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Pilot evaluation of safety and efficacy of an Ayurvedic formulation (AYUSH 64) for accelerating the recovery in cases of Influenza like Illness

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AYUSH 64, a polyherbal Ayurvedic formulation in Influenza like Illness: results of a pilot study

Abstract

Background: Influenza-like illness (ILI)refers to a wide range of viral infections with an important cause of morbidity and mortality worldwide. The global incidence of ILI is estimated at 5-10% in adults and 20-30% in children. In India influenza accounts for 20-42% of monthly acute medical illness hospitalizations during the peak rainy season. AYUSH-64, a poly-herbal drug, is in practice for 40 years for various clinical conditions like fevers, microfilaremia, and inflammatory conditions.

Objectives: A pilot study was conducted to evaluate the safety and efficacy of Ayurvedic formulation AYUSH-64 in clinically diagnosed ILI for accelerating the recovery.

Methods: A prospective, open-label, nonrandomized, single group, single-center pilot clinical study with pre-test and post-test design was conducted at Raja Ramdeo Anandilal Podar Central Ayurveda Research Institute for Cancer, Mumbai an institute of Central Council for Research in Ayurvedic Sciences (CCRAS) between June 2018 and July 2019. A total of 38 participants of clinically diagnosed ILI (18-65 years) were studied with the one-week intervention of 'AYUSH-64' in a dose of 3gm/day and three weeks post-treatment observation period. Assessment of parameters viz. improvement in the symptoms of ILI, frequency of usage of acetaminophen, antihistaminic and cough syrup, hematology, liver function and kidney function tests along-with incidence of secondary complications, and time to return to a normal routine was done.

Results: One-week intervention of AYUSH-64 helped to recover from ILI symptoms with reduced frequency of usage of acetaminophen and antihistaminic. The intervention was safe on hematology and biochemical parameters. No serious adverse effects were observed during the study.

Conclusion:

AYUSH-64 along-with standard care in ILI is safe and efficacious and this may use in other viral infections with pyrexia as add-on to standard care for early recovery and better outcome.

Key words: Influenza-like illness (ILI)**,** *Vata-Kaphaja Jvara*, AYUSH-64, Safety, Efficacy, Ayurveda Intervention

Conflict of Interest: Nil

Clinical Trial Registry of India (CTRI), number: CTRI/2017/10/010145.

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Introduction

Influenza is an important cause of morbidity and mortality worldwide including low- and middle-income countries (LMICs) in tropical regions like India where highest point estimates rates of Influenza-like illness (ILI) and influenza-associated ILI among young and the older adults[1]. ILI refers to a wide range of viral infections with clinical manifestation ranging from fever, malaise and other constitutional symptoms mostly depending upon tissue, organ affected, host defense, age of the patient, and the affecting strain of the virus and bacterial load. Management of uncomplicated ILI remains symptom-based rather than antiviral therapy where Antipyretic, antihistaminic or cough syrup is used for symptomatic relief which again has its own side effects. Patients are advised to take rest and to maintain proper hydration during illness and asked to return to full activity after the illness has recovered gradually. Viruses constantly change their antigenicity as a strategy for evasion from immune-mediated elimination.[2] The classical example is of influenza virus where this strategy leads to the emergence of new strains and the failure of previously generated immunity protect against fresh infections. This has led to the difficulties in generating vaccines against these infections. The unavailability of optimal medication which would effectively hinder the disease pathogenesis while easing the recovery process and simultaneously boosting the immune mechanism has probed the exploration of traditional and such other medicines to promote natural healing in ILI.

The clinical presentation of ILI is similar with *Vata-Kapha Jvara* (fever with predominance of *Vata and Kapha dosha*) as per the description of in Ayurveda classics.[3] The treatment of *Jvara* aims at digestion of *ama* (partially digested metabolic waste) through *langhana* (fasting/ restricted calorie intake) and formulations (single herb/ polyherbal) containing ingredients having *tikta rasa, ushna virya, katu vipaka* and *amapachaka* (anti-inflammatory) property.[4] Many botanicals which are documented in Ayurveda Pharmacopeia viz. *Swertia chirata* (*Kiratatikta), Alstonia scholaris (Saptaparna), Pircrorria kurroa (Kutaki)* etc. are traditionally used for fevers like malaria.[5]

