0.05$*

Leucorrhoea was significantly reduced in both groups (Table 6.3). The initial score was higher in the control group possibly because of a greater number of women with large erosions in this group and remained higher even after treatment.

### Other Local Symptoms:

**a) Itching** was reported by 16 out of 30 cases in Group 1 and persisted, though not severe, in 7 cases. In Group 2 itching was reported by 6/29 cases and persisted in 6 cases. All responded symptomatically to some extent and the mean scores were significantly reduced from 1.23 initially in Group 1 to 0.20 by 1 month, and from 1.42 in Group 2 to 0.39 at 1 month.

**b) Vulvo-vaginal ulcerations** were observed at initial examination in one case with severe fungal infection. She responded very well to the PVK cream treatment followed by oral formulation and became asymptomatic for leucorrhoea and no lesions were seen at follow up examination. However speculum examination showed slight discharge and

fungal hyphae were observed in Pap smear at 1 month, thus indicating the need for Forcanazole at the end of treatment.

#### **Colposcopic assessment of signs and response of cervical erosions:**

None of the cases with abnormal colposcopic findings of polyps, or intraepithelial neoplasias were included in the study. The size of erosions before and after treatment was recorded in both groups and is tabulated in Table 6.4.

Similar response in the form of persistent erosions was seen in both groups- 21/30 responded with improved signs in Group 1, and 19/29 in Group 2.

Total healing of erosions was seen in 4/30 in Gr 1; in 3/29 cases in Gr 2 (Figure 6.4 and 6.5). In 1 case, in both the groups, the size of the erosion increased.

**Table 6.4. Colposcopic findings and size of erosions in Group 1 and Group 2**

	<b>Group1 N=30</b>	<b>PVK Cream</b>	<b>Group 2 N=29</b>	<b>Control</b>
<b>Colposcopy</b>	<b>Before Treatment</b>	<b>After Treatment</b>	<b>Before Treatment</b>	<b>After Treatment</b>
<b>No infection clinically</b>	0	14	0	8
<b>Cervicitis, Vaginitis</b>	18	9	13	11
<b>Total No erosions</b>	<b>12</b>	<b>7</b>	<b>17</b>	<b>12</b>
<b>Large erosions</b>	7	2	13	9
<b>Total No of cases</b>	30	30	29	29

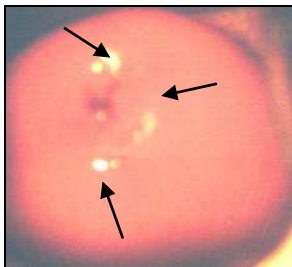
*Note: \*Control group had more severe signs*

**Overall Outcome:** Overall outcome for symptoms and signs is shown in Table 6.5 as cured or improved (positive response) or no change or worse (negative response).

**Table 6.5. Overall outcome in symptoms and signs in two groups of cases**

<b>Groups</b>	<b>Cured or improved</b>	<b>No change or worse</b>	<b>Total</b>
<b>Group1 (PVK cream)</b>			
Symptoms	28	2	30
Signs & Pap smear	28	2	30
Large erosions	5	2	7
<b>Group 2 (Control)</b>			
Symptoms	19	10	29
Signs & Pap smear	17	12	29
Large erosions	4	9	13

*Chi square test:  $p < 0.05$  before vs after treatment in both groups*

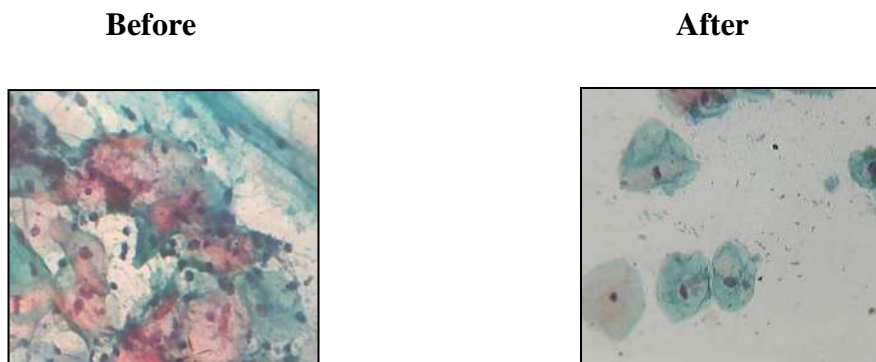
**Figure 6.4. Cervical findings in a case of PVK cream (Group1)****Before treatment-cervix with 30% erosion****After treatment- clean cervix****Figure 6.5. Cervical findings in a control case (Group 2) before & after treatment****Congestion and small erosion****Clean cervix after treatment****Pap smear analysis:**

