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An In-Vitro Study to Evaluate the Anti-Bacterial Effect of Dhupana Yoga in Hospital Area

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ABSTRACT:
Dhupana (fumigation) is a method for prevention and curative purpose to reduce bacterial and fungal effect because Dhupana has an effective disinfectant property. Valeriana wallachi (Tagara), Saussurea lappa (Kushta) Dravyas are used in Dhupana as they have anti-microbial, anti- toxic and anti-oxidant property.
Dhupana or fumigation is a proven anti-microbial process. All the regimens during rainy season strive to attain either anti infectious state or increase immunity or both. Fumigation is one among such regimen.Tagara and Kushta have anti-microbial property, hence Dhupana will be done with the powders of these drugs and the anti- bacterial and anti-fungal effect will be evaluated. To make an effective and easy way of conducting Dhupana, the drugs- Tagara and Kushta will be triturated with Ghrita and made into a form of Dhupa stick and its anti- bacterial and anti-fungal effect will also be evaluated by conducting Dhupana in Hospital area. Hence this study Shows in-vitro anti-bacterial effect of a simple formulation of Dhupana by using Tagara, Kushta along with Ghrita

INTRODUCTION:
Dhupana (fumigation) is a method for prevention and curative purpose to reduce bacterial and fungal effect because Dhupana has an effective disinfectant property[1]. Valeriana wallachi (Tagara), Saussurea lappa (Kushta) Dravyas are used in Dhupana as they have anti-microbial, anti- toxic and anti-oxidant property[2]. Dhupana or fumigation is a proven anti-microbial process. All the regimens during rainy season strive to attain either anti infectious state or increase immunity or both. Fumigation is one amo In the environment billions of micro-organisms are present because of house hold dust and maximum of them are harmful in immune-compromised people. Micro-organisms are dangerous in open wounds, in operation theatre, neonatal wards and in labour room.

OBJECTIVES OF THE STUDY:
To evaluate the antibacterial effect of Tagara (Nata) and Kushta with Ghrita by Dhoopana in hospital area.

RESEARCH DESIGN: In Vitro Anti-bacterial study.

MATERIALS&METHODS:

SOURCE OF DATA:

Literary Source: Library data will be collected form authentic text, relevant research article and other electronic resources

REVIEW OF LITERATURE:

A Dhupana yoga i.e., Ghrita yukta Kushta (Nata) and Tagara Dhupa yoga has been mentioned. Tagara and Kushta are vishghna dravyas. Tagara (Valeriana wallichii) has Katuvipaka, Ushna vishrya, Karma Drik Doshahara, Vishaghna, Doshaghnata, Bhuta and Apasmaranashana properties. Kushta (Saussurea lappa) has Madhura Katu Rasa, Ushnavirya, katu Vipaka, Vishaghna, kandughna, Dadrughna, Kantikruta properties[3].

Goghrita has Madhura Rasa, Madhuravipaka, Vata Pitta Nashaka, Visha Nashaka, Netra Doshanashaka and is Balakaraka[4].

Even though there is dissimilarity in physical constitution of human beings, still there are factors that are common to all individuals, and vitiation of these factors leads to simultaneous manifestation of diseases having the same set of symptoms leading to destruction. Factors which are common for all the inhabitants of a country are air, water, location and seasons[5]. Hospital-acquired infections, or nosocomial infections, are one of the leading causes of death and typically affect patients whose immune systems are compromised. Nosocomial infections are transmitted in hospitals through three main environmental routes - air, surface contact and water. Airborne infections are spread when dust and pathogens are released during hospital renovation and construction activities and due to contamination and malfunction of the hospital ventilation system. Providing clean filtered air and effectively controlling indoor air pollution through ventilation are two key aspects of maintaining good air quality[6].The control of air pollutants (microorganisms, chemicals, dust, and smoke) at the source is the most effective way to maintain clean air. Disinfection in public area especially in hospital, nursing home is required.

