Section A-Research paper
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DEVELOPING AND STANDARDIZING AN AYURVEDIC FORMULATION FOR TREATING SURFACE WARTS

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ABSTRACT:

Background: A wart is the type of solid skin growth caused by the *human papilloma virus* (HPV).

Objective: The aim of the present investigation was to formulate and standardize an Ayurvedic Preparation for surface warts.

Patients and Methods: This clinical trial study are selected on people (Older than 10 years) living in Shirpur city, of Maharashtra, who had warts on their skin in 2018. The sample size will be 50 patients, 25people in the Ayurvedic formulation group and 25 in the placebo group. Group the participants were randomly divided into two group using4-block sampling. Photographs were taken from the warts of Intervention group using digital camera. Then formulation was applied on wart skin and instructed the patient repeated for Three month. All stages in the intervention group were similar to the placebo group. Placebo was used only linseed oil Patients' wart status in both groups was examined Two week and one month after by the physician in terms of improvement or lack of improvement. Data were analyzed using PSPP software version 18, chi-square test, Fisher's exact test, Mann-Whitney test and ANOVA for repeated measure.

Result: Based on the results of the above characterization methods, the optimized batch was selected and was further subjected to a clinical trial on a human volunteer with consent and stability studies at 30 ± 2 ^{0}C and 75 ± 5 % RH. The optimized formulation was found to reduce the wart significantly.

Conclusions: As peoples are unaware about the complications of warts with the increasing age (particularly in females) and compliance with body (non-harmful), majority of them ignore this problem, As it can cause serious health issues, removal of the same is at most important at early age, This research will give a promising breakthrough for the removal of warts which will be benefited to the society in near future.

Keywords: - Human papilloma virus (HPV), warts, ayurvedic formulation.

INTRODUCTION

Background: Warts are common dermatological lesions. They are hard lesions with skinny or gray - brown color and rough surface with a 5 mm of diameter that are single or multitudinous. They can join together and form plaquette and regular, bold and rough surface shapes. They are usually painless; however, they are painful when they are increasing or around the joints and nail or their surface are full of split. Precede studies showed that the prevalence rate of warts is dissimilar based on dissimilar age groups, population and periods of time. In two studies, the pervasiveness rate was 0.84% in the US and 12.9% in Russia. In school children and young adults, the pervasiveness rate was 12% in the UK and 24% in Australia. Warts are caused by layers of cells' infection with human papilloma virus (HPV). Human papillomavirus is a small double-stranded DNA virus (55 - 50 mm), which can infect squamous layers of cells and cause cell procreation.

The most ordinary consequence of HPV infection is to grow the warts. This tumor is created by pleomorphic viruses and can be created in different part such as Hand skin and Feet skin, genitelia skin and mucous, larynx and the mouth mucosa. The virus infects the basal layer of the epithelium. Stem cells can also be infected. However, viral replication happened in location where corneocytes have fully differentiated such as spinosum and granulosum. These viruses replicate inside of the cells since they do not have cover, they are resistant to drought, freezing and solvents. Based on the anatomical distribution, warts are found on the face, hands, nail, foot and genitalia. Warts are contact and inoculated lesions and transmitted by an indirect contact with contaminated materials or walking barefoot. By aging resistance against HPV will develop. Warts are ordinary in children and young adults and rare in the elderly. Some warts that are resistant to treatment and post- treatment relapsed and became more widespread. Histologically, warts have the same changes such as acanthosis and hyperkeratosis. Ordinary warts mainly be generated in children and usually on hands. (1) Common warts have been a frustration for both patients and clinicians since early Greek and Roman times.1 They can greatly affect a patient's quality of life by causing embarrassment, fear of negative appraisal by others and frustration caused by persistence and/or recurrence. Moderate to extreme discomfort is reported in 51.7% of patients, and social or leisure activities are affected to a moderate to extreme degree in 38.8%.2 Warts of the genital tract carry a much more ominous and pernicious threat for women in particular. It has been estimated that up to 70% of sexually active women become infected during their lifetime with human papillomavirus (HPV), the infective agent that causes warts.3-5 A causal role for HPV infections in cervical cancer has been documented beyond reasonable doubt. HPV DNA is present in virtually all cervical cancer cases worldwide, with it being detected in 99.7% of an international series of cervical cancers by genetic amplification techniques and in 100% of cases as confirmed by histological review.6,7 Cervical cancer is the most frequent ordinary cancer in developing countries and the second most ordinary cancer in women worldwide.3 The association between HPV infection and cervical cancer is so strong that HPV is now considered the first positive cause of any human cancer ever identified.3 This raises