It was observed that Nonspecific infections and Bacterial Vaginosis (indicated by the presence of “Clue cells”) were the most common infections in both groups followed by fungal infections. In spite of identical inclusion and exclusion criteria overall all infections were more common and more severe in the control group, as indicated by the leucorrhoea score and size of erosions. The number of cases with cervical erosions was 17 (vs 12 in PVK cream group) and there were 13 cases with large erosions in the control group out of which only 4 responded to treatment. Multiple infections were also more common in the control group. HPV and Trichomonal infections were also more common in the control group.

Response to treatment was similar in both groups and persistent infections of all types except Trichomoniasis were seen in both groups. There were no cytological changes suggestive of Trichomoniasis post therapy in all 5 cases in Group 2 whilst Group 1 showed persistent changes post therapy in 1/2 cases. Clue cells indicative of Bacterial vaginitis were seen only in 2/ 13 cases in Group 1 but in 4/8 cases in Group2 (Table.6.6).

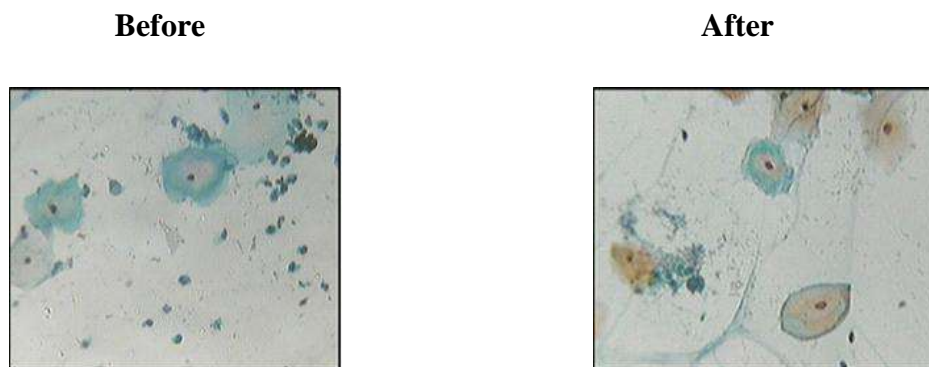
Persistent fungal infection was seen in both Groups in 2 cases.

**Figure 6.6. Bacterial vaginitis in Group 1 before treatment; Negative smear after therapy-Note the presence of Lactobacilli post-therapy**



**Figure 6.7. Papanicolaou smear in a case from control Group 2**

**Clue cells disappeared after treatment, more lactobacilli were seen**



**Table 6.6. Cytological manifestations of RTIs in Group 1 and 2 before & after treatment (Bethesda method)**

<b>Pap smear</b>	<b>Group 1</b>	<b>N=30</b>	<b>Group 2</b>	<b>N=29</b>
<b>Suggestive of</b>	<b>Before</b>	<i>After</i>	<i>Before</i>	<b>After</b>
<b>Nonspecific infection</b>	12	12	7	11
<b>Bacterial Vaginitis</b>	13	2	8	4
<b>Chlamydial Infection</b>	0	0	3	1
<b>Trichomonas Vaginalis</b>	2	1	5	0
<b>Fungal Infection</b>	4	2	5	2
<b>Herpes simplex Virus</b>	0	0	0	0
<b>Human papilloma virus</b>	2	3#	12	9
<b>Multiple infections</b>	4	2	11	2
<b>Negative Smear</b>	1	10	0	1

*\*Total does not add up to group total because multiple infections were seen*

*# In this case koilocytes appeared in the follow up smear. This is possible as the presence of severe infection prior to PVK cream treatment can mask the underlying HPV manifestation of koilocytes in initial smear. Viral infections usually are not cured.*

**Vaginal pH changes:**

Anterior vaginal pH was recorded before and after treatment in 16 cases in Group 1 (Figure 6.8) and 12 cases in Group 2 (Figure 6.9).

