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Research Article

EFFECT OF *BHANDIRA* (*CLERODENDRUM VISCOSUM*) IN CERVICAL INTRAEPITHELIAL NEOPLASIA

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ABSTRACT

Cervical Intraepithelial Neoplasia is precursor or pre-invasive lesions of cervical squamous cell cancers. These are classified based on the degree of disruption in epithelial differentiation. Sexually transmitted HPV are the most important risk factor for CIN and Invasive cervical cancers. A pre-post interventional study was conducted in Govt. Ayurveda College Hospital for Women and Children, Poojappura, Thiruvananthapuram, to assess the effect of *Bhandira* in CIN. Objectives were, to evaluate the effect of *Bhandira churna* pancake and *Bhandira churna yoni pratisarana* on CIN, and also to study its effect on HPV infection and associated complaints like discharge per vaginum, vulval itching, burning sensation, lower abdominal pain and dyspareunia. Females of age group 20-50 years with CIN, confirmed by Pap smear and Colposcopy were included. The patients received treatment with *Bhandira churna* pancake internally and *Bhandira churna* as *Yoni pratisarana* for 3 months and follow up was done after 3 months. Outcome variables were changes in Pap smear and Colposcopy findings- regression, persistence or progression and changes in associated symptoms. Data were analyzed by Wilcoxon's signed rank test and McNemar's test. The treatment was effective with high statistical significance in making changes in Pap smear and Colposcopy findings ($p<0.01$) and also in reducing the associated symptoms.

INTRODUCTION

Cervical intraepithelial neoplasia (CIN) is a histopathologic condition where a part or the whole thickness of cervical squamous epithelium is replaced by cells showing varying degrees of atypia.[1] HPV infection is the most important risk factor attributed for CIN and Invasive Cervical Carcinoma (ICC). Studies in 22 countries, coordinated by the International Agency for Research on Cancer (IARC) identified HPV DNA in almost all (99.7%) cases of cervical cancer.[2] Cervical cancer is increasingly recognized to be a global problem. Most of the cases (85%) presents in advanced and late stages which makes cervical cancer control a challenge.[3] As per the data of World Cancer Research Fund International for the year 2012, cervical cancer is the 7th most common CA in humans which contribute 3.7% of all cancers. It is the 4th most common CA in women worldwide, and the most common CA causing death in developing countries.[4]

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Cervical cancer mortality rates have fallen in most of the developed world during the past 30 years, largely due to screening and treatment programmes. At the same time, however, the rates in most developing countries have risen or remain unchanged, often due to limited access to health services, lack of awareness and absence of screening and treatment programmes.[5] As not common in other cancers, the pre-cancerous changes in cervical tissue can linger for many years before becoming a cervical carcinoma which gives opportunity for early screening and detection. Although HPV vaccination for girls reduces the possibility of developing cervical cancer, the vaccine does not protect against all high-risk HPV types. Current conventional treatment of CIN is limited to local ablative or excisional procedures.

Ayurveda, highlights on two aspects of treatment- *Swasthya samrakshana* and *Vikara prashamana*. HPV infection is an *Aganthu roga* by origin, but later it vitiates the bodily *Dosa* and *Dhatu*

and manifest to a *Nija roga*. Exact manifestation of HPV and CIN are not seen in any *Yoni rogas,* but many symptoms coexist in conditions like *Paripluta, Vipluta, Karnini, Yoni arsas* etc. CIN can in turn be considered as *Sopha* occurring at the cervix which is in turn the *Poorvarupa avastha* of forthcoming *Vyadhi*- cervical cancer. This disease can be prevented through *Sadvritta* and cure can be ensured through the usage of *Krimihara, Vrana- Sodhana- Ropana* drugs both internally as well as through local application. The shortage and price hike of good quality raw drugs are some of the major issues faced by present day Ayurvedic physicians. So, study on cheap and easily available single drugs is the need of the present scenario. Cell line studies have already proven the effect of drug *Bhandira* (*Clerodendrum viscosum*) on the induction of cellular-apoptotic response in HeLa cells that involves activation of effectors protease Caspase 3 in CA cervix.[6] Hence the drug *Bhandira* was selected for internal administration as pancake and the same drug was used for local application i.e., *Yoni pratisarana* with *Madhu* and *Ghrita*. This study is a novel venture to raise awareness and encourage implementation of Ayurvedic drugs in preventing and combating cervical cancer.