the level of concerns over HPV prevention, screening and treatment from that of a nuisance condition to that of a major public health concern. Attention is beginning to focus on the viral factors that determine persistence and neoplastic progression to cancer and the possible role of HPV in other nongenital Cancer (e.g. Skin, upper aerodigestive tract. (2) Papillomaviruses are highly diverse, and likely occur in most mammals and birds. Hundreds of ‘‘PV types’’ have been detected in humans, the only intensively studied host. It took three decades of research of a large number of specialists and sequences from thousands of PV isolates to establish a database that allows us to propose a classification system that will likely be stable while more PV types will be found. Here we review this database and its interpretation by phylogenetic criteria that led to the taxonomic levels ‘‘family’’, ‘‘genus’’, ‘‘species’’, ‘‘types’’, ‘‘subtypes’’, and ‘‘variants’’. (3) The aim of the present investigation was to formulate and standardize an Ayurvedic Preparation for surface warts.

MATERIALS AND METHODS

Patients and Methods

A Randomized, Placebo-Controlled, Clinical Trial:

This clinical trial study are selected on people (Older than 10 years) living in Shirpur city, of Maharashtra, who had warts on their skin in 2018. The Inclusion criteria included person who had not received any drug for their warts treatment in the past two month and Exclusion criteria included pregnancy, oral contraceptive contraindication and cancer. A written informed consent was obtained from the subject or their parents to participate in the study. This study was conducted with assistance of ayurvedic Practioner. The sample size will be 50 patients, 25people in the Ayurvedic formulation group and 25 in the placebo group. Group the participants were randomly divided into two group using4-block sampling. Photographs were taken from the warts of Intervention group using digital camera. Then formulation was applied on wart skin and instructed the patient repeated for one month. All step in the intervention group were similar to the Control group. Control was used only linseed oil Patients’ wart status in both groups was examined one week and one month after by the physician in terms of improvement or lack of improvement. Data were analyzed using PSPP software version 18, chi-square test, Fisher’s exact test, Mann-Whitney test and ANOVA for repeated measure.

Results

Results of this study showed that there was no significant difference between the both groups in terms of gender and location of warts ($P > 0.05$). In terms of change in the Two week after the intervention, results showed that in 72% of the patients of the intervention group the warts were changed, but the control group (placebo group) showed no changes. Also, in follow-up Two month after the intervention, 92% of the warts in the intervention group were removed, whereas in the control group, warts were not removed ($P = 0.0001$). Based on ANOVA for the repeated data, the mean number of warts before, two week and three month after the intervention in the intervention group were 6.0 ± 5.1, 1.9 ± 2 and in the control group were 5.3 ± 5.1, 5.4 ± 5.0, and

5.5±4.9, respectively (P = 0.009). Also, Based on t-test, the mean number of warts in two week and three month after the intervention in both groups were significantly different (P = 0.0001).

Formulation Characterization

Table. I Formulation Characterization Acid value

Values	**1st month**	**2nd month**	**3rd month**
Acid value	0.2.5 to 0.2.6	0.4 to 0.5	0.4 to 0.5
Saponification value	29.22 to 24.56	27.22 to 22.56	29.22 to 22.25
Iodine value	27.89 to 13.37	27.19 to 13.65	26.42 to 13.62
Refractive index	1.478 to 1.479	1.474 to 1.477	1.475 to 1.476
Density	1.016g	1.08g	1.021g

From the above figure stability study of acid value indicates that the acid value was found as 0.2.5 to 0.2.6, 0.4 to 0.5, 0.4 to 0.5 which is indicate that the formulated product was showing better accelerated Stability.

Saponification value

From the above figure stability study of saponification value indicates that the saponification value was found as 29.22 to 24.56, 27.22 to 22.56, 29.22 to 22.25 which is indicate that the formulated product was showing better accelerated Stability.

Iodine value

From the above figure stability study of iodine value indicates that the iodine value was found as 27.89 to 13.37, 27.19 to 13.65, 26.42 to 13.62 which is indicate that the formulated product was showing better accelerated Stability

Refractive index

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Density

From the above figure stability study of density indicates that the density was found as 1.016g, 1.08g, 1.021g which is indicate that the formulated product was showing better accelerated Stability.