DRUG REVIEW:

Drug name	Botanical name	Family	Chemical composition	Part used	Quantity
Kushta	Saussurea lappa	Asteraceae (Compositae)	Costunolide, dehydrocostus, Cynaropicrin, Lappadilactone, germacrenes, Saussureal	Moola	1 part
Tagara	Valeriana wallichii	Valerianaceae	Calarene, Valeranone, ar-curcumene, maalioxide, maalitol	Moola	1 part
Go Ghrita	Ghee				Quantity sufficient

Materials: Clay pot**,** Burning charcoal**,** Karpoora (Camphor), Matchsticks, Ghee,Kushta and Tagara (Nata) Churna, Microscope, Gram stain reagents: Methylene blue, Grams iodine solution**,** Ethyl alcohol**,** Saffranin, Glass slides, Cover slips, Petri plates, Hot air oven, Incubator, Autoclave, Muller Hinton Agar (MHA)

BACTERIAL METHOD:

Preparation of Muller Hinton Agar (MHA) was prepared as per standard protocol[7].

Petri-plate exposure method: Culture media was exposed in the direction of wind blows for about 2 minutes to catch the micro-organisms on solid media, MHA plates was incubated in incubator for 24hours of incubation at 37°C, and plates are at room temperature.

METHOD OF DRUG FUMIGATION:

It was conducted in Post-Operative ward at Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan, the area of the ward is 400sq. ft. MHA is incubated for 24 hours. Later Dhupana will done with Kushta and Tagara (Nata) churna along with Ghrita weighing 25 grams in Post-operative ward for about 20 min in the afternoon, by using charcoal and coconut shells for burning with the help of Karpoora. To affirm uniform distribution of Dhupana dravya in the area, Dhupana yantra will be moved in all directions of the room. After 10 minutes once again Muller Hinton agar will be exposed in the same areas for about 2 min and subsequently incubated for 24 hours.

In similar way, MHA Petri plates was exposed to the air for 2min and incubated for 24 hours in the second group. Later Dhupana will be done with the Dhupa stick weighing 25g prepared with Kushta and Tagara till the Dhupa stick burns completely in Post-Operative ward at Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan. After 10 minutes once again Muller Hinton Petri plate's agar will be exposed in the same areas for about 2 min and subsequently incubated for 24 hours.

GRAM's STAINING:

It will be conducted in Pathology and Microbiology Lab. After incubation for 24 hours observation for the colony morphology and done gram staining separately.

Procedure of Gram staining: Culture smear of colony will be prepared on a clean glass slide, it is allowed to air dry and fixed by flaming. Primary staining: smear is covered with pararosaniline dye and allowed it to act for 30-60 seconds. Application of mordant: primary stain will be poured off and Grams iodine is applied for 1-2 minutes and washed with water. Decolorization: with organic solvent smear is decolorized for 10-30 seconds or until the colour oozes from slide. Then it is washed with water. Counter staining: with dye of contrasting colour it is counter stained for 15-30 seconds. Then it is washed with water and blot dry. It is observed in binocular compound microscope under oil immersion 100 x after putting a drop of cedar wood oil and morphological features of microorganism are identified[8].

INCLUSION CRITERIA:

Indoor Hospital environment in Post-operative Ward

EXCLUSION CRITERIA:

Areas other than Post-operative ward

PLAN FOR STUDY:

The study was conducted by giving Dhupana for consecutive 3 days in Post-operative ward of Sri Dharmasthala Manjunatheshwara Ayurveda Hospital, Hassan. MHA petri plates were exposed to the air for 2min and incubated for 24 hours. Later Dhupana was done with Kushta and Tagara (Nata) Churna along with Ghrita weighing 25 grams in Post-operative ward for about 20 min in the afternoon, by using

charcoal and coconut shells for burning with the help of Karpoora. After 10 minutes once again Muller Hinton Petri plate's agar were exposed in the same areas for about 2 min and subsequently incubated for 24 hours. An average bacterial load of a hospital area was found to be in 10 to 12 different colonies.
The assessment was done every 24 hours for a period of 3 days to evaluate the effectiveness of Dhupana and study the period for which Dhupana was effective as antibacterial.
Hence the effect of Dhupana with the churna of Tagara and Kushta along with Ghrita was evaluated.
Duration of work – 3days.