The mean anterior vaginal pH in the 2 groups before and after treatment is given in Table 6.7 .

**Table 6.7. Mean anterior vaginal pH in Group 1 and Group 2**

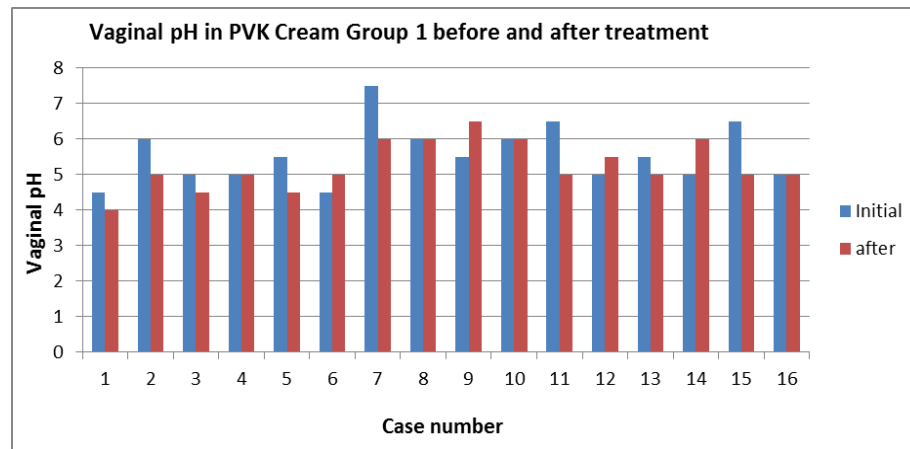
<b>pH</b>	<b>Group 1</b>	<b>N=30</b>	<b>Group 2</b>	<b>N=29</b>
Mean	5.56	5.25	5.67	5.46
SD	0.81	0.68	0.72	0.69
SEM	0.20	0.17	0.21	0.20

*Paired t test p=0.08, NS*

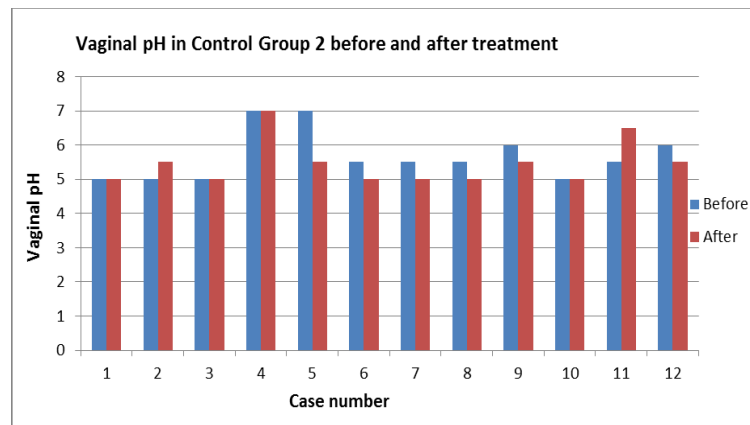
The vaginal pH was marginally reduced after PVK cream treatment in Group 1 but not significantly (paired t test p=0.08, NS).

The mean vaginal pH was not altered after treatment in the control Group 2 (paired t test p= 0.11, NS).

**Figure 6.8. Anterior Vaginal wall pH in PVK cream (Group 1) before and after treatment (N=16)**



**Figure 6.9. Anterior Vaginal wall pH in control (Group 2) before and after treatment (N=12)**



There was a marginal reduction in pH after treatment in both groups but not of any significance.

**Serum Interleukin-6 (IL-6) levels:** Serum IL-6 levels were studied as a proinflammatory cytokine biomarker in 13 cases in Group A and 14 cases in Group B

before and after treatment in both groups. The mean serum IL-6 levels in these groups are given in Table 6.8.

