Research Article

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PHARMACEUTICO-CHEMICAL ANALYSIS OF KHARJOORASAVA AND EVALUATION OF ITS CLINICAL EFFICACY IN PANDU ROGA

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ABSTRACT

The use of fermented products as food and drinks using various raw materials for both intoxicating and therapeutic purpose has been in practice since time immemorial. In due course of time, it was absorbed into Ayurveda and the Acharyas developed the procedure called sandhana kalpana. Shortage of the genuine raw materials is an arising problem in the pharmaceutical industry in the present era. So it is very important to choose medicines containing lesser number of ingredients which are having good clinical efficacy, from the innumerable formulations told in Ayurveda. Pharmaceutical study includes pharmaceutical preparation of kharjoorasava and documenting the steps involved in the procedure. Analytical study emphasized on developing analytical standards of the final product using various chemical analyses. In clinical Study 30 patients fulfilling the inclusion criteria were selected randomly and medicine was given for 28 days at the dosage of 25 ml. Follow up was taken on 56th day of commencement of the treatment. After treatment, the trial drug showed significant action on pandu roga, with the symptoms like Panduta, Dourbalya, Hridspandana, Bhrama, Shunakshikuta, Rukshata, Swasa, Aruchi, Pindikodweshtana, Jwara and decreased Hb levels.

Keywords: Sandhana Kalpana, Kharjoorasava, Pandu Roga

INTRODUCTION

Sandhana kalpana is a unique form of Ayurvedic therapeutics. Naturally fermented formulations like asava and arishta have gained their importance due to lesser dosage, quick therapeutic efficacy, palatability, prolonged shelf life and wider utility. Sandhana kalpana proves more beneficial as it is having medicinal as well as nutritive values. The self-generated alcohol during sandhana prakriya acts as a solvent for maximum extraction from the raw drugs and also as a preservative. In this formulation kharjoorasava[1], the trial drug, 5 Prastha (3.840 kg) of Kharjoora (*Phoenix dactylifera* Linn.) was boiled with 1 Drona (12.288 litre) of water and reduced to ¼ to obtain Kashaya. This was then filtered and taken and added with Hapusha (*Juniperus communis* Linn.) and Dhataki Pushpa (*Woodfordia fruticosa* Kurz) as Prakshepa dravya and kept for fermentation. Kharjoorasava referred in rajayakshma chapter in yogarathnakara was taken for this study. It is having only three ingredients and is indicated in pandu roga.

Pharmaceutical Study

The study was conducted in two batches.

- Batch 1
- Batch 2

The procedures followed for both the batches were same. The difference in observation would be dealt in the discussion part.

Steps involved

- Preparation of the decoction of kharjoora
- Preparation of phanta of dhataki pushpa
- Preparation of the sandhana patra and mixing the wort
- Keeping the wort for fermentation
- Procuring the completely fermented kharjoorasava.

Batch 1

Table 1: Materials used for preparing kharjoora kashaya of Batch 1

Place where study was conducted	Alva pharmacy, Mijar, India
Date of commencement of study	4/4/2013
Quantity of kharjoora used	45 kg
Quantity of water used	144 liters
Quantity of kashaya obtained	36 liters
Date of completion of the procedure	6/4/2013
Vessel used	Stainless steel vessel of 500 liter capacity
Fuel used	Fire wood

The steps involved in the process and observations are listed below

45 kg of kharjoora was taken in a clean stainless steel vessel of 500 liter capacity. 36 liter of water was added to it and the level was marked outside the vessel with a chalk piece and also by putting a cut mark on a long wooden stirrer dipped into the vessel to check the level. The remaining 108 liters of water was then added into the vessel and fire was set. At 8:00 p.m – kharjoora fruits were cooked well and kashaya was very thick in color and consistency. Ants were getting attracted to the drops of kashaya spilled on the floor which shows the high sugar content in it. At 8:30 pm – The marked level was found to reach just above 70 litres. Considering the possible loss as vapor while cooling, the fire was put out and the vessel was closed with an iron mesh and allowed to cool overnight. By 10:00 am (Next day) – The kashaya was

still warm and was in a pulp like consistency. This pulp was showing a level just below 70 liters marked, but above 36 liters (including kharjoora added). So it was clear that more than 36 liters of kashaya and the cooked kharjoora made the pulp. At 2:45 pm 50 litres of kashaya and 24 kg of residue was obtained after filtration. By 3:00 pm – 50 liters of kashaya was poured into another smaller vessel of 60 liter capacity and again heated to reduce it to 36 liters.