METHOD OF PREPARATION:
Authentified raw drugs was prepared into churna form in Teaching Pharmacy, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital Hassan.

ASSESSMENT CRITERIA:
The assessment will be done after 24 hours of incubation in Petri plate's number of colonies and morphology of the bacterias was studied before and after Dhupana.

STATISTICAL ANALYSIS:
This data was statistically compared and tabulated with relevant statistical tests.

OBSERVATION AND RESULTS:
Before doing dhupana air sample of the Post-Operative ward contained bacterial growth. After dhupana there was significant reduction in the growth of both bacteria.
On the first day1 of Dhoopana
There is a much significant result in growth of bacteria before and after Dhoopana.
And total fifteen number of gram +ve bacteria is seen in before dhupana. In this six gram +ve cocci bacteria and nine gram +ve bacilli bacteria are seen
After 10minutes of dhupana significant reduction is observed. Five number of gram + ve bacteria are observed.
It is observed that growth of bacteria is less when dhupana is given with churna .

1st Day Muller Hinton Agar (MHA) plate Before Dhupana: 15 colonies are observed.
1st Day before Dhoopana Gram +ve & Gram Organisms: Table. No: 1

Sl.no	Gram +ve bacteria	No. Of organisms	Name of organisms	Sl.no	Gram –ve bacteria	No. of organisms	Name of organisms
1	Gram +ve cocci	4	Gram +ve pnuemococci spp	1	Gram-ve bacilli	1	Escherichia
2			Gram +ve staphylococci spp	2	Gram –ve diplococci	2	Neisseria spp
3				3	Gram –ve	2	Streptococci

					cocci		

1st Day Muller Hinton Agar (MHA) plate 10 minutes after Dhupana 5 colonies are observed:1st Day after Dhupana Gram +ve & Gram –ve Organisms: Table. No: 2

Sl.n o	Gram +ve organisms	No.of organisms	Name of organisms	Sl.n o	Gram –ve organismsm s	No.organisms	Name of organisms
1	Gram +ve cocci	2	Gram +ve pneumococci	1	Gram –ve diplococci	1	Neisseria spp
2	Gram +ve cocci	2	Gram +ve staphylococci spp	2			

2nd Day Muller Hinton Agar (MHA) plate Before Dhupana, 5 colonies are observed: 2nd Day Before Dhupana Gram +ve & Gram –ve Organisms: Table. No: 3

Sl.no	Gram +ve oragnisms	No. of organisms	Name of organisms	Sl.no	Gram –ve organisms	No.of organisms	Name of organisms
1	Gram +ve bacilli	4	Gram +ve pneumococi	1	Gram –ve bacilli	1	Gram –ve escerichia

2nd Day Muller Hinton Agar (MHA) plates 10 minutes after Dhupana 3 colonies are observed: Table. No: 4

Sl.no	Gram +ve organisms	No.of organisms	Name of organisms	Sl.no	Gram –ve organisms	No. of organisms	Name of organisms
1	Gram +ve bacilli	2	Gram +ve pneumococci	1	Gram –ve bacilli	1	Gram –ve eschericha

3rd Day Muller Hinton Agar (MHA) plate Before Dhupana, 6 colonies are observed: 3rd Day Before Dhupana Gram +ve & Gram –veOrganisms: Tabele. No: 5

Sl.no	Gram +ve organism s	No.of organism s	Name of organisms	Sl.no	Gram –ve organism s	No. of organism s	Name of organism s
1	Gram +ve cocci	3	Gram +ve pneumococci Gram +ve staphylococci	1	0	0	0
2	Gram +ve bacilli	3	Gram +ve pneumococci	2	0	0	0

			spp				

3rd Day Muller Hinton Agar (MHA) plate 10 minutes after Dhupana 2 colonies areobserved:

Table. No: 6

Sl.no	Gram+ve bacteria	No. of organisms	Name of organisms	Sl.no	Gram –ve organisms	No.of organisms	Name of organisms
1	Gram +ve bacilli	2	Gram +ve pneumococci spp	1	0	0	0

RESULTS:

The petriplate before dhupana and the petriplate after dhupana of the same locality iscollected and compared. The results were drawn after following observations:

DHUPANA IS DONE WITH DHUPANA CHURNA PREPARED WITH TAGARAAND KUSHTA DRAVYAS:

Table. No: 7

Days	Before dhupana	After dhupana
1st day	15	5
2nd day	5	3
3rd day	6	2

GROWTH OF BACTERIA BEFORE AND AFTER DHUPANA WITH GHRITA YUKTA TAGARA KUSHTA DHUPANA STICK:

Table. No: 3

Organisms	Before dhupana	After dhupana
Pneumococci spp	7	2
Staphylococci spp	4	1
Streptococci spp	1	1
Clostridium	2	0
Escherichia	2	0
Neisseria spp	0	1

Ayurveda have unique perception and methodologies for the treatment of diseases. Life style of people have brought a lot of changes in environment. Thereby the diseases thatcaused due to poisoned environment have appeared in different form of style. Environment include water, air and lad and the inter-relationship which exists among and between water, air, and land, and human beings, other living creatures, plants, microorganisms and property. Microorganisms, namely bacteria, viruses, fungi and parasites are ubiquitous in soil, water, and atmosphere and on body surface, are responsible for a innumerable infectious diseases in human beings. Microorganisms have a very important role in the biogeochemical cycle, as their task consists in disintegrating organic mass to reusable metabolites [9]. Normal flora for microorganisms is not harmful. But more and more frequency growth conditions like excessive humidity, high water content of building material, seasonal changes and in-door environment

changes are responsible for growth of bacteria's. Bacterial damage in indoor area is caused mostly by pathogenic bacteria present in environment. Sterilization with fumigation is one of the most commonly used techniques in management of individual and social health.

Before doing dhupana air sample of the Post-Operative ward contained bacterial growth. After dhupana there is significant reduction in the growth of bacteria.

On the day1 of Dhoopana, there is a much significant result in growth of bacteria before and after Dhoopana. And total fifteen number of gram +ve bacteria is seen in before dhupana. In this six gram +ve cocci bacteria and nine gram +ve bacilli bacteria are seen

After 10minutes of dhupana significant reduction is observed. Five number of gram + vebacteria are observed.

Significant higher incidence densities of Gram+ve and gram –ve pathogens were observed during before dhupana because there is increasing evidence that different seasonality due to physiologic changes underlies host susceptibility to different bacterial pathogens[10]. Low temperature can influence the response of a microorganism either directly or indirectly. Direct effects include decreased growth rate, enzyme activities, alteration of cell composition and differential nutritional requirements[11]. Benefits of Dhupana include soothing vrana; creating germ free environment and alleviating pain, discharge, odour and microorganisms. Fumigation creates an aseptic environment, kills microbes and thus prevents infection. Plants belonging to Kusthahara, Krimighna, Kandughna and Vranahara Gana have mostly been used for their anti-microbial properties.

Mostly Agni and Vayu Mahabhoota Pradhana dravyas are used for dhupana. These are Laghu, Sheeta, Ruksha in nature. These qualities of the Dravyas help in its rapid spreading and quicker combustion[12]. Drugs are volatile in nature. Volatility would be a distinct advantage in lowering microbial contamination in air and on difficult to reach surfaces. Ghee helps in combustion. When these inflammable drugs are burned, they release a large amount of energy making it useful as a fuel for sustaining the flame and may also help in dispersing the volatile components of the Dhupana formulation. Almost all the Dhupana Dravyas have an inevitable source of combustible ingredient either as a potentiator or activator of the formulation.

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