AYUSH-64, a polyherbal formulation is in practice since 1980s in various conditions like fevers, inflammation and joint pains across CCRAS peripheral institutes. Its efficacy has been reported in conditions like *Vishamjvara* (malarial fever), *Shleepada* (microfilaremia) and chikungunya. During Malaria epidemic in Rajasthan and Assam in 1994 and 1996 respectively, AYUSH 64 was used in 3600 fever cases in Rajasthan and 2294 fever cases in Assam. A decline in infectivity rate was observed when AYUSH 64 was given to all fever cases along with anti-malarial, and a good response was reported in villages where AYUSH 64 administration was done on prophylaxis basis for fever cases. [6,7,8,9,10] *Vishamjvara* and *Vata-kapha jvara* vis-à-vis ILI are the types of *Jvara* and share common pathology, however *Vata* and *Kapha doshas* are predominant in ILI and manifest symptoms as *Shitaka* (Chills), *Gaurava* (Heaviness), *Parvaruka* (Pain in small joints), *Pratishyaya* (Cold/Catarrh), and *Kasa* (Cough), etc. The ingredients in AYUSH-64 are *Jwarahara* (useful in fevers), *Shwasa-Kaasaghna* (useful in asthma, cough), *Shothahara* (relieves inflammation) (Table in supplementary file). On this background this pilot study was aimed to evaluate safety and efficacy of AYUSH-64 in the management of clinically diagnosed ILI.

Methods

Participants

This 4-week single-center pilot study was conducted in a prospective, open-label, non-randomized, single group, pre-test- post-test design at Raja Ramdeo Anandilal Podar Central Ayurveda Research Institute for Cancer, an institute of CCRAS, Ministry of AYUSH, Government of India.

Patients who were clinically diagnosed cases with ILI having axillary temperature ≥38ºC and with at least two constitutional symptoms (headache, chills, myalgia or fatigue) and one respiratory symptom (cough, sore throat or coryza) with the onset of illness since not more than 36 hours, of either sex, age between 18- 65 years who voluntarily signed informed consent to participate in the study were included in the study.

Exclusion criteria were the following: Cases of bronchitis, pneumonia, pleural effusion, interstitial lesions, patients having white blood cells (WBC) count greater than the upper limit of normal (WBC>11.0x10^9L) and lesser than normal (WBC<4.0x10^9/L) or neutrophil count ≥75%, patients coughing purulent sputum or with suppurative tonsillitis, patients with uncontrolled diabetes, COPD, hepatic insufficiency (ALT or AST 2 times above normal or higher); renal

insufficiency (serum creatinine more than the upper lab value); chronic congestive heart failure, psychiatric diseases, patients who already have taken antiviral drugs or related traditional medicine after the onset /before the screening, Women in pregnancy or lactation period, women of childbearing age with a plan of a pregnancy, immune-compromised patients or taking immunosuppressant in last 3 months, with dubious or confirmed alcohol and drug abuse history, suffering from an acute respiratory infection, otitis media or sinusitis 2 weeks before, with history of vaccination for seasonal or new influenza A (H1N1) vaccine 6 months before.

The study was approved by the Institutional Ethics Committee of this institute and registered prospectively at CTRI (CTRI/2017/10/010145 Registered on: 23/10/2017). Recruitment of participants was done as per the inclusion and exclusion criteria after taking informed written consent from the eligible patients.

Intervention

The study drug AYUSH-64 was procured from Indian Medicines Pharmaceutical Corporation Limited (IMPCL) Almora, Uttarakhand, India. Each capsule of AYUSH 64 consists of *Alstonia scholaris* R. Br.(*Saptaparna)* Bark Aqueous Extract 100 mg., *Picrorhiza kurroa* Royle ex. Benth *(Katuki*) Root Aqueous Extract 100 mg., *Swertia Chirata* Pexbex. Karst (*Kiratatikta*) Whole-plant Aqueous Extract 100 mg., *Caesalpinia crista* L.(*Kuberaksha*) Seed powder 200 mg. Quality control and safety parameters of the ingredients and the formulation complied with API limits/ Inhouse limits as appropriate. (Quality Control Analysis enclosed as supplementary files)

The drug was given in a dose of 2 capsules (500mg) thrice daily i.e. 3 gm /day orally after food along-with water for 7 days from baseline. One of the authors, a specialist in Modern Medicine prescribed modern medicines viz. acetaminophen, antihistaminic and cough syrup as per the standard guidelines and monitored the patients along with Ayurveda physicians.