Preparation of Dhataki Phanta

As per the general method of preparation of sandhana kalpanas, the quantity of prakshepa dravya to be added was 1/10th to that of the main drug. In this specific yoga, 45 kg of kharjoora was used. So the total quantity of prekshepa to be used will be 4.5 kg. But as there are two prekshepa dravyas, the quantity of dhataki pushpa to be taken was calculated as 2.250 kg.

Table 2: Materials used for the preparation of Dhataki Phanta Batch 1

Date of commencement	5/4/2013
Dhathaki pushpa	2.250 kg
Water	9 liters
Total output	5.500 liters
Date of completion	6/4/2013

Preparation of the Sandhana patra and mixing the wort

A clay jar of 70 litre capacity was used as the sandhana patra. It was cleaned using hot water and dried, fumigation (dhupana) of this jar was done using maricha, jatamansi and haridra. This process was done 20 minutes before the wort was poured into it. The kharjoora kashaya, dhataki phanta and 2.5 kg of coarse powder of hapusha respectively was poured into the jar and mixed well. The jar was closed using a wooden lid that fits to the mouth of the jar. Then tight packing was given over it using cloth smeared with plaster of Paris, in several layers. Thus the sandhibandhana of the jar was done and the wort was kept in the fermentation room of Alva pharmacy, Mijar, India. After 10 days, clear hissing sound was heard from the closed jar on close observation, indicating the onset of fermentation. This hissing sound was checked every 10 days.

Procuring the completely fermented kharjoorasava

The time period required for the completion of the fermentation of this Yoga was not specifically mentioned by the Acharyas. So considering the climatic conditions and expert opinions, it was calculated as 40 days. The jar was opened on the 42nd day after the sandhibandhana, as there was no more hissing sound heard from the closed jar. The following observations were done on opening the jar.

- A thin black layer was found on the top of the liquid.
- Fine smell of alcohol was arising from the jar.
- Burning candle continued burning inside the jar.
- There was no hissing sound from the jar.
- No bubbles or froth were observed on the surface of the liquid.
- There was no floating of the prekshepa dravya.

The upper thin black layer was removed and the asava was then filtered in clean cloth and measured.

Table 3: Ingredients of the wort along with the date of sandhibandhana, onset of fermentation, completion of fermentation and total output obtained – Batch 1

Date of sandhibandhana	6/4/2013
Kharjoora kashaya	36 liters
Dhataki phanta	5.500 liters
Hapusha (coarse powder)	2.250 kg
Date of onset of fermentation	16/4/2013
Date of completion of fermentation	18/5/2013
Total output	36.200 liters

Analytical Study

The physico chemical analysis of Ayurvedic formulations is necessary in the present era to make the scientific basis of the final product stronger and to make it acceptable in the global market. The standards developed in such analysis helps in the reproducibility of the products of same quality as well. It also helps as a backup for further studies on the product and also to make modifications of the product in future if necessary.

Table 4: Organoleptic characters of Kharjoorasava

Sparsha (Consistency)	Liquid
Rupa (Color)	Bark brown
Rasa (Taste)	Madhura, tiktha, kashaya
Gandha (Odor)	Alcoholic

The chemical analysis and the TLC of a sample of the kharjoorasava were performed at Nagarjuna Herbal Concentrates LTD, Kerala, India. The results obtained after various chemical analysis of the final product are shown in the table below.

