

**RANDOMIZED OPEN LABELLED CLINICAL TRIAL OF HERBAL COOKIES AND HERBAL SOUP ON DYSMENORRHOEA – A GUJCOST SPONSORED MINOR RESEARCH PROJECT****Jasmine Gujarathi¹, Pravin M Ganorkar², Dilip Jani³, K Rukmini⁴**

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Article Received: 10/10/2020 - **Peer Reviewed:** 20/10/2020 - **Accepted for Publication:** 22/10/2020**ABSTRACT**

Introduction: Dysmenorrhoea is one of the gynaecological pathogenesis commonly observed in the society in young and adolescent girls. It is associated with painful cramps during menstruation of uterine origin with association of nausea, vomiting, constipation or diarrhoea. Various methods are practiced relieving pain and such symptoms but the search for herbal nutritive formulations to cure this is in search.

Methodology: The present clinical study was randomized open labelled clinical trial amongst 150 unmarried girls suffering from painful menstruation between age group of 13 to 25. Performa was prepared to analyse data and pain before and after treatment. The population samples were randomly divided into three group with *Rajpravartini Vati* as control group, another two groups were administered herbal soup and herbal cookies.

Results: Percentage relief in pain was 84.9% in cookies, 63.64% in soup group and 45.83% in *Rajapravartni* group. 51.35% girls showed complete relief in dysmenorrhoea in cookies group, 40.54% in soup group and 32.43% in *Rajapravartini* group.

Conclusion: Palatability and acceptability of herbal soup and cookies along with effectiveness in relieving painful menstruation was significant.

Keywords: Dysmenorrhoea, herbal cookies, herbal soup

INTRODUCTION

Dysmenorrhea is still an important public health problem which may have a negative impact on health, social environment, work and psychological status¹. Some of the studies have determined that prevalence of dysmenorrhea decrease with increasing age thus indicating that primary dysmenorrhea peaks in late adolescent by 20s and then the incidence falls with increasing age.² Prevalence studies also have shown several other factors that are associated with dysmenorrhea like body mass index (BMI), smoking, early menarche, prolonged menstrual flow and psychological disturbances.

According to a survey conducted by investigators of this project of about 798 girls of rural and urban areas, 47.02% of girls had dysmenorrhoea and 85.65% of girls missed school / college every month during menstruation in which 40.71% girls remained absent for whole day. The girls were reluctant to take any medications for the fear of side effect of allopathic medicines or palatability of Ayurveda medicines.³

The condition of dysmenorrhoea in young girls is explained in Ayurveda under *Udavartini Yonivyapad* which is similar to spasmodic dysmenorrhoea.⁴ With an aim to provide herbal nutritive, palatable and restorative formulations with properties of balancing *Vata Dosha* causing pain and relieving other symptoms, herbal soup and cookies were designed and clinical study was done on 150 girls.

Materials and Methods

Duration of Study: July 2016 to July 2018 (2 years)

Methodology for New preparations

New dosage form was prepared as herbal soup and herbal cookies from twelve herbs named *Sunthi*, *Jeeraka*, *Krishna Jeeraka*, *Pippali*, *Ajmoda*, *Dhan-yaka*, *Hingu*, *Garjara Beeja*, *Moolaka Beeja*, *Shatava-*

ri, *Yastimadhu* and *Kumari*. Quotations were invited from vendors and were procured after analysis. All herbs were evaluated for quality testing at G J Patel Institute of Ayurvedic Studies and Research, A & R Patel Institute of Biotechnology and Applied Studies (ARIBAS).

Pharmacognostical evaluation of all herbs done at pharmacognosy department of *Indukaka Ipcowala* College of Pharmacy (IICP).

Base material for soup and cookies were also procured similarly.

Methodology of Pharmacognostic evaluation:

Macroscopical studies of aerial parts of plant were done by naked eye and shape, color, taste and odor were determined and reported.

Pharmacognostical evaluation including histochemical study was carried out by taking free-hand sections according to Wallis and powder studies according to Evans. The section was stained with phloroglucinol and concentrated HCl solution and mounted in glycerine. Powder Sieve mesh 60 of the dried aerial parts was used for the observation of powder microscopical character. Photomicrographs were obtained by observing free-hand sections of drug under compound microscope.