Outcome Measure / Efficacy Evaluation

The efficacy evaluation of the drug was done by assessing the ILI symptoms viz. fever, headache, myalgia, running nose, nasal obstruction / congestion, cough, sore throat, fatigue, chills / aversion to cold and sweating. The symptoms were assessed on a scale of 0 to 100 cm using VAS, where 0 represents no symptom and 100 represents the maximum severity of the symptom.[11] These symptoms were assessed at baseline, 3rd day, 7th day, 14th day, 21st day and

28th day. Improvement in the fever was measured as time to defervescence, the time from the first dose of study medication to the time when the body temperature declined to 37.4ºC or below & was sustained for ≥ 24 hours. Severity of the illness was assessed by computing the Area under the curve (AUC) for all the symptoms together. Average score of all the ten symptoms for each patient was computed for calculating the AUC. The area under the curve was evaluated between two consecutive time points. Integration approximating with the trapezoidal rule was used to calculate the areas.[12] The frequency of usage of acetaminophen, antihistaminic and cough syrup and incidence of secondary complications of influenza like illness e.g. bronchitis, otitis media, pneumonitis etc. and adverse reactions related to the intervention were noted at all visits. The time to return to normal state of health and activity was defined as the time (in hours) from study drug initiation to the first 24-hour period in which participants returned to their normal state and remained so for 24 hours . The participants were assessed for Ayurvedic variables with the interest to study diagnostic classifications according to Ayurveda. ~~(supplementary file- Study procedure) ?~~ (supplementary file- Study procedure)

Sample size and Statistical methods

As a pilot study, sample size of 30 + 25% drop out amounting to 38 was considered for study. . The data on discrete variables has been represented as n (%). The data on continuous variables has been represented as Mean (SD). The continuous data has been analyzed by using One-Way Repeated Measure Anova with Bonferroni correction in post hoc analysis. A p-value of <0.05 has been considered significant. The data was analyzed using SPSS Version 15.0.

Results

Between June 2018 and July 2019, a total of 46 participants were screened, out of which 38 were enrolled in the study. (Fig. 1) The patients were given AYUSH-64 for 7 days only and later observed for 21 days. Response assessment was done on day 3, 7, 14, 21 and 28.

Baseline data

Out of a total 34 participants, 14 patients were male and 20 were females. All the participants were residents of Mumbai. No participant was having diabetes mellitus, 01 participant was a known case of hypertension with ongoing medicine. Acute onset of disease was observed in 32 participants and 02 participants had insidious onset of symptoms of ILI.

Outcomes and estimation

Mean Visual Analogue Scale scores for all the ILI symptoms at different time points have been listed in (Table 1.) Statistically significant improvement was seen in all the ILI symptoms from day 3 onwards. Fever was completely relieved in 18 participants on 3rd day and in 10 participants on 7th day of intervention. At day 3, 16 patients took acetaminophen, 8 participants till 7th day and later 6 patients took and out of that only one participant needed it for fever, others had taken for headache or body ache.

Severity of illness as assessed by mean AUC for total symptoms score at 1st time interval of 3 days was 105.30 (95% CI: 94.49 to 116.12), at 2nd time interval of 4 days was 62.85 (95% CI: 48.89 to 76.81), at 3rd time interval of 7 days it was 60.81 (95% CI: 39.30 to 82.32), at 4th time interval of another 7 days it was 45.12 (95% CI: 25.80 to 64.45), further at the 5th time interval of 7 days it was 34.30 (95% CI: 15.77 to 52.82). This shows a continuous decrease in the severity of illness with the passage of time. (Graph as supplementary file)

Antihistaminic and cough syrup were taken by 6 participants till day 7. Normal health was achieved by 6 participants on day 3 while 17 participants achieved it on day 7 and 3 participants on day 14. (Table 2).