**Table 6.8. Serum IL-6 levels (Mean  $\pm$  SEM) in Group 1 and 2 before and after treatment**

Serum IL-6 pg/ml	Before treatment	After Treatment
Group 1 PVK cream N=13	26.1 $\pm$ 7.2 pg/ml	26.9 $\pm$ 7.8 pg/ml
Group 2 Control group N=14	15.9 $\pm$ 17.1 pg/ml	14.2 $\pm$ 8.4 pg/ml

Healthy young controls: <25 pg/ml

*Paired t test:  $p > 0.05$  in both groups ; Not significant*

There was no significant reduction in Serum IL-6 levels in both groups after treatment.

**Prakriti Assessment and response to treatment:** In the PVK cream group prakriti was assessed using a 20 point questionnaire in 21 cases. In all these cases the prakriti was dimorphic. The response was classified as given in the Table 6.8.

**Table 6.9. Overall response to treatment in relation to prakriti**

<b>Prakriti</b>	<b>Cured/ Improved</b>	<b>No change/ Worse</b>	<b>Total</b>
<b>Pitta Kapha or Pitta Vata</b>	10	2	12
<b>Kapha Pitta or Vata Pitta</b>	7	1	8
<b>Vata Kapha or Kapha Vata</b>	1	--	1

Thus 20 cases out of 21 assessed had significant Pitta component, and out of these 12 had Pitta dominant.

#### **b) Biochemical Variables Before & After Treatment in Groups 1 and 2:\***

**Blood tests:** There were no significant changes in all the blood variables studied for organ function tests in both groups as shown in Table 6.10 and Table 6.11.

**Urine examination:** Routine urine examinations before and after treatment did not reveal any abnormalities in both groups indicating absence of urinary infections as a cause of leucorrhoea.

**Table 6. 10. Biochemical Variables in biochemical tests in PVK cream Group 1 before and after treatment**

Variable	Normal Range	Before treatment		After treatment	
		Mean	SEM	Mean	SEM
Haemoglobin gm %	12-16.5	11.2	0.21	11.6	0.20
RBC count millions/cmm	3.5-5	4.5	0.05	4.4	0.07
WBC count /cmm	4000-11000	7036	273	6884	305
PlateletsThousnds/cmm	143,000-500,000	293	10	261	11
ESR mm 1 <sup>st</sup> hr	0-20	24.2	2.2	23.2	2.3
ASAT U/L	10-35	17.1	0.74	18.66	0.75
ALAT U/L	10-45	18.4	1.2	20.2	1.4
ALP IU/L	5-250	195.5	12.4	187.0	10.9
Serum Creatinine mg/dl	0.7-1.4	0.97	0.03	0.97	0.04
Blood Urea mg/dl	10-50	19.7	1.1	20.1	0.8
Blood Sugar mg %	60-110	80.1	1.8	81.2	1.8
Serum Cholesterol mg %	120-200	175.1	4.9	180.1	5.5
Total Proteins gms %	6-8	7.28	0.06	7.28	0.08
S Bilirubin mg %	0.2-1.2	0.449	0.040	0.458	0.027

\* Before vs after treatment- Paired t test-  $p > 0.05$  Not significant for both groups

**Table 6.11. Biochemical variables in biochemical tests in Control Group 2 before and after treatment**

Variable	Normal Range	Before treatment		After treatment	
		Mean	SEM	Mean	SEM
Haemoglobin gm %	12-16.5	11.2	0.23	11.24	0.22
RBC count millions/cmm	3.5-5	4.44	0.08	4.37	0.07
WBC count /cmm	4000-11000	6473	289	6123	245
PltletsThousnds/cmm	143,000-500,000	258458	29344	308470	15687
ESR mm 1 <sup>st</sup> hr	0-20	23.4	3.3	21.5	2.7
ASAT U/L	10-35	17.8	1.7	16.5	1.3
ALAT U/L	10-45	18.7	2.7	17.2	1.7
ALP IU/L	5-250	172.8	15.4	187.2	11.1
Serum Creatinine mg/dl	0.7-1.4	0.89	0.03	0.82	0.04
Blood Urea mg/dl	10-50	24.24	1.48	20.73	1.17
Blood Sugar mg %	60-110	77.1	2.02	78.2	2.06
Serum Cholesterol mg %	120-200	182.9	10.1	192.9	9.4
Total Proteins gms %	6-8	6.9	0.14	7.3	0.13
S Bilirubin mg %	0.2-1.2	0.54	0.04	0.48	0.03