First month stability of sample lemon oil and linseed oil IR

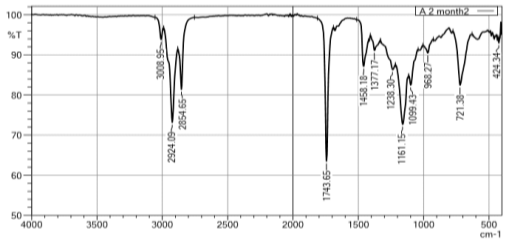


Table. II First month stability of sample lemon oil and linseed oil.

It shown of Anhydride (C=O), Alkene (C-H), Acid C-O, Alkene Stretch Aldehyde CH, Alkene bond present in sample. Describes the sample is stable and confirmed its stability**.**

Third month stability of sample lemon oil and linseed oil IR

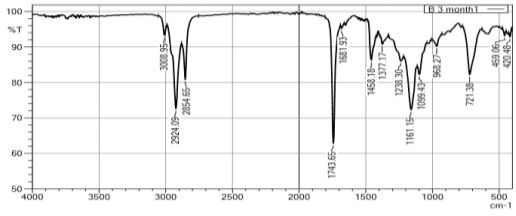


Table. III Third month stability of sample lemon oil and linseed oil

It shown of Anhydride (C=O), Alkene (C-H), Acid C-O, Alkene Stretch Aldehyde CH, Alkene bond present in sample. Describes the sample is stable and confirmed its stability**.**

Second month stability of sample lemon oil and linseed oil IR

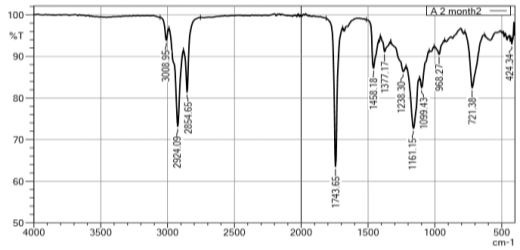


Table. IV Second month stability of sample lemon oil and linseed oil IR

It shown of Anhydride (C=O), Alkene (C-H), Acid C-O, Alkene Stretch Aldehyde CH, Alkene bond present in sample. Describes the sample is stable and confirmed its stability**.**

First month stability of sample lemon oil and linseed oil UV

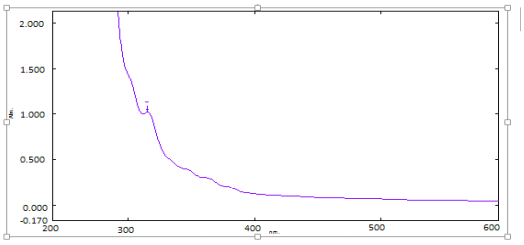


Table. V First month stability of sample lemon oil and linseed oil UV

This shows presence of Lemon oil after 1 month (λ max=314 nm)