Ayurveda variables viz. *Shitaka* (Chills), *Gaurava* (Generalized heaviness), *Tandra* (Lethargy), *Staimitya* (Feeling as if wrapped up / numb), *Parvaruk* (Pain in joints), *Shirograha* (Headache), *Pratishyaya* (Cold), *Kasa* (Cough), *Swedapravartan* (Absence of sweating), MadhyavegaSantapa (Mild fever) seen to have sharp decline by the 7th day of intervention and slow decline in the post treatment phase. (Fig.2)

Hematological parameters including Liver Function Test and Kidney Function tests were within the normal limits during the treatment period and no significant change was observed in either of them. Secondary complications of ILI were not observed during the study period in any participant. No serious adverse reactions related to the intervention were reported in any participant.

Discussion

ILI refers to a wide range of viral infections, quite common in children and old people due to lowered immunity. The conventional management of ILI depends on severity, mild to moderate cases of category A and B are managed by acetaminophen, antihistaminic and severe cases of category C are treated with antiviral, antibiotics, intravenous fluids and other supportive care.[13]

In an internet-based cohort study, it was found that the quality-adjusted life-day (QALD) loss was found to be over three times higher for ILI than for Acute Respiratory Infections.[14] Early recovery is needed for returning to jobs / works along-with arrest to spread of infection. The innate immune response plays a crucial role in the viral elimination and recovery from disease.

It is reported that the symptom scores in influenza infections are directly correlated with IL-6 and IFN-α.[15] Significant production of IL-6, TNF- α, IFN- α, IFN-γ and IL-10 occurs in response to community acquired influenza A illness.[16] Bian J et al has noted that the levels of interleukin (IL)- 6, IL-33 and tumor necrosis factor (TNF)- α were significantly higher in Influenza A patients while IL-6, IL-17A, IL-29, interferon (IFN)- γ and interferon gamma-induced protein (IP)-10 were significantly higher in influenza B patients.[17]

Multiple host-based intervention strategies against influenza are under development and these offer advantages over conventional antivirals. New therapeutic option in the treatment of Influenza infection will be targeting virus-induced metabolic changes to restore host normal metabolism, and research in the immunometabolism field along with studies on modulating immune response.[18] A class of herbal medicines, known as immunomodulators vis-à-vis *Rasayana*, modulate the secretion of multiple cytokines.[19,20,21]

The botanicals in AYUSH-64, reported to have anti-inflammatory, immunomodulatory activity. The aqueous bark extract of *Alstonia scholaris* in BALB/c mouse induced the cellular immune response at 50mg/kg body weight once a day for 7 consecutive days while at 100mg/kg body weight inhibited the delayed type of hypersentivity reaction.[22] Yun-Li Zhao et al studied the effects of indole alkaloids and total alkaloids of *Alstonia scholaris* on post infectious cough in mice and airway inflammation in rats respectively. Indole alkaloids shown down-regulation of inflammatory cells, cytokines (IL-6) and the balance of antioxidants. Total alkaloids inhibited the production of inflammatory cytokines TNF-α and IL-8 in bronchoalveolar lavage fluid and lung.[23,24]

The biopolymeric fraction RLJ-NE-205 from the rhizomes of *Picrorhiza kurroa* improved the immune system of mouse through increase in the proliferation of lymphocytes and cytokine levels (IL-4 and IFN-gamma) in serum, phagocytic index and CD4/CD8 population.[25] Pretreatment with *Picrorhiza kurroa* rhizome extract exhibited anti-inflammatory activity through the suppression of macrophage-derived cytokines (TNF-α, IL-1β, IL-6, IL-10) and mediators via suppression of NF-κB signaling.[26] Picroside II, active ingredient from *Picrorhiza*

scrophulariiflora showed the promising effects of anti-inflammation in cells and animals through decrease in concentrations of TNF-α, IL-1β, and IL-6. It suppressed the activation of p65 NF-κB signaling pathway compared with lipopolysaccharide (LPS) stimulation. The pathologic changes of lung tissues had been alleviated and lung wet/dry weight ratio was decreased after Picroside II treatment.[27]