Heavy metal analysis done at Sophisticated Instrumentation Centre for Applied Research and Testing (SICART)

Methodology of preparation of herbal soup and herbal cookies

All the herbs were powdered separately at Pharmacy of G J Patel institute of Ayurvedic studies and Research. The powders were prepared with the help of Pulvarizer and fine powder They were stored in airtight bags properly at room temperature. Batches of

laboratory scale soup and cookies were prepared at Food technology department of ADIT college. Sensory evaluation was carried out by a panel of ten judges. Hedonic rating test was employed using 9-point hedonic scale (from like extremely -9 to dislike extremely -1). Sensory parameters such as color, taste, texture and overall acceptability were evaluated. Nutritional evaluation was done for fat, protein and carbohydrate content. Scale up formulation of 110 kg cookies and 110 kg soup were prepared in Pharmacy of G J Patel Institute of Ayurveda studies and research.

Clinical Study

Criteria for Inclusion

1. Age group between 13 to 25 unmarried complaining of either painful and or irregular menstruation
2. Pain with scanty or average amount of menstruation

Criteria for Exclusion

1. Girls suffering from excessive menstruation
2. Girls below 13 years and above 25 years
3. Any chronic illness
4. Any uterine pathology like fibroid, adenomyosis, endometriosis

Posology:

Table 1: The dose and duration of all groups

	Group C (Trial group)	Group S (Trial group)	Group R (control group)
Formulation	Herbal cookies	Herbal soup	Tablet Rajapravartini vati
Dose	2 cookie approx 23 gm twice a day (2.5 gms herbal mixture in one serving)	30 gm twice a day (2.5 gms herbal mixture in one serving)	2 tablets (approx 500 mg) twice a day
Route	Oral	Oral	Oral
Duration 3 consecutive cycles	1 st cycle – 15 days before Menstruation 2 nd Cycle – 10 days before menstruation 3 rd Cycle – 7 days before menstruation		
Time period	Morning before breakfast – Evening before dinner		

Criteria of Assessment: To assess the effect of therapy, subjective scoring pattern was used with signs and symptoms of painful menstruation.

Statistical Analysis: Statistical evaluation of subjective and objective parameters was done with Sigma Stat Software by applying paired t test and unpaired t test.

The girls were provided with detailed information sheet and informed consent was taken after making them understand about the study. A detailed performa was prepared for data collection and analysis of the study.

Study Design

The present clinical study was randomized open labeled clinical trial. The randomization method used was simple random technique through numbering.

Ethical approval: The proposal of clinical study was approved by institutional ethical committee. IEC-3/GJPIASR/2015-16/1(15)

CTRI Registration: Clinical trial was registered prospectively. CTRI/2018/02/011980

Investigations: Before and after treatment Routine hematological investigations like complete blood count, differential blood count, haemoglobin, packed cell volume, serum calcium were done. **Urine:** Routine and microscopic examination

Ultrasonography (USG): USG for uterine and adnexal study was done only for cases suspected of any pathology.

