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The Study Protocol for Evaluation of the Concept of Anupana as Bioavailability Enhancer Effect with Special Reference to SaindhavaLavana along with Haritaki (TerminaliaChebula Retz.)

Jaydeep Kumar Sahu[1], Renu Rathi[2]

[1]PhD scholar , Department of Dravyaguna, Mahatma Gandhi Ayurved Collage, Hospital &Research Centre, Salod(H) , DattaMeghe Institute Medical Sciences, Wardha
[2]Professor, Department of Kaumarbhritya, Mahatma Gandhi Ayurved Collage, Hospital &Research Centre, Salod(H) , DattaMeghe Institute Medical Sciences,Wardha

Corresponding Author:
DrJaydeep Kumar Sahu. M.D (Ayu) , PhD scholor , Department of Dravyaguna, Mahatma Gandhi Ayurved Collage, Hospital &Research Centre, Salod(H) , DattaMeghe Institute Medical Science Wardha
Email ID-drjaydeepsahu26@gmail.com
Orcid ID- 0000-0001-5941-0934

Abstract:

Background:*Ayurveda* medicines are prescribed to be taken with various media of intakes like warm water, honey, ghee, milk etc. Such substances for taking medicines are called as *Anupana.* It is also known as vehicle or adjuvant. It may enhance the absorption, action and therapeutic effect of the principal drug. **Objectives:** The present study aimed to evaluate the bioenhancer activity of*Saindhavalavana* (Rock salt) as a*Anupana* of *Haritaki* (*Terminaliachebila* Retz.). **Material and methods:** The present study is a experimental study in which 30 healthy volunteers will be enrolled in two groups. In group A *Haritaki* (*Terminaliachebula*Retz.) fruit *Kwatha*(Decoction)will administered as control and group B will administered *Haritakikwatha*along with *Saindhavalavana*as trial. The dose of *kwath* should be 48 ml and 3gm of *Saindhavalavana* as *Anupana* once after breakfast. At a predetermined interval of 0, 30, 60, 90, 120, 180, 240, 300 and 360 min after administration of *Haritaki*, (2ml) blood will be collected from pre-inserted cannula in the vein and immediately transferred to the EDTA tube. Then blood serum will be collected and analysis will be done. Objective parameters like Phenolic compounds will be assessed through HPLC method. With the help of unpaired t-test using SPSS statistics software and p values less than 0.05 will be noteworthy.**Result and Discussion:** Results and observation will be presented in the form of graph, chart and tables with statistical values of significance. The obtained values will be discussed with suitable scientific interpretations. **Conclusion:** Suitable conclusion will be drawn from the obtained observation and results.

Keywords: Anupana, Bioavailability enhancer, Haritaki, SaindhavaLavana, Varsharitu

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INTRODUCTION:

Ayurveda medicines are prescribed to be taken with various media of intakes like warm water, honey, ghee, milk etc. Such substances for taking medicines are called as *Anupana.* It is also known as vehicle or adjuvant.[1]*Anupana* is complimentary substance taken afterwards or along with the principal drugs. May be it enhances the absorption, action and therapeutic effect of the principal drug. Certain drug may act specifically and effectively when administered with specific *Anupana*. [2]. The *Anupana* is claimed to distribute the drug throughout the body within no time as '*Yogavahi',* serve as catalytic agent. It spreads like oil drop on water i.e spreads in all directly swiftly. The drug will be reaching all parts of the body by its strength and potency. *Anupana* facilitate the bioavailability as well as relieve the side effects of medicine. They help to act direct effect of medicine to the deeper and subtler tissue to the body. According to *AcharyaSharangadhara,* the *Anupana*helps in spreading the drug very fast in body like the oil spreads over the water[3]. Importantly, any specific drug will exhibit a therapeutic effect when it maintains the minimum effective concentration in systemic circulation. Therefore, the action of specific drug can be enhanced by increasing its bioavailability. That means, increasing the bioavailability, reduces the dosage regimen, reduces the drug toxicity and importantly reduces the regular consumption of expensive drugs. This unique but important concept of *Ayurveda* would helpful to retainthe effective concentration of drug in systemic circulation in order to increase the bioavailability of active drugs.

