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STANDARDIZATION OF VASA GHRTA AND ITS EXTRACT FORM AND THEIR COMPARATIVE PHAMACO-CLINICAL STUDY WITH SPECIAL REFERENCE TO SWASA ROGA

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This is the era of science and technology as well as commercelization.The manufacturing of ayurvedic drugs also became a part of industrial business and more and more pharmaceuticals are introducing a lot of herbal drugs daily. Nowadays thus drug manufacturing becomes a business and hence chances of manipulations and discriminations are increased to derive more benefits in addition to this, there are no standard parameters to check the quality of drugs coming into the market. Secondly different types of new dosage forms like extract in different solvent or solvent systems of classical ayurvedic formulations are coming into the market, merely to enhance the palatability and to reduce dose without following any classical basics about the same. This particular work is a modest attempt in this direction and in which development and establishment of parameters of standardization for routine analysis of vasa ghrta and vasa extract are tried through physico-chemical evaluation (Pharmaceutical and Analytical studies) and biological evaluation (Pharmacological and Clinical studies) of both the dosage forms of vasa and in addition to this data generated by these various studies are utilized to establish the guide lines for further development of new dosage forms.

The present study entitled " Standardization of Vasa Ghrta and its Extract form and their comparative Pharmaco-Clinical Study with special reference to Swasa Roga " is presented in six sections; Conceptual Study, Pharmaceutical Study, Analytical Study, Pharmacological Study, Clinical Study and Discussion & Conclusion after introducing the subject and planning of the work and humble attempt is done to recap the whole study under the chapter summary.

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Introduction. The subject is introduced on the basis of some references research institutes are reported and analysed critically and based on the and other facts regarding the same. The previous works done at various lacunas and other facts regarding the subject, a brief introduction of scientific planning and presentation of the work has been provided to fulfill the following **Aims and Objective** selected of the study scientifically.

1. To procure drug after proper identification or from the authentic source.
2. To prepare Vasa Ghrta and Vasa Extract by following standard methods and to observe the steps and treatments given objectively as far as possible.
3. To carry out physico-chemical and chromatographic studies with a view to standardize and if possible to quantity vasicine, the main alkaloid of Vasa in both preparations.
4. To conduct pharmacological study to evaluate and compare the test drugs for immunomodulation and bronchodilator activities.
5. To carry out clinical study to evaluate and compare the clinical efficacy of both dosage forms of Vasa in the patients of Swasa to broaden the utility of the study.
6. To collect and systematize the data generated and evaluate them by proper statistical methods to present and discuss the study objectively.

Conceptual Study : This section of study is dealt in 7 chapters. The chapters on Pancavidha Kasaya Kalpana, Ghrta Kalpana, Extractions and Standardization are devoted to the comprehensive description of **fundamentals of ayurvedic pharmaceuticals.** Pancavidha kasaya kalpanas are not only the five types of dosage forms; svarasa, kalka, kwatha, hima and phanta, but also the same terms is used to described the type of process and basis behind the use of that particular method of preparation in addition to this; points towards their use in particular type and / or disease and diseased state. The concept of upakalpana might have been developed by ancient acaryas considering the problems of palatability, non-availability of raw drugs every time; and also to enhance the potency as well as to extract the maximum concentration of required ingredients in a particular more useful dosage form so as to produce selective therapeutic effects, the same is reflected from the fact; the use of different dosage forms from a same formulation or drug in different disease or

diseased. All these facts are reviewed and discussed elaborately in the light of modern basic sciences approach about the same. Fundamentals regarding the ghrta paka, from different ayurvedic acaryas point of view is presented and discussed in the chapter on Ghrta Kalpana. The facts about the process of extraction and its comparable entity rasakriya of ghana kalpana from ayurvedic point of view is reviewed alongwith the methods, selection of solvent or solvent systems and types of extracts and ghana in addition the scope of extraction of herbal formulations is also accounted in the chapter on extraction with the emphasis, that if the extracts of herbal drugs are derived by selecting the solvent or solvent systems more or less equal to that used in the manufacturing of reference formulations or drugs; the process of the preparation and the process of extraction is carried out under similar conditions; then the extract might have retain equivalent physico-chemical properties and biological activities. By centralising the same idea, the extraction of the ingredient of vasa ghrta (except ghrta and madhu) is done in this study.