OBJECTIVES

1. To evaluate the effect of *Bhandira churna* as pancake and *Bhandira churna pratisaranam* in CIN.
2. To study its effect on HPV infection and associated complaints like discharge per vaginum, vulval itching, burning sensation, lower abdominal pain and dyspareunia.

METHODOLOGY

Study Design: Interventional study- Pre and Post. Patient's condition after treatment was compared with the status before treatment.

Study Setting: OPD and IPD, Govt. Ayurveda College Hospital for Women and Children, Poojappura, Thiruvananthapuram.

Study Population: Females of age group 20-50 years diagnosed as CIN, attending the OPD and IPD, Govt. Ayurveda College Hospital for Women and Children, Poojappura, Thiruvananthapuram.

Inclusion Criteria

- Females of age group 20-50 years diagnosed as CIN, confirmed by Pap smear and Colposcopy.
- CIN I, CIN II grades.

Exclusion Criteria

- CIN III/ Carcinoma in situ (CIS)
- CA cervix
- 3rd degree uterine prolapse
- Pregnancy
- Patients under prolonged medications for systemic illness- Diabetes mellitus and Hypertension

Sample Size: Sample size was 15

Sampling Technique: Consecutive cases satisfying the inclusion criteria and till sample size is attained.

Data Collection: Primary data collected by interview method.

Study Tool

- Case proforma
- Lab investigations- Blood and Urine R/E, LFT
- Speculum examination, Visual Inspection with Acetic acid (VIA), Visual Inspection with Lugol's Iodine (VILI)
- Colposcopy
- Pap smear

Procedure- Selection Procedure

Patients attending OPD of Women & Children Hospital, Poojappura, with vaginal symptoms were posted for gynecological examination. Patient suggestive of CIN were send for Pap smear and Colposcopic examination. For uniqueness of examination both these tests were done at Regional Cancer Center, Thiruvananthapuram, for all the patients. From those patients diagnosed as CIN I or II informed consent was taken and they were included in the study.

Drug Administration

20gm *Bhandira* root powder and 100gm rice flour were mixed together and made into 120gm drug packets. Such drug packets were dispensed to the patients each month, along with a written advice on how to prepare pancake and were advised for internal administration twice daily. Patient was advised to come after each menses for a period of 1 week for *Yoni pratisarana* with *Bhandira churna* in the consecutive 3 cycles.

Fig 1: *Clerodendrum Viscosum* Plant and Root Powder

Duration of Administration

Internal administration of drug started from 8th day of LMP and was continued for 3 consecutive cycles. *Yoni pratisarana* was done for 7 days from 8th day of LMP for consecutive 3 cycles.

Follow up: Follow up was done after 3 months.

Assessment

Patients were advised to report on the 8th day of LMP. Change in symptoms like discharge per vaginum, vulval itching, burning sensation, lower abdominal pain and dyspareunia were noted in the 4th, 8th, 12th, 16th, 20th and 24th week. At the end of follow up period Colposcopy and Pap smear tests were repeated.

Duration of Study: 6 months

Method of Preparation

20gm *Bhandira* root powder was mixed well with 100gm rice flour using the required amount of water to get a semi-solid consistency. It was spread uniformly over banana leaves and covered well. These were then steamed to make four pan cakes which were consumed twice daily.

Method of *Pratisarana*

After emptying the urinary bladder, patient was made to lie in dorsal position on the treatment table. Sterilized Cusco's Bivalve speculum was inserted in the vagina to visualize the cervix. The mucous over cervix was cleaned properly with a cotton swab by a sponge holding forceps. 5gm of the root powder was mixed with 5ml of honey and 2.5ml of ghee and was made into a paste. This paste was spread evenly on the cervix using a cotton swab held by a sponge holding forceps. Medicine was retained for a period of 30 minutes. Then the medicine was washed well with distilled water. This procedure was repeated for 7 days, in 3 consecutive cycles.

Outcome Variables

- Changes in Colposcopy findings assessed as regression, progression or persistence.
- Changes in Pap smear test findings assessed as regression, progression or persistence.
- Changes in discharge per vaginum, vulval itching, burning sensation, lower abdominal pain and dyspareunia assessed by grading system.

Parameters for Assessment

CIN lesion was assessed after six months by using Colposcopy and Pap smear test done at Regional Cancer Center, Thiruvananthapuram. By using the modified Reid Colposcopic index a score was given to the lesion after evaluating the features of lesions on application of acetic acid and Lugol's iodine.