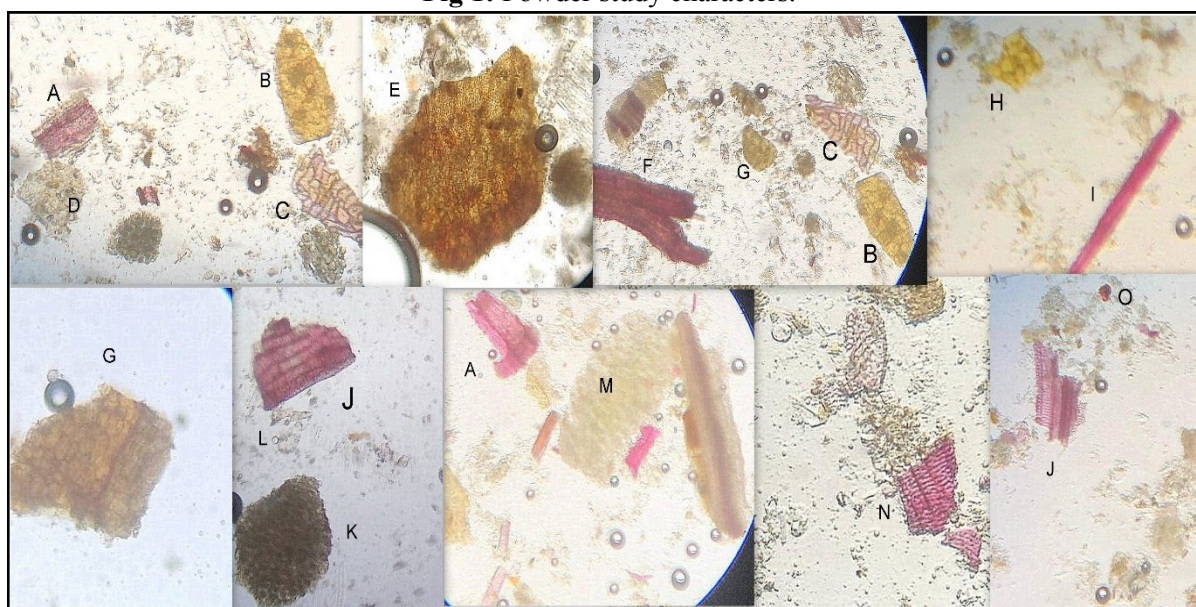
Observation and Results:

Standardization of Raw materials

Organoleptically all procured herbs were found to be as per API.

Pharmacognostical study of herbal mixture.

Fig 1: Powder study characters.



A: Lignified parenchyma of *Shatavari* Root **B:** Vittae of Ajmo **C:** mesocarp of carrot seed
D: Endosperm of Ajmo **E:** Cork of Liquorice Root **F:** Xylem fragments of Liquorice Root
G: Endocarp of Ajmo **H:** Cork of Ginger **I:** phloem fibre of liquorice root

J: Annular vessel of *Shatavari* root **K:** mesocarp of piper fruit **L:** starch grains of ginger
M: endosperm of Ajmo **N:** Scalariform xylem vessel of *Shatavari* Root

Finished Products

Table 2: Scale up formulation of cookies with base material

SN	Ingredients	100 kg Batch Size Quantity
1	Refined wheat flour	43 kg
2	Ayurvedic herbs blend	7 kg
3	Hydrogenated fat	25 kg
4	Sugar	25 kg
5	Skim Milk Powder	5 kg
6	Baking Powder	0.4 kg
7	Vanilla flavour (optional)	100 ml

Table 3: Scale up formulation soup with base material

SN	Ingredients	1 st batch size (50 Kg)	2 nd batch size (60 Kg)
1	Tomato powder	22.5	27
2	Ayurvedic herbal mixture	3.750	4.5
3	Corn starch	7.500	9
4	Sugar	10	12
5	Coriander	1.5	1.8
6	Dehydrated Onion powder	1.5	1.8
7	Dehydrated Garlic powder	1.5	1.8
8	White pepper	0.600	0.720
9	Salt	3	3.6
Total		51.85	62.22

Fig 2 Herbal soup and cookies Lab scale



Fig 3 Herbal soup and cookies final with packaging



Table 4: Proximate composition of scale up products

SN	Parameters	Tomato soup powder	Cookies
1.	Moisture content, %	9.15± 0.69	5.1 ± 0.2
2.	Ash, %	14.4± 1.2	3.2± 0.3
3.	Protein (%N x 6.25), %	4.7± 0.6	6.7± 0.6
4.	Fat, %	0.8± 0.2	30.4± 1.4
5.	Carbohydrate*, %	70.95	54.6

*Carbohydrate content is estimated by difference method

Heavy Metal Analysis of scale up products

Five heavy metals (Zinc, Lead, Cadmium, Arsenic and Mercury) were quantitatively analysed in both samples. Results are depicted in Table 7.