Table 5: Results of chemical analysis

Test Requirements	Protocol	Results
Total solids	Pharmacopoeial Standards For Ayurvedic Formulations	23 %
pH		3.98
Specific gravity		1.089
Total acidity		1.39 %
Alcohol content		9.5 %
Reducing sugar		19.69 %
Non reducing sugar		0.44 %
Brix value		29 %

TLC Result

The proposed trials revealed close similarities between Dhatakipushpa and Hapusha, under TLC separation which is possibly due to chemical similarities between secondary metabolites in each of them. The TLC done on the final product Kharjoorasava revealed only minute presence of each of the ingredients.

Clinical Study

Clinical study is an essential part of research work intended at evaluating the efficacy of the drug. This helps to determine the dosage, duration of the treatment and adverse effects on administration of the drug. This study

was approved by Institute Clinical Ethical Committee Clearance No AAMC/2013/OL/165/003.

Inclusion criteria

- Patients more than 16 and less than 60 years of age of both sexes.
- Patients presenting with the classical symptoms of pandu roga.
- Patients with Hb level in the range of 7-12.5 g/dL in males and 7-10.5 g/dL in females.

Exclusion criteria

- Patients of pandu roga associated with other systemic disorders
- Pregnant and lactating women
- All types of secondary, genetically acquired anemia, due to any cancer and hormonal imbalances.
- Patients who have undergone any recent surgeries
- Patients suffering from menorrhagia.

Criteria for assessment

The improvements in patients were assessed based on following points:

- Increase in hemoglobin percentage.
- Improvement in signs and symptoms of the disease (Panduta, Daurbalya, Hritspandana, Bhrama, Shunakshikuta, Rukshata, Swasa, Aruchi, Pindikodweshtana, Jwara)

Diet

Drug was advised to be taken after having food in order to avoid the complications such as burning sensation of abdomen. The patients were advised to follow normal diet that they were usually taking.

Hematological Parameters

Blood was collected from each patient for performing hematological tests like Hb %. TC, DC and ESR were also done to exclude any infective conditions.

RESULTS

After treatment, the trial drug showed highly significant action on pandu roga. After the follow up, the effect of the trial drug seemed to be sustained on some of the symptoms of pandu roga. A small percentage of improvement was noted in some symptoms even though it was statistically insignificant. Considering the overall improvement shown by the patient in signs and symptoms, the total effect of the therapy was assessed. It was done on the basis of percentage of relief obtained.

>75 % : Marked improvement
51 – 74 %: Moderate improvement
26 – 50 %: Mild Improvement
<25 % : No improvement

Table 6: Overall assessment of treatment

Assessment	14th day		28th day		56th day	
	No. of patients	%	No. of patients	%	No. of patients	%
No improvement	30	100 %	0	0	0	0
Mild improvement	0	0	3	10 %	1	3.33 %
Moderate improvement	0	0	15	50 %	15	50 %
Marked improvement	0	0	12	40 %	14	46.6 %

Overall significance level

Table 7: Statistical evaluation of overall significance level using one way ANOVA

Comparison	M.D	t value	p value	Significance
BT Vs AF	1.182	5.458	< 0.001	Highly significant
BT Vs AT	1.118	5.163	< 0.001	Highly significant
14th Day Vs AT	0.993	4.584	< 0.001	Highly significant
BT Vs 14th Day	0.125	0.579	> 0.05	Not significant
AT Vs AF	0.063	0.295	> 0.05	Not significant

Overall significance level is $p < 0.05$, which shows mild significance; BT – Before treatment, AT – After treatment, AF – After follow up