Colposcopy was assessed using the following grading

Grade 0- Normal
Grade 1- HPV infection
Grade 2- HPV+ CIN I
Grade 3- CIN II

Pap smear was assessed using the following grading

Grade 0- NILM
Grade 1- Inflammation
Grade 2- Koilocytic atypia/**ASCUS**
Grade 3- LSIL

Vaginal Discharge

Grade 0 (Absent) - No vaginal discharge
Grade 1 (Mild)- Enough discharge to lubricate the vagina and vulva but without staining undergarments
Grade 2 (Moderate)- Under garments are deniably soiled and the patient complains of discharge
Grade 3 (Severe)- Excessive degree of vaginal discharge, require some extra-absorbent pad or cloth.

Vulval Itching

Grade 0- Absent
Grade 1- Present

Burning Sensation

Grade 0- Absent
Grade 1- Present

Lower Abdominal Pain

Grade 0- Absent
Grade 1- Present

Dyspareunia

Grade 0- Absent
Grade 1- Present

Analysis

Pre-post comparison of qualitative variables will be analyzed by McNemar's test and pre-post comparison of ordinal data will be analyzed by Wilcoxon's signed rank test.

OBSERVATION AND RESULTS

Data related to Effectiveness of Treatment

The effectiveness of treatment was assessed using Wilcoxon's signed rank test and McNemar's test.

Effectiveness of Treatment on Pap Smear Results

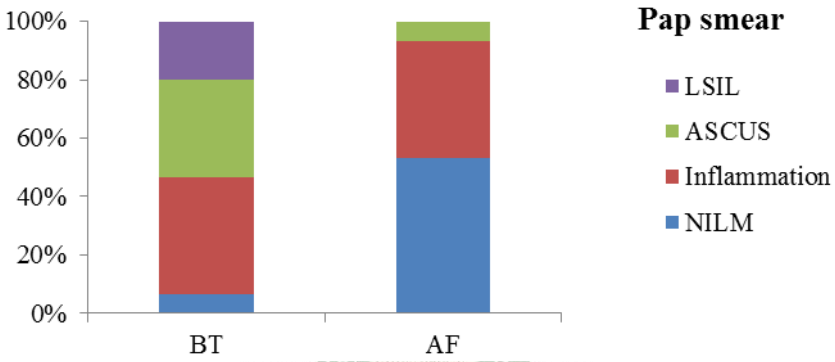
Before treatment 20% cases suffered from LSIL, 33.3% from ASCUS, 40% from inflammation and 6.7% were negative for intraepithelial lesion or malignancy (NILM). After the follow up 53.3% were in NILM grade i.e., got complete relief. 40% had persistence of inflammation and 6.7% were with ASCUS, while no cases reported LSIL. As per Wilcoxon signed Rank test the changes in Pap smear test after follow up is found to be statistically highly significant at the levels of $p < 0.001$.

After the follow up period there was disease regression in 7 patients while another 7 patients were with disease persistence (no change).

Table 1: Effectiveness of treatment on Pap smear results

Pap smear	BT		AF	
	N	%	N	%
NILM	1	6.7	8	53.3
Inflammation	6	40	6	40
ASCUS	5	33.3	1	6.7
LSIL	3	20	0	0
Total	15	100	15	100

Fig 2: Effectiveness of the treatment on Pap smear results



Effectiveness of treatment on Colposcopy results

Before treatment 20% cases were with CIN II lesions and 80% with HPV+CIN I lesions. After the follow up period 0% cases presented with CIN 2 lesions, 13.3% with HPV+CIN I lesions, 66.7% were with HPV infection and 20% got complete cure. As per Wilcoxon's signed rank test the result was of high statistical significance at the levels of p=0.001.

After follow up period there was disease regression in 3 patients while 12 patients were with persistence (but there was reduction in the severity of grading).

