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Role of Yavasarshpadichurnadhupan (as antimicrobial and antifungal agent) in operation theatre sterilization.

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Abstract :

Fumigation is one of the most effective sterilization modality used to prevent sepsis in biological environment. It is the process in which a lethal chemical was released in an enclosed area to kill the manifestation by pests. It is important to develop asepsis in hospital wards, operation theatres to prevent nosocomial infections. Since long time, *Ayurveda* has mentioned a modality called as *dhupana* to maintain asepsis and antisepsis in *Vranitagara*. In present era, fumigation is mainly done by formalin gas which has been proved aa potent carcinogen. So, it is timely need to use herbomineral compound over synthetically derived formalin gas fumigation as a safe way of sterilization. In this study an attempt has been made to prove the role of *Yavasarshapadichurnadhup yoga* as compared to formalin gas fumigation.

Key words : Fumigation , YavasrshapadiChurnadhupan yoga as santimicrobial andantifungal agent, Formalin as carcinogen.

Introduction:

Dhupan karma is one of the classical ancient remedy of sterilization to maintain healthy biological environment all over the world. It is essential to maintain good asepsis to do various karmas. Kashyap Samhita have mentioned 40 dhupan yoga in dhupkalapadhyay; Sushrutacharya mentioned dhupan karma of Shalya karma mandir in Vranitopasaniyaadhyay ,whileCharakacharya mentioned dhupan of vastras and vranitagar in Jatasutriyashariradhyay .

Dalhana explained procedure of sterilization in Sushrut Chikitsasthan.[1] Before surgery Shalyakarmamandir must be fumigated or disinfected to avoid infections. The source of most hospital epidemics is infected patients i.e. patients contaminated with pathogenic organisms. These microorganisms are often released into environment in very high numbers, exceeding the minimal infective dose & contaminate others who subsequently develop hospital acquired infections. Yavasarshapadichurnadhupyog was used as antimicrobial and antifungal agent and its effect was compared with action of formalin gas fumigation on four specific groups of microorganisms which are highly responsible for creating fatal sepsis in operative theatres. Formalin gas fumigation has been proven to cause irritation to the mucus membrane affecting nose, eyes, lungs and can lead to asphyxia and carcinoma of the lungs. It is also responsible for abortions. So, *Yavasarshapadichurnadhup yog*[2]herbomineral compound which is equally effective and can be used instead of formalin fumigation for operation theatre sterilization.

MATERIAL :

1. *Yavasarshapadidhoopa yoga*
2. Two planned scale down models prepared by wooden box (size=2ft x 2 ft x1 ft) of 1/10th of the size of operation theatre no. 1 of our institute i.e.3200sq ft.
3. Specially designed *Dhupanyantra*
4. *Yavasarshapadichurna*=8gm
5. *Goghrut*= 8 gm
6. Formalin = 6ml
7. $KMnO_4$ = 2 g
8. Species of staphylococcus aureus, Pseudomonas aeurigenosa, Ecoli, Candida albicans
9. Dry distillation apparatus.
10. T.L.C. Machine.
11. The aceutof GCV JMST100GCV machine.
12. Nutrient , mac conkey and SDA agar Culture medias'

Method of preparation of dhupayog :

All the drugs were identified, standardised and authentified.

Following are the 14 constituent *Dhupana yoga* as mentioned by *Charak*-[2]

*Charak*has not mentioned the exact amount of each drug to be taken so by using followingreference of *sharangadhar samhita,*[4]

Ingredients	Quantity
Yavachurna	600mg.
Sarshapachurna	600mg.
Atasichurna	600mg.
Hinguchurna	600mg.
Guggulu	600mg.
Vachachurna	600mg.
Chorakachurna	600mg.
Bramhichurna	600mg.
Golomichurna	600mg.
Jatamansichurna	600mg.
Lakh churna	600mg.
Kutakichurna	600mg.
Sarpanirmokchurna	600mg.
Ghrut	8gm.

1) Raw material needed to prepare *Yavasarshapadichurna* i.e. *YavaSarshapa, Atasi, Hingu,Guggulu, Vacha, Choraka, Bramhi, Jatamansi,Laksha, Kutaki, Sarpanirmok, Ghrut*werecollected.