The concept of bioavailability enhancer (*Anupana*) has not been reported along with any active *Ayurveda* medicines to confirm their importance while co-administration with active drug.Bioavailability enhancer term first time used by Indian scientist at Regional Research Laboratory, Jammu which is now known as the Indian Institute of integrative Medicine. In 1979 Piperine was discovered and scientifically validated as the world's first bioavailability enhancer.[4]Bioavailability enhancer themselves do not show any typical drug activity alone or used together, these are drug facilitators, they enhances the activity of drug molecules in several ways including increasing bioavailability of the drug. A 'bioenhancer' is capable of increasing bioavailability and bioefficacy of a particular drug with which it is combined, without any typical pharmacological activity of its own at the dose used. These are also termed as absorption enhancer, which are functional excipients included in formulations to improve the absorption of a pharmacologically active drug.

Bioavailability of a drug is defined as the amount or percentage of drug that is absorbed from a given dosage forms and reaches the systemic circulation following non-vascular administration[5].The concept of bio enhancer was reported in Ayurvedabut no one has attempted it practically to understand its effects of active metabolites from *Ayurvedic* medicines. Supporting the similar work performed by Bose et al in 1929, reported the increasing anti-asthamatic effect of *Vasaka*leaves after adding *Pippali* to it[6].

Hence, the objectives of this study is to understand the effectiveness of selected *Anupana* and its interaction with studied active components from species; *Terminaliachebula*Retz. (*Haritaki*).

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AIM AND OBJECTIVES

AIM: Evaluate the bioavailability enhancer effect of *Saindhavalavana* with *Haritaki(Terminaliachebula*Retz.*).*

OBJECTIVES:

1. To asses and characterize chemical constituents of *Haritaki(Terminaliachebula*Retz.*) kwatha* by using HPLC- diode array detector (DAD) technique.
2. To asses and characterize chemical constituent of *Haritaki(Terminaliachebula*Retz.*) Kwatha* with *Saindhavalavana* by using HPLC- diode array detector (DAD) technique.
3. To evaluate and compare the bioavailability enhancer effect of *Haritaki (Terminaliachebula*Retz.*) with anupana (Saindhavalavana)* and without anupana in *Varsharutu*(Mid July to mid September)

METHODOLOGY:

Study design: Prospective,randomized controlled experiment on healthy volunteers.

Study setting: Healthy volunteers will be recruited from Swastharakshan O.P.D of Mahatma Gandhi Ayurved College, Hospital and Research Centre, Salod(H), Wardha (Maharashtra).

Registration no: CTRI/2020/05/025061

IEC no:DMIMS(DU)/IEC/Sept-2019/8335

Inclusion criteria:-

Volunteers of either sex age group between 20 to 40 years and having liver function tests, renal function tests, lipid profile, blood report within normal limits.Also volunteers shouldnot have history of any major illness and Hypertension,BMI index should be 18.5 to 24.9 will be included in this study.

Exclusion criteria:-

Volunteers with any medication or any acute or chronic disease, history of drug or alcohol abuse, liver, kidney, disorders, hypertension, diabetes and BMI index less than 18.5 and more than 24.9

Interventions:

Sample selection technique: - Internet generated random number table.

Posology:-The *Kwatha* of fruit will be prepared as per reference mentioned in *Sharangadharasamhita.* One part of drug with eight part of water will be boiled in low flame and $1/4^{th}$ will be collected.[7]

i. **Dose:**1pala (48ml)
ii. **Anupana:***Saindhavalavana*- 1 shana(3gram) [8,9]
iii. **Period of intervention:-** Once after food

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Table 1: Sample size, grouping and intervention					
GROUP	**NO OF PARTICIPANTS**	**RECEIVING DRUG**	**RUTU**	**Route**	**Duration**
A	30	*Haritaki* (positive control)	*Varsharutu*(Mid July to mid September)	Oral	Once
B	30	*Haritaki* with *Saindhavalavana*	*Varsharutu*(Mid July to mid September)	Oral	Once

Volunteers will be randomly divided into two groups. Each group will be consisting of 30 participants. Group A will receive only *Haritaki* fruit *kwatha*in *Varsharutu*as a positive control. Group B will receive *Haritaki*fruit *kwatha*with *Saindhavalavana*in*varsharutu*. The prepared drug will be administered orally.