Table 2: Effectiveness of treatment on Colposcopy results

Colposcopy	BT		AF	
	N	%	N	%
Normal	0	0	3	20.0
HPV	0	0	10	66.7
HPV+CIN I	12	80.0	2	13.3
CIN II	3	20.0	0	0.0
Total	15	100.0	15	100.0

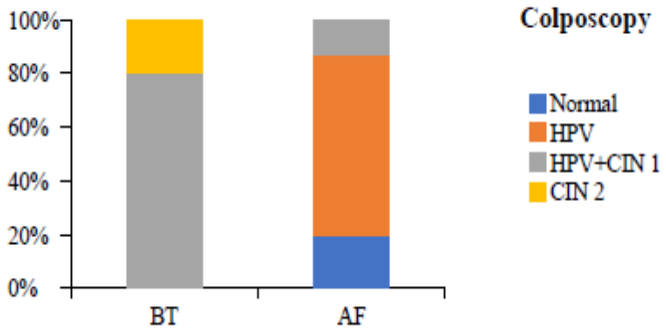


Fig 3: Effectiveness of treatment on Colposcopy results

Effectiveness of treatment on Associated symptoms

Changes in the outcome variables such as severity of discharge per vagina, vulval itching, burning sensation, lower abdominal pain and dyspareunia were assessed and statistically analysed before treatment, during each month of the study period, and during 3 months of follow up period. For convenience 3 main assessments are only explained- before treatment (BT), after 3 months of treatment (AT3) and after 3 months of follow up (AF3).

Effectiveness of Treatment on Severity of Discharge per Vagina

Before treatment 66.7% and 33.3% were with severe and moderate discharge p/v respectively. After treatment (AT3) 60% were with no discharge p/v, 40% were with mild discharge p/v and 0% suffered from moderate or severe grades. After follow up (AF3), 20% were with no discharge p/v, while 53.3% and 26.7% suffered from mild and moderate discharge p/v respectively. As per Wilcoxon's signed rank test, on comparison of the AT and AF with BT in reducing the severity of discharge p/v, both AT3 and AF3 were highly significant at the levels of p< 0.01.

Table 3: Effectiveness of Treatment on Severity of Discharge per Vagina

Discharge PV	BT		AT1		AT2		AT3		AF1		AF2		AF3	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Absent	0	0	0	0	3	20.0	9	60.0	7	46.7	5	33.3	3	20.0
Mild	0	0	6	40.0	11	73.3	6	40.0	8	53.3	10	66.7	8	53.3
Moderate	5	33.3	9	60.0	1	6.7	0	0.0	0	0.0	0	0.0	4	26.7
Severe	10	66.7	0	0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Total	15	100.0	15	100.0	15	100.0	15	100.0	15	100.0	15	100.0	15	100.0
Wilcoxon signed rank test z (comparison with BT)			3.557		3.535		3.578		3.573		3.499		3.482	
p			<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	

Effectiveness of Treatment on Severity of Vulval Itching

Before treatment vulval itching was present in 80% cases and absent in 20% cases. After treatment (AT3) vulval itching was absent in 100% while after follow up (AF3) vulval itching was absent in 73.3% and present in 26.7%. As per McNemar's test, on comparison of the AT and AF with BT in reducing the vulval itching, p value could not be computed in AT3 due to high significance and AF3 was significant at the levels of p< 0.01.

Table 4: Effectiveness of treatment on severity of vulval itching

	Vulval itching				McNemar test (Comparison with BT)- p
	Absent		Present		
	N	%	N	%	
BT	3	20.0	12	80.0	
AT1	11	73.3	4	26.7	.008
AT2	14	93.3	1	6.7	.001
AT3	15	100.0	0	0.0	-
AF1	14	93.3	1	6.7	.001
AF2	13	86.7	2	13.3	.002
AF3	11	73.3	4	26.7	.008

Effectiveness of Treatment on Severity of Burning Sensation

Before treatment burning sensation was present in 73.3% cases and absent in 26.7% cases. After treatment (AT3) burning sensation was absent in 100% while after follow up (AF3) burning sensation was absent in 73.3% and reappeared in 26.7%. As per McNemar's test, on comparison of the AT and AF with BT in reducing the severity of burning sensation, p value could not be computed in AT3 due to high significance and AF3 was significant at the levels of p< 0.05.

Table 5: Effectiveness of treatment on severity of burning sensation

	Burning sensation				McNemar test (Comparison with BT)- p
	Absent		Present		
	N	%	N	%	
BT	4	26.7	11	73.3	
AT1	9	60.0	6	40.0	.063
AT2	13	86.7	2	13.3	.004
AT3	15	100.0	0	0.0	-
AF1	15	100.0	0	0.0	-
AF2	13	86.7	2	13.3	.004
AF3	11	73.3	4	26.7	.016

Effectiveness of Treatment on Severity of Lower Abdominal Pain

Before treatment lower abdominal pain was present in 86.7% and absent in 13.3%. After treatment (AT3) lower abdominal pain was absent in 100% while after follow up (AF3) lower abdominal pain was absent in 66.7% and present in 33.3%. As per McNemar's test, on comparison of the AT and AF with BT in reducing the severity of lower abdominal pain, p value could not be computed in AT3 due to high significance and AF3 was significant at the levels of $p< 0.01$.