DISCUSSION

Acharyas have mentioned rakthalpata in the case of pandu roga. This mainly indicates the decrease in the quality of blood, than the quantitative decrease. Majority of the symptoms of pandu roga are attributed to this rakthalpatha. In the modern aspect also, most of the symptoms are directly due to the decrease in the hemoglobin level, thereby decrease in the oxygen carrying capacity of the blood[2]. Nutritional deficiency, malabsorption, unhygienic food habits, worm infestation etc are some of the commonest reason behind anemia. Almost all the general causes and symptoms of this disease told by the acharyas and the modern aspect can be closely co related. Kharjoora[3] is having carminative and anthelmintic action. It is a good cardio tonic. It has high nutritive values and contains 87 % sugar. Dhataki pushpa[4] is a well-known natural sandhana preraka dravya (fermentation initiator) and also a sandhana ranjana dravya (coloring agent). It is krimikhna in action and is capable of curing yakrith vikaras. Hapusha[5] is sugandhita, pachaka and uthejaka in karma. It cures krimi and pliharoga. It is also used in the preservation of certain fermented products. During the preparation of kharjoora kashaya, the proportion of water added was lesser than 4 times to that of the kharjoora. This might be probably because acharyas might have calculated the amount of

water content already present in kharjoora, which will be released during the process of kwathana. It is only the sugar content present in kharjoora, that helps in the fermentation as no other madhura dravyas like jaggery is used in this formulation. A sample of 100 ml of the kashaya obtained was stored in a tightly closed bottle, leaving no much space for air in the bottle. This sample is still self-preserved without the help of any other preservatives. This might be due to the presence of concentrated sugar content in the kashaya. This also indicates that the amount which was reduced during the process of kwathana was correct as mentioned in the yoga, to obtain the desired concentration of the kashaya and thereby attaining proper fermentation. As the kashaya prepared has a tendency of self preservation, the role of strong fermentation initiator is very important for the successful completion of fermentation. This highlights the importance of dhataki pushpa in this yoga. The use of dathaki pushpa itself as a fermentation initiator is most commonly practiced. But in this study, dhataki phanta was used as there are chances of growth of unwanted microbes over floating dhataki pushpa and thereby resulting in spoilage of the product. Previous study by Kroes *et al*[6], titled "Fermentation in traditional medicine: the impact of *Woodfordia fruticosa* flowers on the immune modulatory activity and the alcohol and sugar contents of nimba arishta" explains that dhataki pushpa promotes fermentation by releasing enzymes like invertase. This effect can be attained more efficiently by following phanta kalpana method as the necessary enzymes can be extracted to water and at the same time, deactivating the unwanted microbes in the hot water. The first batch took 42 days from the day of sandhibandhana for completing the fermentation, whereas the second batch took 60 days. The difference in this time duration may be probably due to climatic variations as the two batches of the product was prepared in two different seasons. Batch 1 was prepared during greeshma ritu and batch 2, during sharath ritu. Fumigation (dhupana) of the sandhana patra is having a very important role in promoting proper fermentation. The unwanted microbial flora in the fermentation jar is destroyed during this process. The drugs used in fumigation are having anti microbial action and the volatile active principles are released when burnt and get mixed with the fumes and reaches the minutest pores of the vessel. Analytical standards developed help in the reproducibility of product of same quality and may provide back up for further studies. The TLC of the final product revealed only traces of individual ingredients because sandhana kalpana involves very slow chemical process during which marker compounds in the ingredients might have undergone chemical changes. On the 14th day of treatment, when the percentage of improvement was calculated, none of the patients had a percentage of score more than 25 %. So it was considered no improvement, even though some of them had very minute improvement. On statistical evaluation, the symptoms pandutha and swasa showed a moderate significance. Dourbalyata, hridspandana and bhrama showed mild significance. The symptoms shunakshikuta, rukshata, aruchi and jwara showed insignificance. There was a 1.18 % increase in Hb level and showed mild significance on statistical evaluation. On examining the patients on 28th day revealed that the patients were recovering from the symptoms. After statistical calculations, the p value of the signs and symptoms except jwara was found to be highly significant. An increase of 8.3 % was noted as increase in Hb level and the p value corresponding to it showed high significance. The percentage of improvement in jwara was noted as 100 % even though its statistical evaluation showed insignificance. This was because there was only four patients presenting with jwara and all of them got relief. Any rise in body temperature associated with pandu roga will appear when the Hb level falls to 7 g/dL or below. As most of the patients chosen for this study were manual labors and economy of them was poor, the symptoms manifested in them were more prominent due to lack of nutrition and hard labor at the same time. Most probably, because of this reason, even the patients with Hb level of 9 g/dL, 8.5 g/dL, 8.4 g/dL and 7.6 g/dL presented the symptom jwara. The medicine was stopped on the 28th day of commencement of treatment. The follow up was taken on 56th day. The statistical evaluation showed that all the symptoms except jwara was highly significant. Jwara showed insignificance statistically even though there was a 100 % improvement. A statistical evaluation between after treatment and after follow up was then taken to get more clear information on how much the effect of the drug was sustained 28 days after its stoppage. The symptoms pandutha and dourbalyatha showed a mild significance at $p < 0.05$. All the other symptoms and the Hb % showed insignificance at $p > 0.05$. The symptoms like hridspandana, shunakshikuta, rukshata, pindikodweshtana and jwara showed no change between after treatment to after follow up and the p value was found to be 1. This means that the effect of the drug on those symptoms was sustained without any change on the follow up. Because of this, there was zero percentage improvement and also statistically insignificant. Although the effect of the drug after treatment was found to be sustained, symptoms pandutha and dourbalyatha showed statistical significance as the patients were more relieved from those symptoms even after withdrawing the trial drug. Some percentage of improvement was found in swasa, aruchi and bhrama even though it was statistically insignificant.