Concept of standardization and different probable areas viz. raw material, formulation, methods of preparation and finished product with the main emphasis on the standardization of finished product because discrimination or manipulation done at any other stage of drug manufacturing might change the quality of finished product and that can be checked also if we have parameters to analyse the finished product only; and parameters like physical constants, chemical analysis, organoleptic tests, biological methods and others like TLC, HPTLC, HPLC and spectrophotometry; for the same from the literature available on the subject from books on modern pharmaceuticals and analysis and some important points regarding the same from ayurvedic texts and the scope of standardization for ayurvedic formulations/drugs are reviewed and discussed scientifically in the chapter on Standardization.

Vasa ghrta and vasa extract as a whole and their ingredients individually with their upto date phytochemical, pharmacological and clinical studies have been presented and discussed alongwith their detailed description accounting their synonyms, classification, rasapancaka, actions/uses, number of dosage forms; available from various ayurvedic classics; in the chapter on Drug Review. It came into light from the literary review of vasa that the various claimed therapeutic effects in ayurvedic classics are confirmed by various experimental

and clinical studies. Under the chapter of Swasa, the concept of swasa in general and tamaka swasa in particular with its derivation, definition, etiological factors, pathogenesis, clinical presentation, diagnosis, prognosis, therapeutic diets and principles of management is reviewed comprehensively from various available ayurvedic classics and its comparable disease entity bronchial asthma; which is no longer considered as a condition with isolated acute episodes of bronchospasm. Rather, asthma is a chronic inflammatory disorder of the airways that involves complex interactions among inflammatory cells, mediators and the cells and tissues in the airways. The interactions result in airflow limitation from acute bronchoconstriction, swelling of the airway wall, increased mucus secretion, and airway remodeling. The inflammation also causes an increase in airway responsiveness; from modern medical science is presented in a separate chapter on asthma with its upto date references collected from internet services and various modern texts and journals available on the subject.

Vasa ghrta and vasa extract were prepared by following the method described in Caraka the ingredients were used; vasa pancanga: ghrta: vasa puspa kalka in 4:1:1/8 and madhu 1/2 of ghrta in vasa ghrta and only vasa pancanga and vasa puspa (in the quantity as in vasa ghrta) in the preparation of vasa extract; mainly and the various factors like temperature, time of heating etc. utilized during the processings are observed carefully and presented in the section of Pharmaceutical Study with a view to utilize them in checking the variation in quality and yield of drugs from batch to batch. The vasa ghrta was obtained 94.17% w/w and vasa extract 9.84% w/w. Each 10 gms. of vasa ghrta contains active ingredients from averagely 28.73 gms. of vasa (vasa pancanga+ vasa puspa). The pills of vasa extract were prepared with pills making machine, weighing each 400 mg. The packing and labelling of both drugs were done properly. In addition to this, the collection and preparation of raw drugs with their source and methods are described briefly.

Analysis was carried out at two stages i.e. **in process** and **finished product,** with the aim to evolve and establish the parameters for standardization of two dosage forms of vasa i.e. vasa ghtra and vasa extract. Various methods like organoleptic, physico-chemical tests were utilized for in process analysis(vasa kwatha) and addition to these all, other like TLC, HPTLC and spectrophotometric methods were utilized for finished products (vasa ghrta

and vasa extract) analysis. The non-specific parameters like organoleptic and physico-chemical tests were carried out with a view to evaluate for general characteristics of both the dosage forms. TLC and spectrophotometric studies indicate the absence of vasicine, the main alkaloid of vasa in vasa ghrta but confirms the presence of vasicinone, another important pharmacological active alkaloid of vasa in both the doses form of vasa. To know the fact about the same; TLC of residue left after the filtleration of ghrta was also carried out and the presence of vasicine was observed in it. But in HPTLC study presence of vasicine in vasa ghrta(0.006% w/w)was detected which was about 33 times less than that in vasa extract (0.2% w/w). The analytic study carried out proved to be very frutile for the standardization of vasa ghrta and vasa extract and the parameters used in this study specially TLC, spectrophotometric and HPTLC can be utilized for routine analysis of these two dosage forms of vasa as well as other formulations containing vasa as one of the ingredients.

Pharmacological study was carried out with the aim to evaluate the quantum of pharmacological activities of both the dosage forms of vasa in the models related to bronchial asthma; comparable disease entity to swasa and to swasa utilize the data obtained in standardization as well as in establishing the concept of development of new dosage forms of vasa (vasa extract in this study) through pharmacological efficacy.