Table 6: Effectiveness of treatment on severity of lower abdominal pain

	Lower Abdominal Pain				McNemar test (Comparison with BT)- p
	Absent		Present		
	N	%	N	%	
BT	2	13.3	13	86.7	
AT1	8	53.3	7	46.7	.031
AT2	14	93.3	1	6.7	.000
AT3	15	100.0	0	0.0	-
AF1	13	86.7	2	13.3	.001
AF2	11	73.3	4	26.7	.004
AF3	10	66.7	5	33.3	.008

Effectiveness of Treatment on Severity of Dyspareunia

Before treatment dyspareunia was present in 73.3% and absent in 26.7%. After treatment (AT3) dyspareunia was absent in 100% while after follow up (AF3) dyspareunia was absent in 66.7% and present in 33.3%. As per McNemar's test, on comparison of the AT and AF with BT in reducing the severity of dyspareunia, p value could not be computed in AT3 due to high significance and AF3 was significant at the levels of $p< 0.05$.

Table 7: Effectiveness of Treatment on Severity of Dyspareunia

	Dyspareunia				McNemar test (Comparison with BT)- p
	Absent		Present		
	N	%	N	%	
BT	4	26.7	11	73.3	
AT1	13	86.7	2	13.3	.004
AT2	15	100.0	0	0.0	-
AT3	15	100.0	0	0.0	-
AF1	15	100.0	0	0.0	-
AF2	12	80.0	3	20.0	.008
AF3	10	66.7	5	33.3	.031

DISCUSSION

Even though there are no exact references regarding CIN in our classics, certain area point towards similar condition that coexisted during the period such as- *Oupasargika vyadhi, Aganthu/ krimijanya roga, Granthi- Arbuda & Yoni vrana, Yoni roga* etc. In the context of *Yoni roga nidana* it is well explained that weak or very young woman having excessive coitus may suffer with *Yoni roga*.[7] Young age of first intercourse and pregnancy is considered as one of the most important risk factor of cervical cancer.[8] In addition, *Mithyahara, Brisha maithuna* (excessive sex), *Apadravya*[9] (foreign objects), *Adaksha purusha* (unhygienic sex by husband), *Sapathni* [10] (extramarital relationship) are all said to be involved in etiopathogenesis of HPV infection and CIN lesions.

It begins as an *Aganthu roga,* further leads to development of *Sopha* at the site of *Sroto vaigunya*, the cervix. Due to *Ahitha ahara seva* leads to *Agni mandhya* and *Ama* formation, which hampers the *Dhatu Pushti* and *Ojas,* further leads to *Oja kshaya*. Depletion of the *Vyadhi kshamatwa* or *Bala*, further aids the progression of the disease to cancer cervix.

Treatment of CIN was planned by giving prime importance to *Sadvritta*, improving *Vyadhikshamatwa* and definitive management through *Vrana sopha chikitsa*. *Sodhana* and *Ropana* are the prime modalities in *Vrana chikitsa*. *Bhandira churna* is an excellent drug which serves the purpose of *Vrana sodhana* and *Ropana*, via both internal administration as well as external application. *Pratisarana karma* and *Madhu sarpi* belongs to the 60 *Upakramas* which makes them the preferable treatment procedures in CIN

Discussion on Data Related to Response of Treatment

Pap smear and Colposcopy were the most reliable measurements of the effectiveness of treatment. These tests were conducted before the treatment and after follow up, at a time gap of 6 months.

Regarding Pap smear, Negative for Intraepithelial Lesion or Malignancy (NILM), inflammation, Atypical Squamous Cells of Undetermined Significance (ASCUS) and Low Grade Squamous Intraepithelial Lesion (LSIL) were the four grades. Before treatment 20% cases suffered from LSIL, 33.3% from ASCUS, 40% from inflammation and 6.7% were NILM. After the follow up 53.3% were in NILM grade i.e., got complete relief. 40% had persistence of inflammation and 6.7% were with ASCUS, while no cases reported with LSIL. As per Wilcoxon's signed Rank test the changes in Pap smear test after follow up was found to be statistically highly significant.