\* Before vs after treatment- Paired t test-  $p > 0.05$  Not significant for both groups

**Discussion :**

Reproductive Tract Infections are a major cause of morbidity and mortality in women and neonates. Genital infections may be present in as many of 1/3<sup>rd</sup> of women in young age group even with sexually low risk behaviour group, eg housewives. Although it is customary to treat RTIs with allopathic line of treatment, in India the option of treating with traditional Indian system of Ayurveda is open. There is however paucity of published information of the efficacy and side effects of Ayurvedic therapies, eg Panchavalkal in leucorrhoea. Panchavalkal, prepared from the barks of 5 medicinal plants is described in all Ayurvedic texts and nighantus and is recommended for the treatment of Pittajayonivyapad. Alternative plants, *shirisha*, *vetas* and *nimba* also have been mentioned. However in the absence of objective authentic published information and to facilitate global acceptance of Ayurveda we first undertook a study with the conventional kwath based on the experiential and exploratory path of Reverse Pharmacology and knowledge of phytochemistry and phytopharmacology and “in vitro” antimicrobial experiments by Dr RM Bhatt. Using objective criteria like Pap smear, vaginal pH and colposcopy these studies as described in Chapter 3 provided evidence that the Modified Panchavalkal kwath (N=42) provided symptomatic relief or cure in 85 % of women with uncomplicated leucorrhoea (absence of Pelvic inflammation, fibroids etc). In a subsequent pilot study efficacy of Ayurvedic treatment, Kwath + oral systemic treatment, (N=20) was comparable to a small number of cases treated with standard allopathic treatment (N=16) (Chapter 3).

Need for Vaginal Formulation: Daily vaginal douche with the PVK kwath, although effective, is not convenient for the following reasons: **i)** Fresh kwath had to be prepared every day in the hospital - hence time consuming **ii)** Standardisation of the barks was a problem **iii)** Patient had to make a daily visit to the hospital **iv)** Doctors (gynecologist or registrar under supervision) had to attend to the patient alongwith an attendant for daily douche on all 14 days **v)** Batch to batch variation of the kwath was bound to occur **vi)** Acceptability and regularity of treatment will be affected.

**Formulation development:** This project therefore aimed at the development of a user friendly vaginal formulation (cream) which was developed in collaboration with Viridis

BioPharma Pvt Ltd as described in Chapter 5 and the cream had the following advantages:

**i)** It was prepared in a single batch at the company with a manufacturing license **ii)** Phytochemistry and standardisation was carried out for the PVK plants and hydroalcoholic extracts of barks **iii)** The purity, toxicity and heavy metal, pesticide and bacterial load, aflatoxin contents were within the specified limits of CCRAS & WHO. **iv)** Women were demonstrated how to use the cream and could carry the cream to their home for daily use **v)** Acceptance and compliance was very good **vi)** Cream remains in contact with the vaginal mucosa for a longer period than the Kwath which is administered via a douche.

The global demand for safe and effective microbicides is increasing and some of these of herbal origin have undergone clinical testing. In the present study an internationally acceptable carbopolymer was used. Vaginal cream with petroleum jelly, a common cream base, can interact with the latex used for condoms and in women using the cream and condom simultaneously may resulting in unwanted pregnancy and can be a risk factor for HIV and other STDs (Rosen & Rosen, 1999; European Working Group on HIV Infection, 1993).

Herbal therapies are not without hazards hence in this study the safety of PVK cream was evaluated, both preclinically, as well as clinically and biochemically with organ function tests before and after treatment.

In view of the common prevalence of the RTIs in Indian studies the syndromic approach was recommended for the treatment of the control group and the partners of women who were sexually active.