Both the test formulations i.e. vasa ghrta and vasa extract were evaluated for haemagglutination titre against S_{RBC} in rats, S_{RBC} induced immunological paw edema in pre-sensitized mice, cerraggenin induced paw edema in rats, substance 40/80 induced degranulation of rat mesentric mast cells, antihistaminic and anti-cholenergic activity and in addition to this effect of vasa ghrta on total cholesterol and triglycerides. The basis of selection of various models with their interpretation with ayurvedic concept of management of swasa are also discussed elaborately. The results and observation from various experiments are discussed and presented with their probable interpretation to ayurvedic concept in the section in pharmacological study. The summary of pharmacological activity profile in various selected experimental models is presented in table-1.

Table-1

Pharmacological Activity profile of vasa formulations

Drug Preparation	Test Carried Out							PC	
	AIF	IMN	CMI	MDG	SLP	AH	BS	Thymus	Spleen
Vasa Extract	NE	↑	↓	↓	NE	↓	↓	NE	↓
Vasa Ghrta	NE	NE	BP (↑↓)	NE	NE	NE	NE	NE	↓

NE-No significant effect, BP-Biphasic, ↑-Increase, ↓-Decrease, AIF-Anti inflammatory, IMN-Immunomodulation, MDG-Mast Cell degranulation, CMI-Cell mediated immunity, AH-Anti-histamine, BS-Bronchial spiral, SLP-Serum lipids, PC-Ponderal changes.

Clinical study was carried out in 121 patients of swasa; out of them 115 (27 in VG and 88 in VE group) patients completed the full duration (21days) of treatment. Vasa ghrta, and vasa extract pills were administered 10 gm and 2 gm. b.d. respectively with luke warm milk or water as per the convenience of the patient. The criteria of selection and assessment adopted were based on both ayurvedic classical line, and modern methods of clinical diagnosis and assessment. The quantification of various subjective signs/symptoms of swasa as well as atura bala was done by adopting scoring pattern based on parameters of severity of the same. The biological constants wise distribution was done are observations are discussed elaborately and data obtained related to the frequency of signs and symptoms as well as that of etiological factors of swasa which was in accordance with the reference available in ayurvedic classics, were also presented and discussed. The summary of the effects on various parameters related to sign and symptoms of swasa, pathological findings, biochemical findings, PFT findings and atura bala is presented in table-2.

Table-2

Improvement observed by the use of Vasa Ghrta and Vasa Extract on various parameters related to disease and diseased

Parametered	% Improvement		Unpaired	
	Group G	Group E	t	p
Chief Complaint				
Swasa kastata	95.07↓	96.97↓	-.708	>.10
Kasa-(Dry)	94.12↓	91.80↓	+.661	>.10
-(Wet)	80.81↓	92.96↓	-.020	>.10
Ghurghurakam	53.65↓	50.00↓	+.708	>.10
Associated Symptoms				
Grivasirah samgraha	86.54↓	95.60↓	+.275	>.10
Pinasam	77.51↓	89.05↓	-3.199	<.01
Kanthodhvansa	80.00↓	91.38↓	-.825	>.10
Ucchritaksa	90.04↓	77.06↓	-.492	>.10
Lalata Sweda	63.78↓	74.58↓	+.132	>.10
Visuskasya	83.34↓	70.20↓	+.638	>.10
Atura Bala				
Sensitivity towards EF	77.62↓	75.01↓	-.618	>.10
Saririka Bala	85.73↑	82.20↑	+.109	>.10
Manasika Bala	36.85↑	10.97↑	+2.934	<.01
Vyadhikshamtva	77.78↑	41.81↑	+2.022	<.05
Agni Bala	90.64↑	101.03↑	-.408	>.10
O/E of R/Ss				
Rerpiratory Rate	19.16↓	19.42↓	+.491	>.10
Chest expansion	37.13↑	42.63↑	-2.4	<.05
Crepitations	100.00↓	98.38↓	-1.80	<.06
Rhonchi	98.56↓	97.20↓	-1.545	<.10
Spirometrie findings				
FVC (%Predicted)	67.30↑	47.89↑	+.792	>.10
FEV1(%Predicted)	71.96↑	55.16↑	+1.394	>.10
FEV1/FVC(%Predicted)	26.28↑	42.31↑	-.214	>.10
MVV (%Predicted)	43.05↑	50.00↑	-.550	>.10
Pathological Findings				
Eosinophill	40.49↓	47.05↓	-.879	>.10
Biochemical findings				
S. cholesterol (above 250)	30.16↓	26.16↓	+.636	>.10
S. cholesterol (all)	12.98↓	10.66↓	+.616	>.10

Statistically all these changes as improvement on different parameters were found significant at $p<0.001$ except reduction of eosinophill in vasa ghrta group, which was found significant at $p<0.05$.

Overall effect of both the dosage forms of vasa was observed as shown in table 3.