Table 5: Heavy Metal analysis of scale up products

Heavy Metal	Instrument Detection limit	Ayurvedic herbs incorporated cookies (mg/kg)	Ayurvedic herbs incorporated tomato soup powder (mg/kg)
Zinc, mg/L	0.0059 mg/L	9.4785	7.8588
Lead, mg/L	0.0420 mg/L	Not Detected	Not Detected
Cadmium, mg/L	0.0027 mg/L	0.0139	0.0200
Arsenic, mg/L	0.0530 mg/L	Not Detected	Not Detected
Mercury, mg/L	0.0610 mg/L	Not Detected	Not Detected

Clinical study observations

Table 6: Number of participants registered

	Group C (Cookies)	Group S (Soup)	Group R (<i>Rajapravartini</i>)	Total
Registered	50	50	50	150
Completed	38	45	35	118
Dropped out	2	2	0	4
LAMA (Left against medical advice)	10	3	15	28

Table 7: General information

Age in years	Group C (Cookies)	Group S (Soup)	Group R (<i>Rajapravartini</i>)	Total	%
13-19	10	13	14	37	24.66
20-25	40	37	36	113	75.34
Haemoglobin < 12 gm %	13	17	18	48	32%

Table 8: Onset of pain

Onset of pain	Group C (Cookies)	Group S (Soup)	Group R (<i>Rajapravartini</i>)	Total	%
Before menstruation	15	18	17	50	33.33
First day	41	43	44	128	85.33
1-2 nd day	0	0	3	3	2

Table 9: Site of pain in dysmenorrhea

Site of pain	Group C (Cookies)	Group S (Soup)	Group R (<i>Rajapravartini</i>)	Total	%
Low back	33	31	31	95	63.33
Hypogastrium	33	29	32	94	62.66
Thigh	21	20	21	62	41.33
Legs	28	23	22	73	48.66
Calf	3	1	3	7	4.66
Breast Tenderness	1	1	1	3	2
Vulva	1	1	6	8	5.33

53 (38.13%) girls remained absent during menstruation due to severe pain, whereas 24 girls (17.27%) took medications and other treatment to relieve pain. 29 girls (20.87%) girls suffered from pain during menstruation but continued their routine work, whereas in 33 girls (23.75%) menstruation was not painful.

127 (84.67%) girls complained of scanty menstruation, 95 girls suffered from abdominal pain (66.33%), 84 girls (56%) had irregularity in menstruation, 71 (47.33%) girls suffered from fatigue during menstruation, 55 (36.66%) girls also complained of anorexia at the time of menstruation.