At a predetermined interval of 0, 30, 60, 90, 120, 180, 240, 300 and 360 min after administration of *Haritaki*, (2ml) blood will be collected from pre-inserted cannula in the vein and immediately transferred to the EDTA tubes. Then the serum will be separated and sent for analysis.[10]

Criteria for discontinuing or modifying allocated interventions: The subject will be withdrawal from the study, if it found to be unanticipated incidence characters of drug sensitivity or any adverse reaction arises. The cure will be offered free of cost till the symptoms disappears.

Follow up: 2nd day after drug administration, if any complaint raised by volunteers.

Primary outcome: We are going to evaluate the bioenhancer effect of Rock Salt on *Terminaliachebula* Retz. Fruit.

Secondary outcome: We will see any other effect of Rock salt on *Terminaliachebula* Retz. Fruit during the study period.

Statistical Analysis: The obtained data will be analysed by using unpaired t-test using SPSS statistics software.

Time duration till follow up: the volunteers will be under observation till the last sample collected and will be examined next day if any complaint or adverse effect of drug is observed.

Time schedule of enrolment, interventions: Volunteers enrolled in the present study should fulfil the inclusion criteria and undergo investigation of liver function tests, renal function tests, lipid profile report from Mid July to mid September (*Varsharutu*).

Sample Size: 30 per group

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The sample size calculation for a bioavailability and bioequivalent study depend onmultiple factors like power, intra subject coefficient of variation, expected geometric mean ratio. According to C. Bhupathi and V.H. Vajjha. (STATISTICA, anno LXXVII, n. 1, 2017),power of 85% would be reasonable for a bioavailability study to conduct on healthy volunteers. By considering the values of Lower Bound (LL) =0.80, Upper Bound (UL)=1.25, Alpha=0.05, Geo Mean Ratio (GMR)= 0.947, Coefficient of Variation (CV) = 0.239 as fixed, the sample size can be calculated. So, 30 individuals are considered for one group based on 85% power.
Recruitment: The patient will be enrolled by randomly through computer generated number.

Implementation: The principal invigilator will allocate and round up the Volunteers.

Methods: Data collection, management and analysis.

Data collection methods: Research Proformafill-up by interview, examination and lab analysis report.(Attached Annexure 1)

Table 2: Blood collection time

Group	**Blood collection time in minutes after administration of drug**								
A	00	30	60	90	120	180	240	300	360
B	00	30	60	90	120	180	240	300	360

Objective criteria: Phenolic compounds will be assessed from blood serum through HPLC method.

Data management: The coding of patients and samples will be done by principal investigator.

Table 3: Coding of blood sample of Group A

Group A (n=30)	**Blood collection time in minutes after administration of drug**								
	00	30	60	90	120	180	240	300	360
A1	A1-00	A1-30	A1-60	A1-90	A1-120	A1-180	A1-240	A1-300	A1-360
A2	A2-00	A2-30	A2-60	A2-90	A2-120	A2-180	A2-240	A2-300	A2-360
A3	A3-00	A3-30	A3-60	A3-90	A3-120	A3-180	A3-240	A3-300	A3-360
A4	A4-00	A4-30	A4-60	A4-90	A4-120	A4-180	A4-240	A4-300	A4-360
A5	A5-00	A5-30	A5-60	A5-90	A5-120	A5-180	A5-240	A5-300	A5-360
A6	A6-00	A6-30	A6-60	A6-90	A6-120	A6-180	A6-240	A6-300	A6-360