Table-3

Overall Effect of Therapy on 115 patients of Swasa

Effect	Group G		Group E	
	No.	Percentage	No.	Percentage
Marked Improvement	25	92.59	83	94.32
Improvement	02	7.41	05	5.68
Mild Improvement	00	00.00	00	00.00
No Improvement	00	00.00	00	00.00

92.59% patients in ghrta group were markedly improved and 7.41% were improved whereas from extract group 94.32% patients were markedly improved and only 5.68% patients got improvement. None of the patients remained unchanged or got mild improvement among any group.

Correlation between data obtained in experimental pharmacological and clinical studies. As far as the clinical efficacy of the vasa extract is concerned, it can be explained on the basis of presence of CMI suppression, anti-histaminic, histamine bronchoconstriction antagonizing and mast cell degranulation inhibition effects observed in the experimental study. This activity profile can be presented as the basis for the observed clinical efficacy. However, the data obtained with vasa ghrta are not in conformity with the clinical findings. The biphasic effect on CMI in SRBC paw edema test was the only notable effect observed. It can not be considered as the sole basis for explaining the observed clinical efficacy of the vasa ghrta. Three speculative explanations can be offered - **i** species difference in the biological activity, this can be ruled out in the light of the results observed with vasa extract. **ii** Clinical evaluation depends predominantly on subjective evaluation while the converse is true with regards to experimental study. This again does not seem to be plausible for vasa ghrta offered apparently significant clinical improvement as evident

from the parameters measuring respiratory signs and symptoms and spirometry which cannot be ignored. Moreover, the clinical efficacy assessment of vasa extract is also based on the same set of parameters. **iii** There are many additional factors involved in the initiation and sustenance of airway inflammation and other pathophysiological features observed in asthma that have not been taken into consideration due to inadequate infrastructural facilities to carry out relevant experimental studies. Some of the important features which have not been studied are - **a.** neural control of airways. **b.** neuropeptide regulation of airways **c.** possible involvement of other neurotransmitters like nitric oxide. Evaluation of test formulations on the above aspects may provide useful data to come to an unequivocal conclusion. Another explanation may be that ghee being a good lipid solvent might have facilitatory effect on absorption of certain lipid constituents of the drug which might be responsible for the observed clinical efficacy and which might not have been detected in the present study.

concluding remarks. Vasa ghrta containing Vasa Pancanga:Ghrta: Vasa Puspa: Madhu (4:1:1 / 8:1/2) prepared by following the method described in C.Ci. 5/126-127, after collecting the vasa pancanga and vasa puspa in the months of March and April from the surrounding areas of Jamnagar and Junagadh (Gujarat); yield 94.17% w/w. To have a control over quality and yield factors like temperature(Av.97C) and time etc. are kept constant in all the batches and analysis of intermediate product, vasa kwatha was also carried out with a view to have control over quality as well as yield.The vasa aqueous extract was also prepared under more or less equal conditions and obtained 9.84% w/w. TLC and spectrophotometric analysis confirms the presence of vasicinone in both the dosage forms of vasa but in vasa ghrta vasicine could not be traced out, which was present in very minute quantity as confirmed by HPTLC study (about 33 times less than in vasa extract).

In pharmacological study ghrta proved to be almost inert on the models; antibody titre against S_{RBC}, immunological edema in mice and as anti-histaminic and anti-cholenergic tests, on which vasa extract produced significant effects except in immunological edema; which may be due to technical error or other modes of action of vasa ghrta. The prepared by aforesaid method and tested, vasa ghrta and vasa extract in the dose of 10 gms. and 2 gms. b.d.used in clinical study in the patients of swasa, produced comparable effects on almost

all of the signs and symptoms of swasa (bronchial asthma) assessed by using classical ayurvedic and modern developed methods of clinical evaluation. On the basis of the results and facts obtained from physico-chemical and biological evaluated profile regarding the selected parameters in this study, both the dosage form are comparable and on the basis of the same it can be recommended that vasa extract can be used as an alternative kalpana of vasa ghrta and may have benefits over vasa ghrta mainly in terms of palatability, cost etc. Thus, if we prepare the extract of ayurvedic formulations by selecting more or less equal solvents to the reference formulation and adopt same method of preparation then the extract can produce comparable results but only as part this study, is an first attempt in this direction and carried out only in one reference formulation and dosage form, therefore more and more studies are required to confirm the hypothesis. Parameters used for the purpose of standardization in analytic study proved to be useful and can be adopted for the analysis specially TLC, HPTLC and spectrophotometric and quality control of vasa ghrta and vasa extract as well as for other formulations having vasa as one of the ingredients.

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