**Safety:** This cream used by the women in the study Group 1 provided symptom relief and was safe. The clinical and biochemical safety was documented for the first time with clinical and blood tests carried out before and after treatment.

**Objective evaluation of efficacy:** This was carried out with semiquantitative scoring (Visual pictogram) , vaginal pH, colposcopy and Pap smear evaluation for type and severity of infections.

**Control Therapy:** It our policy to inform women and their husbands that if they experience some discomfort after the first 2 doses of forcanazole and azithromycin they should take the secnidazole on the next day after dinner so that side effects are minimised. It is also our practice to advise this modified regiment to women or men with history of acidity and dyspepsia or intolerance to drugs and those who have low weight or a delicate constitution. This may have been the reason why the compliance was 99% for allopathic therapy also.

Haematological and other organ function and urine examination did not change after treatment in both groups indicating safety of both modes of treatment.

Although the same criteria were used for enrolment, on analysis of symptoms and signs it was seen that the women in Group 2 had marginally more severe disease. Though cases with PID were excluded from both groups, more women in Group 2 had large erosions and slightly more severe symptom of leucorrhoea.

Statistically significant clinical symptomatic improvement was seen in >98 % of cases in Group 1. Similar significant symptomatic relief was seen in >95% of cases in Group 2.

This was associated with reduced signs like cervicitis and reduced size of erosion in both groups. There were few resistant cases in both groups and 1 case worsened in each of the groups. This may be explained by infection with resistant or multiple organisms.

Persistence of specific infections was also seen in both groups. Hence the importance of a follow up examination and partner treatment in both groups cannot be overemphasised for complete holistic treatment and for prevention of complications like chronic PID and infertility.

**Serum Interleukin-6 (L-6) levels:** Serum IL-6 is a proinflammatory cytokine and is raised in some acute or chronic inflammations or in cancers. Its association with gynecological cancers has been shown by Heikkela et al (2008). Some investigators have

shown association of high serum or local tissue IL-6 levels with cervical precancer and correlation with progression to invasive cancer and metastasis (Pardo-Govea, 2005; Bustamam et al, 2008). In view of this we studied serum IL-6 levels in women with inflammatory smears and with abnormal smears (atypia and low grade lesions) and observed a significant difference. We have reported a reduction in serum IL-6 levels in women with Low Grade Intraepithelial Lesions (LSIL in Pap smears) when treated with a standardized Turmeric oil extract, a plant extract, indicating anti-inflammatory role of Turmeric and its possible role in arrest or regression of low grade precancer (Paradkar et al 2010; Joshi et al, 2011). However in all cases in the present study with inflammatory smears the serum IL-6 levels remained within the normal range and did not decrease with either PVK cream + Oral Ayurvedic formulation (Group 1) or with standard of care (allopathic) treatment (Group 2). There was some variation in levels of IL-6 in both groups and the levels did not change significantly in both groups after treatment of infections. In both groups the mean serum IL-6 levels were below 25 pg/ml, both before and after treatment. The mildly high IL-6 levels could be explained by some persistent infections as well as the age since IL-6 levels are known to be higher in older women as compared to young adults (<10 pg/ml). It was observed that mean serum IL-6 level was significantly lower than the mean level of  $248 \pm 156$  (SEM) pg/ml observed in women with Pap smears diagnosed as Low-Grade Squamous Intreepithelial Lesion as reported by us in the same period (Joshi et al, 2010, 2011). These cases with abnormal Pap smears were excluded from the current study.

In conclusion we observed that Serum IL-6 levels were within normal limits for this age in both Groups I and II and did not find a change in serum IL-6 levels in women with inflammatory smears, either with Ayurvedic (Group 1) treatment or with Allopathic treatment (Group2).

**Prakriti assessment:** In standard Ayurvedic text books Panchavalkal is advised preferably for the *pitta prakopa*. As in our previous study (Chapter 3) in the present study also we observed a better response in *Pitta* dominant *prakriti* cases. The numbers are small but the *Prakriti parikshan* does show that majority of cases of chronic leucorrhoea

had *Pitta* component. Our previous analysis of *prakriti* in earlier project (Chapter 3) indicated a similar trend. Those with *Pitta* dominant *prakruti* responded best to Panchavalkal treatment as in the earlier study confirming the sutra *Panchavalkastu pittarta* (Charak Samhita).