Clinical Study Results

Chart 1: Comparative result in severity of pain

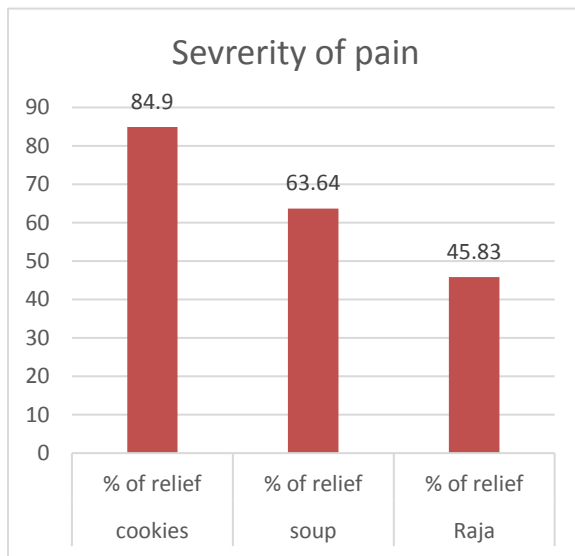


Chart 2: Comparative relief in duration of pain

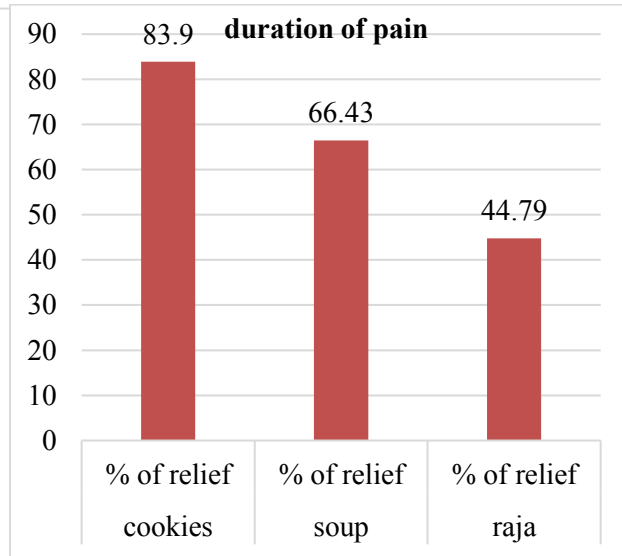


Chart 3: Comparative relief in associated symptoms

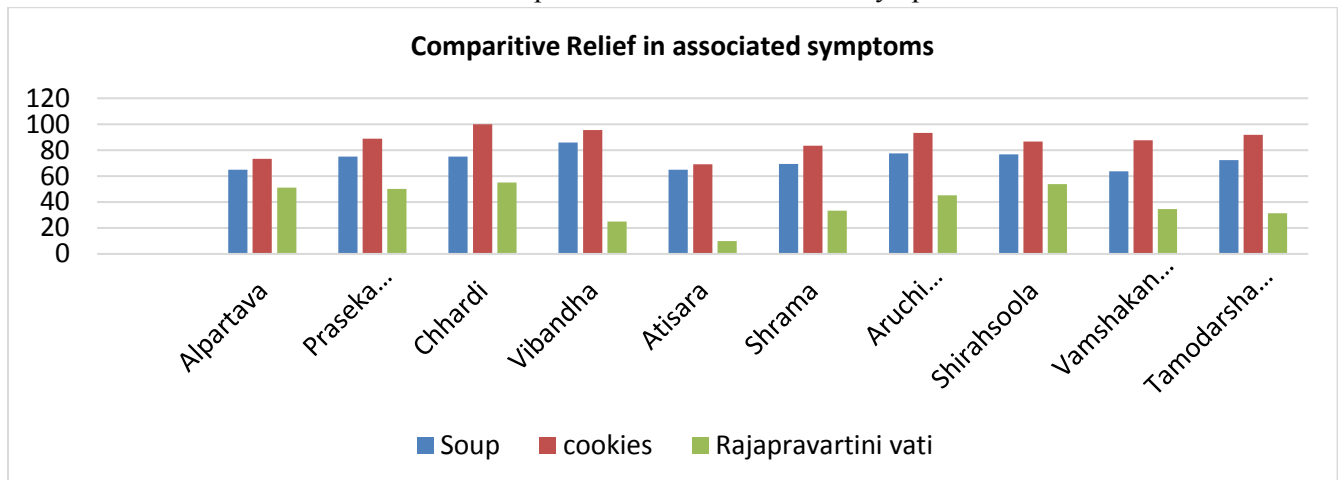
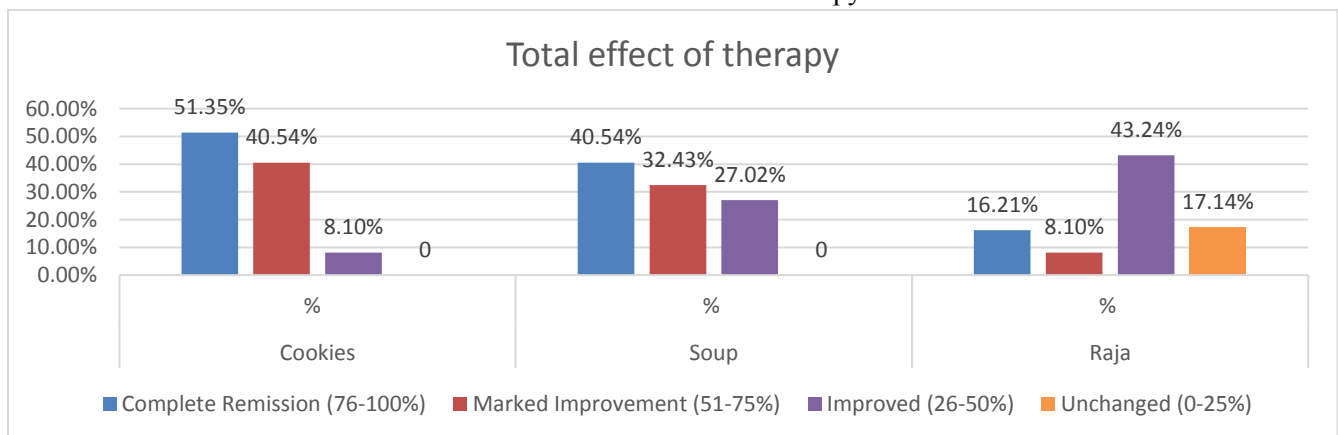