A7	A7-00	A7-30	A7-60	A7-90	A7-120	A7-180	A7-240	A7-300	A7-360
A8	A8-00	A8-30	A8-60	A8-90	A8-120	A8-180	A8-240	A8-300	A8-360
A9	A9-00	A9-30	A9-60	A9-90	A9-120	A9-180	A9-240	A9-300	A9-360
A10	A10-00	A10-30	A10-60	A10-90	A10-120	A10-180	A10-240	A10-300	A10-360
A11	A11-00	A11-30	A11-60	A11-90	A11-120	A11-180	A11-240	A11-300	A11-360
A12	A12-00	A12-30	A12-60	A12-90	A12-120	A12-180	A12-240	A12-300	A12-360
A13	A13-00	A13-30	A13-60	A13-90	A13-120	A13-180	A13-240	A13-300	A13-360
A14	A14-00	A14-30	A14-60	A14-90	A14-120	A14-180	A14-240	A14-300	A14-360
A15	A15-00	A15-30	A15-60	A15-90	A15-120	A15-180	A15-240	A15-300	A15-360
A16	A16-00	A16-30	A16-60	A16-90	A16-120	A16-180	A16-240	A16-300	A16-360
A17	A17-00	A17-30	A17-60	A17-90	A17-120	A17-180	A17-240	A17-300	A17-360
A18	A18-00	A18-30	A18-60	A18-90	A18-120	A18-180	A18-240	A18-300	A18-360
A19	A19-00	A19-30	A19-60	A19-90	A19-120	A19-180	A19-240	A19-300	A19-360
A20	A20-00	A20-30	A20-60	A20-90	A20-120	A20-180	A20-240	A20-300	A20-360
A21	A21-00	A21-30	A21-60	A21-90	A21-120	A21-180	A21-240	A21-300	A21-360
A22	A22-00	A22-30	A22-60	A22-90	A22-120	A22-180	A22-240	A22-300	A22-360
A23	A23-00	A23-30	A23-60	A23-90	A23-120	A23-180	A23-240	A23-300	A23-360
A24	A24-00	A24-30	A24-60	A24-90	A24-120	A24-180	A24-240	A24-300	A24-360
A25	A25-00	A25-30	A25-60	A25-90	A25-120	A25-180	A25-240	A25-300	A25-360
A26	A26-00	A26-30	A26-60	A26-90	A26-120	A26-180	A26-240	A26-300	A26-360
A27	A27-00	A27-30	A27-60	A27-90	A27-120	A27-180	A27-240	A27-300	A27-360
A28	A28-	A28-	A28-	A28-	A28-	A28-	A28-	A28-	A28-

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	00	30	60	90	120	180	240	300	360
A29	A29-00	A29-30	A29-60	A29-90	A29-120	A29-180	A29-240	A29-300	A29-360
A30	A30-00	A30-30	A30-60	A30-90	A30-120	A30-180	A30-240	A30-300	A30-360