Probable Mode of Action

As per Ashtanga sangraha, alcoholic preparations made using a mixture of many raw materials will acquire the qualities of those which are predominant in quality[7]. In this yoga, kharjoora is the main ingredient. It is having following properties[8].

Rasa - Madhura
Guna - Snigdha, Guru
Virya - Sheetha
Vipaka - Madhura
Dosakarma - Vatapittasamaka

All the properties, except guru guna are very much ideal for the management of pandu roga. After fermentation, the guru guna is transformed to lakhu, thereby making it

more absorbable by the body. Therapeutically, kharjoora is hridya, carminative and anthelmintic also. The other two ingredients are commonly used in alcoholic beverages for proper fermentation, flavoring and preservation purposes. In this formulation dhataki pushpa not only serves as a fermentation initiator, it also exhibits its therapeutic actions for curing pandu roga as it is capable of curing yakrith vikaras (liver disorders). As yakrith is the moola of rakthavaha srothas, dhataki pushpa would be having a role in the therapeutic action of kharjoorasava on pandu roga. Hapusha is having its therapeutic action on pleeha (spleen) and it is also an uttejaka (stimulant). So it can be considered that hapusha is capable of stimulating the spleen. Again pleeha is also a moola of rakthavaha srothas. Pandu roga is a pitta pradhana vyadhi[9,10], raktha is the dhatu which is affected, and liver, spleen etc organs are directly involved in this condition. The trial drug is having qualities capable of curing all those conditions. krimighna property is that which is common in all the three ingredients. Lack of education, in the patients taken for the study might have resulted in maintaining poor hygienic environment and unhygienic food habits, resulting in worm infestation which is one of the reasons for pandu roga.

CONCLUSION

The trial drug was an asava kalpana, only in nomenclature. It can also be classified under arishta, pakwarasa sidhu or varuni. Kharjoorasava is a very unique sandhana kalpana that no other madhura dravya like guda or sharkara is added. The sugar content in the kharjoora itself is used in the process of fermentation. The total output of final product obtained from 90 kg of deseeded kharjoora was 72.3 liters which means there was 80.33 % yield. Among the two batches prepared for the study, Batch 1 was having better organoleptic characters probably because of ideal climatic conditions. The statistical evaluation of clinical study revealed that kharjoorasava have mild significant action on pandu roga, with the symptoms like panduta, dourbalya, hridspandana, bhrama, shunakshikuta, rukshata, swasa, aruchi, pindikodweshtana, jwara and decreased Hb levels.

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