Chart 4: Total effect of therapy



Statistical evaluation of three groups on haematological parameters showed no significant change in soup and cookies group in haemoglobin values. Whereas in Rajapravartini group statistically significant (P=0.005) improvement was seen in haemoglobin levels. This is because of content ferrous sulphate in the preparation which is haematinic. Statistically significant improvement was also found in Mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration in cookies and soup group. This is because of haemopoetic factors present in herbal mixture as content in soup and cookies. The improvement in serum calcium levels was statistically significant in cookies group. Whereas, in soup and *Rajapravartini Vati*, the change was not significant.

DISCUSSION

To improve palatability of Ayurveda preparations and to design nutritional and restorative formulation, herbal cookies and herbal soup with common 12 Ayurveda ingredients were formulated, and clinical trial was done on Young girls with dysmenorrhea.

The cookies and soup group were readily acceptable by girls rather than *Rajapravartni Vati*. Total 15 girls out of 50 in *Rajapravartini* group left the treatment without information. They were reluctant to take tablets. In cookies and soup number of LAMA were 10 and 3 respectively. The new formulations also provided nutritional supplement and improvement in regularity of cycles. The mode of action of twelve herbs used as ingredients of new formulations is as under.

Table 10: Mode of action

Sr No	Name	Mode of action
1	<i>Sunthi</i>	Produces analgesic effect by COX -1 inhibition. ⁵ Its supplementation not only increases absorption of iron but also increases serum ferritin, total iron binding capacity which in turn corrects anaemia. ⁶ It is also rich in protein and has anti-inflammatory activity. ⁶
2	<i>Jeeraka</i>	It regulates menstrual function through hormonal balance as it has anti estrogenic properties, ⁷ maintains calcium levels in body and hence has anti osteoporotic effect. ⁸ excellent source of minerals like iron, copper, calcium, potassium, manganese, selenium, zinc and magnesium. It also contains very good amounts of B-complex vitamins such as thiamin, vitamin B-6, niacin, riboflavin, and other vital antioxidant vitamins like vitamin E, vitamin A and vitamin C. ⁹ It also shows anti spasmodic activity and hence used therapeutically for spasmodic pain as in dysmenorrhoea. ¹⁰
3	<i>Krishna Jeeraka</i>	Effective in relieving pain and inflammation through its antispasmodic property. ¹¹ Caraway seeds are enriched with many important nutrients like vitamin A, B-complex, C and E. Mineral contents such as calcium, magnesium, copper, iron, zinc, potassium, selenium and manganese ¹²
4	<i>Pippali</i>	It has anti spasmodic action as well increases bioavailability and absorption of other herbs. ^{13,14} It also has immunomodulatory, antioxidant and adaptogenic effect, improves digestion. ¹⁵
5	<i>Ajmoda</i>	It is used as a regulatory agent for women with irregular menstrual cycle by balancing estrogen and producing estrous effect in ovarian cycle. ¹⁶ Anti spasmodic effects of its extracts due to blockade of voltage dependent calcium channels. ¹⁷ Mineral elements such as calcium, potassium, magnesium, phosphorous, sodium, vitamin A, and niacin. ¹⁸
6	<i>Dhanyaka</i>	Antispasmodic activity ¹⁹ and induces estrous cycles for menstruation. ²⁰ also induces regular menstrual cycles. Also possess antioxidant activity. ²¹ It possesses analgesic activity. ²²
7	<i>Hingu</i>	It reduces contractile activity and hence causes antispasmodic effect due to its partial inhibitory effect on histamine (H1) and potent inhibitory effect on muscarinic receptor. ^{23,24} In other study it also showed relaxatory effect on pre contracted tissue. ²⁵
8	<i>Gajar Beeja</i>	It is antioxidant ²⁶ and possess nutritional value because of present of molybdenum helping in absorption of iron. Also is good source of magnesium, which is helpful for bone, protein, relaxing nerves and muscles ²⁷ The seeds have especially shown antifertility – abortifacient effect by inducing menstruation. ²⁸
9	<i>Moolaka Beeja</i>	Specifically shows antibacterial and antifungal activity invitro. It also shows antispasmodic activity. ²⁹