Since we wanted an objective evaluation as per the inclusion and exclusion criteria we used the same treatment for all cases, and yet we observed >90 % response for partial or complete relief of symptoms. It is expected that the use of Ayurvedic medicines as per *prakriti* and *doshaprakopa* will give more responses in the “cured” category than in the partial category however this can be confirmed only by a large sample size.

**Other clinical studies on Ayurvedic therapies for leucorrhoea:** Usha Rani et al (1995) have evaluated a vaginal cream with *Azadirachta indica*, *Curcuma longa*, *Pongamia glabra*, *Glycyrrhiza glabra* and *Santallum album* for vaginitis in a randomized placebo controlled double blind multicentric study. They observed an overall efficacy of 76% in treated vs 24 % in placebo group. Salhan et al (2002) studied the efficacy of the same cream in women with vaginitis. Kulkarni et al (2012) studied another polyherbal cream in women with clinical symptoms and signs and wet vaginal smear. They observed persistent discharge in 10/30 cases. Only subjective and clinical evaluation was carried out. Symptoms and signs of discharge were persistent in >31% of cases at the end of the study.

Salhan and coworkers (2009) also studied another polyherbal formulation in cream form as well as the convenient vaginal tablet form. The authors found that 92% of women using herbal formulation were relieved of their symptoms of abnormal vaginal discharge (AVD) as against 81.6% women using Betadine. It has been developed principally for HIV and STD prevention and hence requires long term use.

In the present study the PVK cream was used symptomatically for a limited period only and the acceptability as well as symptom relief was very good.

Talwar and coworkers (2008) have also evaluated another polyherbal cream using diferuloylmethane (curcumin), extracts of *Emblica officinalis* (Amla), saponins from *Sapindus mukorossi*, *Aloe vera* and rose water. Curcumin is extracted from *Haridra* (*Cucuma longa* Linn). It was shown to be safe in clinical and preclinical studies and was found to inhibit the growth of *Neisseria gonorrhoeae*, including those resistant to penicillin, tetracycline, nalidixic acid and ciprofloxacin. *In vitro* it had inhibitory action against *Candida* species resistant to azole drugs and amphotericin B. It also had anti HIV and anticancer activity in HELA cell culture. We have also observed anticancer activity of a supercritical extract of Turmeric (*Curcuma longa* Linn) oil in cervical precancer with reduction in serum IL-6 levels however these cases were not evaluated for treatment of leucorrhoea (Joshi et al, 2011).

It is important to realise that most of these studies, including our study, have excluded women with pelvic inflammation (PID) as this requires a multipronged therapy, sometimes with hospitalisation. It is remarkable how the Ayurvedic physicians have recognised dyspareunia and PID, which are more advanced stages of leucorrhoea, as different entities as *vipluta* and *paripluta yoni* as given below:

विप्लुता  
विप्लुता नित्यं रुजावती परिप्लुता  
पुनर्व्यावाये अथ पीडावती इति अनयोः  
भेदः । सु. उ. २८-१० टीका

शूना स्पर्शाक्षमा सार्तिनीलपीतमसृक् स्रवेत् ।  
श्रोणिवंक्षणपृष्ठार्ति जरातार्याः परिप्लुता॥ च.चि. ३० - २३, २४

The treatment of PID will require multiple drugs as well as therapies like Panchkarma.

**Conclusion:** Modified PVK cream (2%) applied as a vaginal formulation had similar clinical efficacy as standard of care allopathic treatment symptomatically. A higher proportion of larger erosions and specific infections, and persistence of specific infections in a small number on post-treatment examination was observed in the control group. Both

therapies caused cure or partial relief of symptoms in >95% cases. Allopathic treatment also did not cure all cases and this is corroborated by world literature as antibiotic is recorded in 5 -15% of cases. The present study was unique in that there was standardisation, formulation, preclinical and clinical as well as biochemical safety demonstration and evaluation using objective methods like semiquantitative symptom scoring, clinical examination, colposcopy, vaginal pH and Papanicolaou smears.