Swertia chirata is found to inhibit NF-kB/DNA interactions and also reduced pro-inflammatory IL- 8 expression in cystic fibrosis cells at IC_{50} concentrations.[28] Bellidifolin and Swerchirin, the two main xanthones from *Swertia chirata* inhibit the production of the proinflammatory cytokines IL-6 and TNF- α. Bellidifolin potently inhibited the prostaglandin E_s (PGE_2) by suppressing the protein expression of cyclooxygenase-2 (COX-2).[29] The $CHCl_3$ soluble, crude extract of the whole *Swertia chirata* inhibited the expression of Viral protein R (Vpr) in Hela cells harboring the TREx plasmid encoding full-length Vpr (TREx-HeLa-Vpr cells).[30] Crude extract of *swertia* plant showed antiviral properties against Herpes simplex virus type-1.[31]

Administration of methanolic extracts of *Caesalpinia crista* reduced TNF- α, IL-1β and IL-6 mRNA expression in hippocampus and the frontal cortex brain areas in rats.[32] *C. bonducella* seed extract produced dose dependent increase in both the parameters, i.e. antibody production and delayed type hypersensitivity in rats indicating promising immunostimulant properties.[33] A water-soluble gluco-arabinan isolated from the alkaline extract of the endosperm of seeds of *Caesalpinia bonducella* showed immunostimulant activity through splenocytes and thymocytes activations.[34] Whole ethanolic seed extract of *Caesalpinia bonducella* seeds in experimental albino rats displayed anti-inflammatory, antipyretic and analgesic activities.[35,36]

As per the ayurvedic understanding, the weakened *Agni* in *Jvara* leads to formation of *Ama (*proinflammatory undigested substance*)* leading to *amavastha* that results into weak immune status. The ayurvedic management aims at correction of *Agni* status through *Agnideepana* and *Amapachana* resulting into *niramavastha* (devoid of ama) i.e. reversing the pathology and improving the immune status. The ingredients in AYUSH-64 are having *Tikta rasa* which is *Agnideepak and Amapachak* (anti-inflammatory). AYUSH 64 acts as *amapachaka* and converts the status from *sama* to *nirama. Niramavastha* results into restoring the normal metabolism and strengthening immune status.

In this study the declining trend in symptoms of ILI is seen from the 3rd day of intervention to 7th day. The combination effect of botanicals is *Jwarahara* (relieves fever), *Kasahara* (useful in

cough), and *Shwasahara* (useful in asthma). This effect is seen from the reduction in the requirement of acetaminophen from 3rd day onwards after the intervention and minimal use of antihistaminic and cough syrup during the study period. The recovery time and returning to normal life was 3rd day in 6 patients, 7th day for 17 patients and 14th day for 3 patients. The seven-day treatment of AYUSH 64 along-with standard care has shown reduction in symptoms of majority patients. Symptoms like fever, *svedapravartana*, *shitaka*, *staimitya*, *tandra*, *parvaruk*, *kasa* have shown sharp decline by 7th day of intervention. The post treatment follow-up period has seen mild symptoms and requirement of antihistaminic and acetaminophen in 7 patients. Declining trend was seen in all the symptoms of ILI with fairly early recovery, however a longer duration of AYUSH 64 may have shown better results in terms of the restoration of metabolism and further strengthening the immune status. As far herb-drug interaction is concerned, AYUSH 64 was used along-with standard antimalarial treatment in earlier malaria epidemic. In this study the seven-day administration of AYUSH 64 along-with standard care was tolerated well and no adverse events were recorded.

Limitations

This is single-group pretest-posttest design study with a small sample size and was focused on clinically diagnosed ILI condition with only clinical safety related biochemical parameters. However, for substantial evidence, a larger multicentric study with a robust design with standard diagnostic investigations and cytokine response will be helpful to validate the efficacy of this drug.

Conclusion

AYUSH-64 along-with standard care in ILI is safe and efficacious and it may be used in other viral infections with pyrexia as add-on to standard care for early recovery and better outcome.