2) All the above substances were taken in equal amount.

3) *Churnas*were prepared. These*churnas (*powders) were mixed thoroughly with the help ofmortal and pistali.e.*KhalvaYantra*.

4) Above mixture of *churnas* was mixed together and *Yava-sarshapadichoorna* was prepared and stored in plastic containers kept at room temperature in dry place.

5) Amount of *dravyas* in dhupayoga and duration of *Dhupana* karma was not mentioned in *Samhita grantha.*

Method of fumigation with both dhupyog and formalin gas:

6) With the reference mentioned in plant pathology; material taken in equal quantity of *dhupanadrvya*with respect to combination of formalin (6ml) + KMnO4(2gm) forfumigation(fumigation quantity=2ml/cuft) to study their action comparatively. [15,17]

7) Hence it was decided to give *Dhupana*to scale down model of *Vranitagara* with 8gm of *Yava-sarshapadichurna*with equal quantity of*goghrut*for 30 mins.(as whole*Churna*completely burnt out in 30 mins observed in pilot study) and chamber was kept remain closed for next 4 hours.

Efficacy of Yavasarshapadidhupan yoga on given species of microorganisms wasevaluated with 24 experimental cycles and an attempt was done to prove its action on these microorganisms with the help of thin layer chromatography along with gas chromatography and mass spectrometry.

Results and Discussion :

COMPARATIVE STATISTICAL STUDY OF TWO GROUP

A = DHUPAN B= GAS FUMIGATION

No.	Organisms	Group		Mean	S.D.	S.E.	t value	P value	Remark
1	STAPHYLOCOCCUS AUREUS	A	B.T.	644.91	438.50	89.508	6.909	< 0.0001	Extremely Significant
			A.T.	111.70	100.63	20.541			
		B	B.T.	644.91	438.50	89.508	5.736	< 0.0001	Extremely Significant
			A.T.	273.04	23.04	41.44			
2	PSEUDOMONAS AERUGINOSA	A	B.T.	825.125	421.42	86.022	8.80	< 0.0001	Extremely Significant
			A.T.	174.58	131.13	26.76			
		B	B.T.	825.125	421.42	86.022	6.733	< 0.0001	Extremely Significant
			A.T.	350.70	246.10	50.23			
3	CANDIDA ALBICANS	A	B.T.	101.667	75.566	15.425	5.86	< 0.0001	Extremely Significant
			A.T.	7.916	9.315	1.901			
		B	B.T.	101.667	75.566	15.425	4.689	< 0.0001	Extremely Significant
			A.T.	28.166	19.016	3.882			
4	E-COLI	A	B.T.	2083.083	1348.3	275.23	3.995	< 0.0001	Extremely Significant
			A.T.	913.416	642.28	131.11			
		B	B.T.	2083.083	1348.3	275.23	2.666	< 0.0001	Significant
			A.T.	1455.87	905.96	184.93			

STATISTICAL ANALYSIS TABLE

1) Effect of Dhupan on STAPHYLOCOCCUS AUREUS

	Mean	S.D.	S.E.	t value	P value	Remark
B.T	644.91	438.50	89.508	6.909	< 0.0001	Extremely Significant
A.T.	111.70	100.63	20.541			

Since P value is less than 0.0001, we reject Ho. Hence the Dhupan is significantly effective on this *Staphyalococcus Aureus*.

Effect of Fumigation on STAPHYLOCOCCUS AUREUS

	Mean	S.D.	S.E.	t value	P value	Remark
B.T	644.91	438.50	89.508	5.736	< 0.0001	Extremely Significant
A.T.	273.04	203.04	41.44			

Since P value is less than 0.0001, we reject Ho. Hence Fumigation is significantly effective on this Staphylococcus Aureus.

2) Effect of Dhupan on *PSEUDOMONAS AERUGINOSA*

	Mean	S.D.	S.E.	t value	P value	Remark
B.T	825.125	421.42	86.022	8.80	< 0.0001	Extremely Significant
A.T.	174.58	131.13	26.76			

Since P value is less than 0.0001, we reject Ho. Hence Dhupan is significantly effective on this *Pseudomonas Aeruginosa*.