The activity of PVK was more evident in bacterial vaginitis and for healing of erosions. The addition of systemic Ayurvedic therapy appears to have improved marginally the effectiveness of PVK cream as the kwath had 85% symptom response rate vs 99% in this study however it will not be correct to compare the data in 2 different studies carried out sequentially. An important finding in this study was the persistence of symptoms and signs in both groups and the absolute need for a gynaecological check up in all cases of leucorrhoea despite symptomatic relief. This requires further management with either Ayurvedic or Allopathic or integrated therapy so that chronic pelvic inflammation and its complications are avoided. The possibility of side effects is more with allopathic treatment. Systemic Ayurvedic treatment was not associated with any side effects, maybe because a few selected plants were included instead of several plant extracts or bhasmas as is common with some commercial formulations. It is possible that failures in the Ayurvedic therapy can be reduced by advising treatment as per prakriti and doshaprakopa just as the failures in Allopathic therapy can be reduced by using alternative antibiotic combinations.

Since infections are often polymicrobial it is recommended that both therapies have a role and may have to be used sequentially depending on the severity of disease ,and preference of individual patients. However since Allopathic therapy is microbicidal we recommend Allopathic therapy first, to be followed up by Ayurvedic therapy for the residual infections and erosions. In cases with contraindications to Allopathic therapy Ayurvedic therapy or in Ayurvedic hospitals or clinics Ayurvedic therapy alone can be given. The critical aspect, for both therapies, is the partner treatment which is essential for preventing recurrences, PID, and complications.

The effectiveness of Ayurvedic PVK cream therapy was seen only partially in the clearance of Chlamydia, Trichomoniasis and Fungal infection whilst there was significant relief from symptoms (leucorrhoea score) or signs (erosion size).

## 6. Future Research

Reproductive Tract Infections are a major cause of morbidity and mortality in women and neonates. Genital infections may be present in as many of 1/3rd of women in young age group even with sexually low risk behaviour group. Although it is customary to treat RTIs with allopathic line of treatment, in India the option of treating with traditional Indian system of Ayurveda is open. There is however paucity of published information of the efficacy and side effects of Ayurvedic therapies, eg Panchavalkal in leucorrhoea. However in the absence of objective authentic published information and to facilitate global acceptance of Ayurveda we first undertook a study with the conventional kwath based on the experiential and exploratory path of Reverse Pharmacology and knowledge of phytochemistry. Using objective criteria like Pap smear, vaginal pH and colposcopy these studies provided evidence that the Modified Panchavalkal kwath provided symptomatic relief or cure in 85 % of women with uncomplicated leucorrhoea (absence of Pelvic inflammation, fibroids etc). In a pilot study the efficacy of Ayurvedic treatment was comparable to a small number (N=16) cases treated with standard allopathic treatment. Objective methods of evaluation included history, general and gynecological examination, symptom scoring, vaginal pH, Papanicolaou smear, colposcopy, microbiology and relevant blood tests before and after therapy. About 80% had symptomatic relief whilst remaining showed persistent infection.

The global demand for safe and effective microbicides is increasing (Rencher,2001; Reddy, 2004; Ravel, 2012) several vaginal creams of herbal origin have undergone clinical testin (Talwar 2008; Salhan, 2009; Palep 2003, 2004). In the present study an internationally acceptable carbopolymer was used for formulation development. Vaginal

cream with petroleum jelly, a common cream base can interact with the latex used for condoms and women using the cream and condom simultaneously resulting in unwanted pregnancy.

Herbal therapies are usually without hazards however the safety must be demonstrated. In this study the safety was evaluated, both clinically as well as biochemically with organ function tests before and after treatment. Unlike other reports on Ayurvedic or herbal therapies of leucorrhoea in the present study with Modified Panchavalkal the partners of women all women who were sexually active were treated with syndromic Allopathic therapy to prevent reinfections and prevention of complications of PID.

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