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Table 1. Effect of trial drug on chief complaints

Chief Complaints	Assessment Stages					
	Baseline	3rd day	7th day	14th day	21st day	28th day
Headache	57.65 (32.127)	29.12 (24.385)**	10.88 (14.846) **	10.44 (18.273) **	4.85 (8.302)**	6.91 (14.356) **
Myalgia	67.35 (26.975)	31.32 (28.744) **	11.03 (15.801) **	11.03 (18.537) **	4.85 (8.918) **	7.21 (14.934) **
Running Nose	33.68 (32.921)	15.29 (25.254) **	8.09 (20.114) **	5.00 (15.076) **	5.15 (13.953) **	4.12 (10.185) **
Nasal Obstruction / Congestion	36.62 (32.769)	18.97 (21.943)*	6.47 (14.063) **	5.29 (11.930) **	6.62 (15.750) **	2.65 (6.989) **
Cough	36.91 (29.362)	21.91 (27.192)*	17.50 (23.940) **	9.71 (19.304) **	11.76 (24.676) **	5.15 (14.061) **
Sore Throat	45.88 (31.754)	17.79 (22.737) **	12.65 (20.197) **	8.24 (16.091) **	7.94 (17.927) **	3.97 (12.540) **
Fatigue	73.38 (17.655)	42.35 (26.834) **	23.68 (24.812) **	14.56 (19.124) **	9.85 (13.953) **	9.26 (13.714) **
Chills	39.12 (31.273)	8.97 (17.046) **	2.50 (6.770) **	1.47 (4.357) **	1.03 (4.569) **	0.59 (2.388) **
Sweating	33.53 (29.427)	11.76 (18.459) **	2.94 (7.084) **	4.03 (10.656) **	1.53 (4.907) **	1.32 (4.816) **

Values have been expressed as Mean (SD). *p-value <0.01, **p-value<0.001

Table 2. Effect on other parameters

Assessment Criteria: n	Baseline	Day 3	Day 7	Day14	Day 21	Day 28
Frequency of use of Acetaminophen	1	16	8	1	3	2
Frequency of use of Antihistaminic	2	5	3	0	1	0
Frequency of use of Cough syrups	-	1	3	0	0	0
Time to return to normal health	-	6	17	3	5	0
Complete defervescence	-	18	10	4	1	0

FIG.1. CONSORT 2010 Flow Diagram

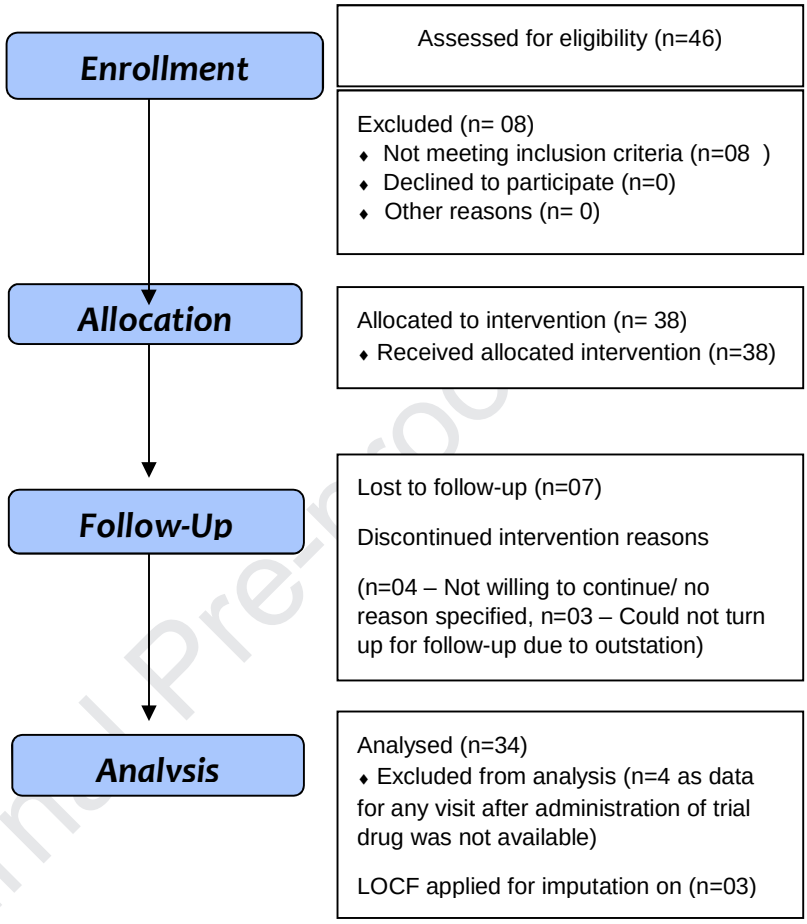

Fig 2: Effect on Ayurvedic Parameters