Second month stability of sample lemon oil and linseed oil UV

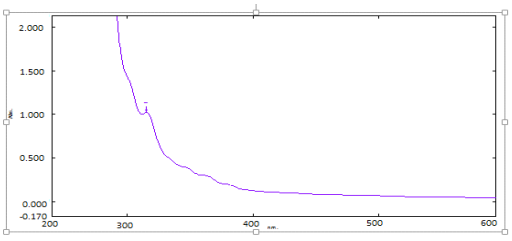


Table. VI Second month stability of sample lemon oil and linseed oil UV

No any change in the concentration of Linseed oil and Lemon oil

Third month stability of sample lemon oil and linseed oil UV

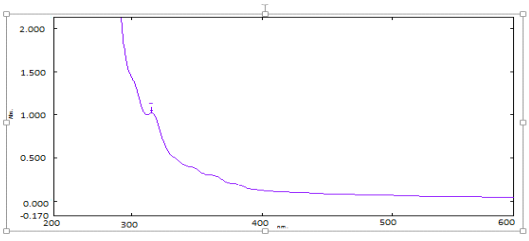
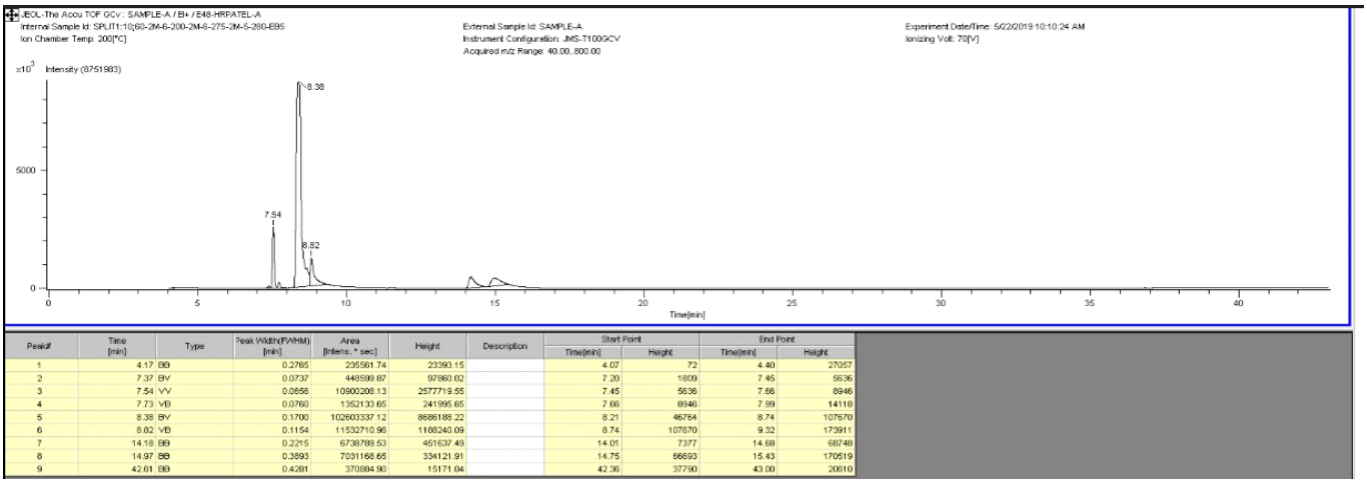


Table. VII Third month stability of sample lemon oil and linseed oil UV

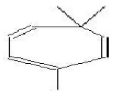
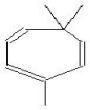
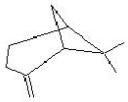
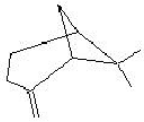
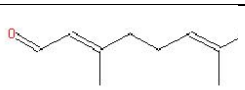
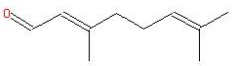
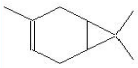
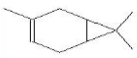
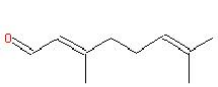
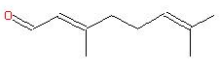
No any change in the concentration of Linseed oil and Lemon oil

Stability GC-MS



Peak#	Time [min]	Type	Peak Width(FWHM) [min]	Area [Intens. * sec]	Height	Description	Start Point		End Point	
							Time[min]	Height	Time[min]	Height
1	4.17	BB	0.2765	235561.74	23393.15		4.07	72	4.40	27057
2	7.37	BV	0.0737	448598.87	97860.02		7.20	1809	7.45	5636
3	7.54	VV	0.0656	10900208.13	2577719.55		7.45	5636	7.66	8946
4	7.73	VB	0.0760	1352133.65	241995.65		7.66	8946	7.99	14118
5	8.38	BV	0.1700	102603337.12	8686188.22		8.21	46764	8.74	107670
6	8.82	VB	0.1154	11532710.96	1168240.09		8.74	107670	9.32	173911
7	14.18	BB	0.2215	6738788.53	451637.49		14.01	7377	14.68	68748
8	14.97	BB	0.3893	7031168.65	334121.91		14.75	66693	15.43	170519
9	42.61	BB	0.4281	370894.90	15171.04		42.36	37790	43.00	20610

Table.VIII Stability of GC-MS

Before Stability	After stability	Before Stability	After stability
 Retention time : 7.37 min	 Retention time: 6.14 min	 Retention time : 7.54 min	 Retention time: 6.27 min
 Retention time : 8.15 min	 Retention time: 11.12 min	 Retention time : 8.39 min	 Retention time: 6.92 min
 Retention time : 14.98 min	 Retention time: 11.71 min		

In, Gas chromatographic profile Before and after stability, Retention given of constituents are as followed table (Before and After stability) the change in retention time of constituents may be due to Analytical parameter like, Flow rate of mobile phase, temperature variation, temperature rate, Analyst so stability study by GC-MS. Further need to be improved.

Table 1. Frequency Distribution of Variables in the Intervention and Control Groups

Variables	Intervention group	Control group	P value
	Gender		
Male	16	9	**0.15**
Female	09	16	
Age	25.32±2.6	25.64±2.6	
	Place of warts		
Hand	7	6	**0.33**
Hand and Face	10	11	
Hand and Foot	9	8	
	After Two-week treatment		
Changed	18	0	**0.001**
No changed	7	25	
	After Three-month treatment		
Removed	23	0	**0.001**
Not removed	02	25	

The values are presented as No. (%) or mean ± SD.