In Colposcopy, before treatment 20% cases were with CIN II lesions and 80% with HPV+CIN I lesions. After the follow up period 0% cases presented with CIN 2 lesions, 13.3% with HPV+CIN I lesions, 66.7% were with HPV infection and 20% got complete cure. As per Wilcoxon's signed rank test the result was of high statistical significance.

Associated Symptoms

Regarding the major symptom white discharge p/v, before treatment, 66.7% and 33.3% subjects suffered with severe and moderate discharge p/v respectively. After the third month of treatment (AT3), there were 0% cases with severe and moderate discharge, 40% with mild discharge while 60% were with absent discharge p/v. The treatment has shown a decline in the quantity of vaginal discharge and no cases were suffering from severe or moderate quantity discharge p/v. After follow up, the cases with mild and moderate discharge p/v were 53.3% and 26.7% respectively and no discharge p/v 20%. On statistical analysis both AT3 and AF3 were highly significant, which indicated sustained drug action

Before treatment vulval itching was present in 80% cases and absent in 20% cases. After third month of treatment (AT3) vulval itching was absent in 100% cases. After the third month of follow up vulval itching was present in 26.7% and absent in 73.3% which shows a slight increase during follow up period. But on statistical analysis both AT3 and AF3 were significant. Before treatment burning sensation was present in 73.3% cases and absent in 26.7% cases. After third month of treatment (AT3) burning sensation was absent in 100%. After third month of follow up burning sensation reappeared in 26.7% and remained absent in 73.3% cases, which was statistically insignificant. Before treatment lower abdominal pain was present in 86.7% and absent in 13.3%. After third month of treatment (AT3), lower abdominal pain was absent in 100% cases. Before treatment dyspareunia was present in 73.3% and absent in 26.7%. After treatment, dyspareunia was absent in 100% cases which reappeared in 33.3%. But on statistical analysis, both AT3 and AF3 were significant indicative of sustained drug action.

Probable Mode of Drug Action

In the present study *Bhandira churna* was used internally in combination with rice flour as pancake. The drug *Bhandira* being *Thikta katu rasa, Usna veerya, Laghu teekshna guna, Krimihara* and *Dushta vrana hara* [11] has special action in HPV infection and CIN lesions. Owing to its *Usna veerya, Katu vipaka, Deepana pachana* and *Amavata hara* property it improves the *Agni* and arrest formation of *Ama* in the body. Being *Katu* in *Rasa* and *Vipaka, Bhandira* is *Vrana avasadanakara, Vrana sodhana,* and *Sophajith.* The

drug was administered in combination with rice flour as pancake. *Shali* being *Madhura rasa, Kasaya anurasa, Laghu snigdha guna, Seeta virya* and *Madhura vipaka* the combination makes *Bhandira* more *Soumya* in property. The *Tridosha hara* property attained by this combination helps in checking the pathogenesis of CIN by correcting the macro and micro cellular abnormalities in cervical epithelium caused by the three *Dosha.*

Local treatment modality is inevitable as the disease manifest at the particular site- cervix. The drug *Bhandira* was used for *Pratisarana* in combination with *Madhu* and *Ghrita*. The *Laghu teekshna guna* of *Bhandira* along with *Madhu-ghrita* combination helps in easy penetration and healing at the cervical tissue. The *Kasaya madhura rasa, Yogavaahi, Sukshma margaanusari, Krimihara, Sleshma-visha- pittanut, Vrana- Sodhana- Sandhana- Ropana* property of *Madhu* and *Vata pitta hara, Vrana ropana* property of *Ghrita* aids in healing CIN lesions. Studies states the wound healing properties of honey which include stimulation of tissue growth, enhanced epithelialization, and minimized scar formation.[12] Ghee based formulations are also proven for wound healing.[13] Lipophilic action of ghee facilitates its transportation to a target organ and final delivery inside the cell, because cell membranes are also made up of lipids.

CONCLUSION

The drug *Bhandira* is highly effective in managing CIN, limiting its progression to CA cervix and to a certain extent in cure of CA cervix. The drug is also effective in reducing HPV infection, but not in totally removing HPV. HPV could not be totally eradicated. The drug being cheap and widely distributed in Kerala, is a cost effective and non-invasive preventive measure of CA cervix. Hence this formulation is a real boon to the whole medical community and a promising breakthrough in Ayurveda.

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