10	Kumari	It contains vitamin A B C E, Calcium, amino acids and enzymes. ³⁰ In vitro study data shows that it directly acts on enzymes 3βHSD modulating the flux towards estradiol formation. Hence for regularization of menstruation in cases with polycystis ovarian syndrome. ³¹
11	Shatavari	Known female rejuvenative, adaptogenic. Regularizes menstrual function because of presence of steroidal saponins Shatavarin I – IV which acts as phytoestrogen. It also contains vitamins A, B1, B2, C, E. It is source of magnesium, Calcium and Iron. ^{32,33} It shows estrogenic effect. ³⁴ One clinical data also shows effect of <i>Shatavari</i> in minimizing risk of postmenopausal osteoporosis. ³⁵
12	Yashtimadhu	Contains isoflavones, a type of phytoestrogen for regularizing menstrual function. It decreases serum testosterone on women and helps in reducing anaemia. ^{36,37}

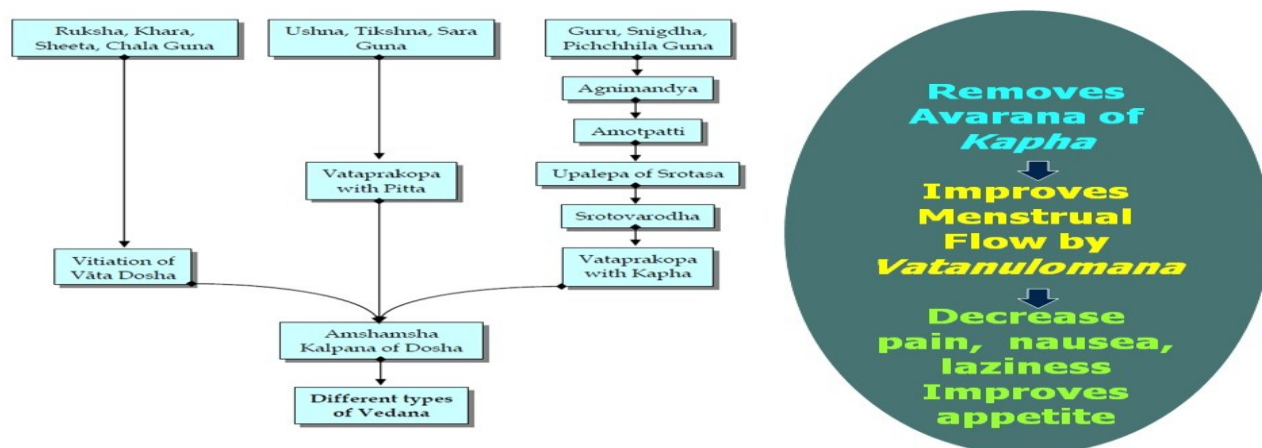


Fig 5: Pathology and mode of Action through Ayurveda concepts

CONCLUSION

Irregularity of menstrual cycle with abnormal BMI was observed with other associated symptoms of dysmenorrhea like scanty bleeding, fatigue, anorexia and others. Soup and cookies were palatable amongst girls and they were happy to take as a nutritional supplement also. Further studies can be done with large number of sample size as this was a pilot study of newly designed products.

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