Effect of Fumigation on *PSEUDOMONAS AERUGINOSA*

	Mean	S.D.	S.E.	t value	P value	Remark
B.T	825.125	421.42	86.022	6.733	< 0.0001	Extremely
A.T.	350.70	246.10	50.23			Significant

Since P value is less than 0.0001, we reject Ho. Hence the Fumigation is significantly effective on this Pseudomonas Aeruginosa.

3) Effect of Dhupan on *CANDIDA ALBICANS*

	Mean	S.D.	S.E.	t value	P value	Remark
B.T	101.667	75.566	15.425	5.86	< 0.0001	Extremely
A.T.	7.916	9.315	1.901			Significant

Since P value is less than 0.0001, we reject Ho. Hence, Dhupan is significantly effective on Candida Albicans.

Effect of Fumigation on *CANDIDA ALBICANS*

	Mean	S.D.	S.E.	t value	P value	Remark
B.T	101.667	75.566	15.425	4.689	< 0.0001	Extremely
A.T.	28.166	19.016	3.882			Significant

Since P value is less than 0.0001, we reject Ho. Hence, Dhupan is significantly effective on Candida Albicans.

4) Effect of Dhupan on *E-Coli*

	Mean	S.D.	S.E.	t value	P value	Remark
B.T	2083.083	1348.3	275.23	3.995	< 0.0001	Extremely
A.T.	913.416	642.28	131.11			Significant

Since P value is less than 0.0001, we reject Ho. Hence,Dhupan is significantly effective on E-Coli.

Effect of Fumigation on *E-Coli*

	Mean	S.D.	S.E.	t value	P value	Remark
B.T	2083.083	1348.3	275.23	2.666	< 0.0001	Significant
A.T.	1455.87	905.96	184.93			

Since P value is less than 0.0001, we reject Ho. Hence, Fumigation is significantly effective on E-Coli.

From the above results growth of microorganisms of staphylococcus aureus, pseudomonas aeruginosa, Candida albicans was found to be extremely significant in both the groups while in case of E coli, result observed after *dhupan* was found to be extremely significant as compared to formalin gas fumigation.

In this study, the trial was conducted on four species of microorganisms staphylococcus aureus, pseudomonas aeruginosa, candida albicans&Ecoli; divided in two groups. The microorganisms were selected for 24 experimental cycles. Their colony count was done before and after dhupan and fumigation. Yavasarshapadichurnadhupa yoga was found to be equally effective as compared to formalin gas fumigation. *Yava-sarshapadiDhupan yoga* for the fumigation of scale down model of Vranitagara was selected for the present study by considering its significance mentioned in Ayurvedic texts. *Dhupana* should be done for *ArishtagaraVastra, Shayya, Asanadi*of*Bala*to avoid Neonatal sepsis.[3]

Effectivity of *dhupana:*It has been observed from the results derived from all 24 cycles ofexperiments that action of *dhupan yoga* was approximately similar to that of formalin gas fumigation in case of 3 species i.e. staphylococcus aureus, pseudomonas aeruginosa and candida albicans. But amongst these experiments i found that *dhupan yoga* showed marked effect on growth of e coli as compared to formalin gas fumigation. Hence, considering all the parameters it was proved that *Yavasarshapadichurnadhupa yoga* was efficient to reduce the growth of 4 species of microorganisms i.e.staphylococcus aureus, pseudomonas aeruginosa, Ecoli and candida albicans on planned scale down model of the *vranitagara*.