Table 2. Comparison of the Mean Number of Warts in Both Groups

Stage	Intervention group	Control group	P value
Before	6.0 ± 5.1(4)	5.3 ± 5.1(2)	**0.43**
After Two week	1.9 ± 1.2 (1)	5.4± 5.0 (3)	**0.002**
After Three month	0.4 ± 0.7 (0)	5.5±4.9 (3)	**0.001**

The values are presented as mean ± SD (Median)

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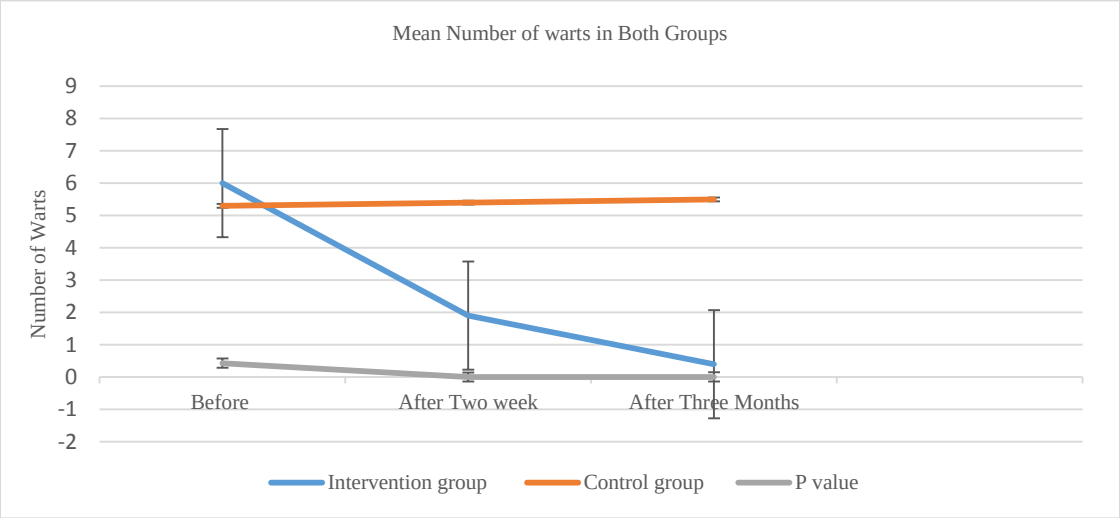
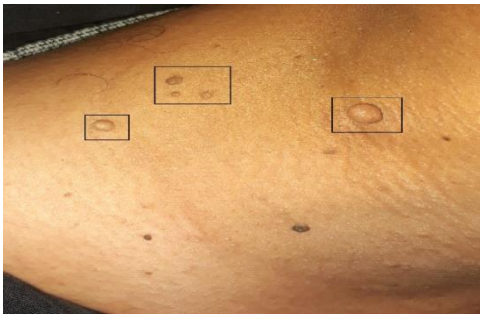


Figure.1 The Means Number of warts in Both Groups

Discussion

The important of this study was to determine the therapeutic effect of topical application of Natural oil on dermal warts. In this regard, this method is important because not any way of the treatment methods used to remove dermal warts had been completely effective. Moreover, treatment of dermal warts has more complications, while with Ayurvedic oil treatment would be more cost-effective with less complications. Our findings showed that in the Two week after the intervention, in 72 % of the patients in the intervention group, warts changed; however, there was no change in the warts' status in the control group. In the intervention group, 92 % of warts were removed Three month after, whereas in the control group no warts disappeared. In addition, in the intervention group, the mean number of warts in two week and three month after the treatment was reduced significantly compared to the Control group. No any side effects were observed in the Intervention group of the study. In this study, we observed that in the intervention group some of wart surface tissues became necrotic and finally the entire wart became necrotic and removed without leaving scar; therefore, we can say that the skin warts have improved by a similar mechanism.



(A) Before

(B) After

Figure: 2 The Patient's Neck in Intervention Group A Before, B, After

Conclusions: As peoples are unaware about the complications of warts with the increasing age (particularly in females) and compliance with body (non-harmful), majority of them ignore this problem, As it can cause serious health issues, removal of the same is at most important at early age, This research will give a promising breakthrough for the removal of warts which will be benefited to the society in near future.

Acknowledgments

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