Table 4: Coding of blood sample of Group B

Group B (n=30)	**Blood collection time in minutes after administration of drug**								
	00	30	60	90	120	180	240	300	360
B1	B1-00	B1-30	B1-60	B1-90	B1-120	B1-180	B1-240	B1-300	B1-360
B2	B2-00	B2-30	B2-60	B2-90	B2-120	B2-180	B2-240	B2-300	B2-360
B3	B3-00	B3-30	B3-60	B3-90	B3-120	B3-180	B3-240	B3-300	B3-360
B4	B4-00	B4-30	B4-60	B4-90	B4-120	B4-180	B4-240	B4-300	B4-360
B5	B5-00	B5-30	B5-60	B5-90	B5-120	B5-180	B5-240	B5-300	B5-360
B6	B6-00	B6-30	B6-60	B6-90	B6-120	B6-180	B6-240	B6-300	B6-360
B7	B7-00	B7-30	B7-60	B7-90	B7-120	B7-180	B7-240	B7-300	B7-360
B8	B8-00	B8-30	B8-60	B8-90	B8-120	B8-180	B8-240	B8-300	B8-360
B9	B9-00	B9-30	B9-60	B9-90	B9-120	B9-180	B9-240	B9-300	B9-360
B10	B10-00	B10-30	B10-60	B10-90	B10-120	B10-180	B10-240	B10-300	B10-360
B11	B11-00	B11-30	B11-60	B11-90	B11-120	B11-180	B11-240	B11-300	B11-360
B12	B12-00	B12-30	B12-60	B12-90	B12-120	B12-180	B12-240	B12-300	B12-360
B13	B13-00	B13-30	B13-60	B13-90	B13-120	B13-180	B13-240	B13-300	B13-360
B14	B14-00	B14-30	B14-60	B14-90	B14-120	B14-180	B14-240	B14-300	B14-360
B15	B15-00	B15-30	B15-60	B15-90	B15-120	B15-180	B15-240	B15-300	B15-360
B16	B16-00	B16-30	B16-60	B16-90	B16-120	B16-180	B16-240	B16-300	B16-360
B17	B17-00	B17-30	B17-60	B17-90	B17-120	B17-180	B17-240	B17-300	B17-360
B18	B18-00	B18-30	B18-60	B18-90	B18-120	B18-180	B18-240	B18-300	B18-360
B19	B19-00	B19-30	B19-60	B19-90	B19-120	B19-180	B19-240	B19-300	B19-360
B20	B20-00	B20-30	B20-60	B20-90	B20-120	B20-180	B20-240	B20-300	B20-360
B21	B21-00	B21-30	B21-60	B21-90	B21-120	B21-180	B21-240	B21-300	B21-360
B22	B22-00	B22-30	B22-60	B22-90	B22-120	B22-180	B22-240	B22-300	B22-360
B23	B23-00	B23-30	B23-60	B23-90	B23-120	B23-180	B23-240	B23-300	B23-360
B24	B24-00	B24-30	B24-60	B24-90	B24-120	B24-180	B24-240	B24-300	B24-360
B25	B25-00	B25-30	B25-60	B25-90	B25-120	B25-180	B25-240	B25-300	B25-360
B26	B26-00	B26-30	B26-60	B26-90	B26-120	B26-180	B26-240	B26-300	B26-360
B27	B27-00	B27-30	B27-60	B27-90	B27-120	B27-180	B27-240	B27-300	B27-360
B28	B28-00	B28-30	B28-60	B28-90	B28-120	B28-180	B28-240	B28-300	B28-360
B29	B29-00	A29-30	B29-60	B29-90	B29-120	B29-180	B29-240	B29-300	B29-360
B30	B30-00	B30-30	B30-60	B30-90	B30-120	B30-180	B30-240	B30-300	B30-360

Ethics and dissemination: The research ethics were approved from institutional ethics committee and the approval no is - **DMIMS(DU)/IEC/Sept-2019/8335**

Consent: The written informed consent will be obtained from the participants before starting the study. During the study, the confidentiality of each participant will be maintained.

Dissemination policy: The data will be disseminated by paper publication, authorship eligibility guidelines and any other intended use of professional writer.

Inform consent materials:A model consent form and other related documentation will be given to the participants and authorized surrogate with all the information.

Expected Results: At the time of protocol writing the analysis is not completedso,the expected result of this study is that group B will have enhanced bioavailability as compared to group A.

DISCUSSION:

In this protocol the group A is receiving *Haritakikwath*without*Anupana*, which is considered as standard and group B is the test group receiving *Saindhavalavana* as *Anupana* with *Haritakikwath*. The evaluation will be done on the basis of objective parameters. After the collection of all data, it will be analysed by using statistical test and will be presented in the form of table and chart. Many articles related to different aspect of this study were reviewed[11-15].

CONCLUSION:

Haritaki with *Anupana* is having more bioavailability than without *Anupana* of *Saindhavalavana*.

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Conflicts of interest: There are no conflicts of interest.

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