Probable mode of action of *YavasarshapadichurnaDhupa Yoga*:

The mode of action of *Yavasarshapadidhupa yoga* was probably due to *Agni sanmskar* on *dhupadravya*were used for*dhupan karma*.*Dhupa*dravya has *Vayu, Akash* and *Agni Mahabhootadhikya*.*Sukshmastrotogamitva*attained owing to the combination of these

mahabhootas. So, the sterilization property of drugs is maintained in every corners and at microbiological level.[1]

Action of indigenous drugs :

*Yava, sarshapa, Atasi, Hingu, Guggulu,Vacha,Choraka,Bramhi, kutaki*are mentioned to bekrumighna in *Samitas* and *Nighantus. Ghrut* is essential for combustion of all the constituents.*Sarpanirmok* is mentioned in *Kashyap Samhita DhupakalpaAdhyaya* four times as *Rakshoghna*.*Laksa* is also mentioned in *Kashyap Samhita* in two *Dhupakalpas* as *Rakshogna karma*.[3,5,6]

Action of drug in accordance to modern perspective:[7,8,10,11,18,19,20,21,22]

26 volatile constituents were found after Gas chromatography and mass spectrometry study and some of them found to have microbiocidal and fugicidal action over the four species which i had taken under the study.

I referred literature of individual constituents, some of them were appeared to be presented in a raw material of my herbs and they were likely to come in the atmosphere when separated during *dhupan* process.

Out of 26 volatile constituents some of them were found to be present in the drugs of my *dhupan yoga* which are listed below,

Decyl, trifluoracetate has fungicidal and microbiocidal action. Dodecane which was found to be present in a drugs belonging to family vulgare from which yava belongs, likely to have fungicidal and antimicrobial activity.Hexacosyltrichloroacetate has antimicrobial action.Hepatocosyltrifluoroacetate which is effective against candida albicans, e coli, psalmonellaspecies.Oxirane (oxacyclopropanes), 2 butyl,3 methyl cis and pthalic acids (pthalides) found in choraka proven as a good fungicide and bactericide.Phenol, 2,4 bis (1,1 dimethylethyl) found in kutaki also has the same action.Pentanoic acid is a good antimicrobial and antifungal agent.Hexadecane , nonadecane, hexadecanoic acid, methyl ester, Pentadecanoic acid and octadecanoic acid most likely found in drugs belong to the family vulgare.3 chlorpropionic acid is most likely found in shwetadurva. Mustard oil is potent mutagenic, so it might act as genetic modulator in microbes.So no such study has been conducted and it is further scope of research.Linseed oil have antimicrobial activity obtained from seeds of *Atasi*. Volatile oil from roots of Vacha inhibited the growth of Mycobacterium

tuberculosis in a concentration of 10 mcg/ml. Ethanolic extract of *Bacopamonnieri* antibacterial activity seen in more effective in Gram –vebacteria.The aqueous extract of bramhi showed moderate antibacterial activity against Staph. Aureus and Salmonelatyphi, and marked inhibition against Esch.Coli. Volatile oils from roots of *Acoruscalamus* Linn. inhibited the growth of gram-vegativeorganisms.Volatile oils from gum-resin of *Guggulu* shows antibacterial activity.The aqueous extract showed moderate antibacterial activity against Staph. Aureus and Salmonelatyphi, and marked inhibition against Esch. Coli.

Conclusion:

One of the mechanisms of action was explored in the current study to find out volatile constituents in the atmosphere which were responsible for antimicrobial and antifungal action on the microorganisms.

- Temperature was noted and observed to remain constant at 39^0c .
- Amount of *dhupandravya*(by weight) required to fumigate scale down model of vranitagara (whose dimension was $1/10^{th}$ of hospital OT no. 1 dimension i.e. 3200 cu ft) was 8 grams for every cycle of dhupan.
- It has been proved that *Dhupan* given for 30 minutes a day followed by keeping the scale down model of *vranitagar* of size 2x2x1cu ft. enclosed for 4 hours was effective to reduce load of microorganisms.
- Amount of *dhupandravya* (by weight) was taken as 8 grams with equal quantity of *goghrut*which was taken as per the mentioned fumigation quantity required forformalin gas fumigation.
- As the global scenario is now changing towards the use of non toxicherbomineral drugs over synthetically derived chemical compounds for sterilization, development of Ayurvedic formulations like *Yavasarshapdhupyog* should be emphasized to prevent and control of peri and intra operative sepsis. In fact, time has come to use Indian Traditional knowledge through modern approaches of development of fumigation as per ancient classics of Dhupan karma.

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