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ANTI-MICROBIAL AND WOUND HEALING EFFICACY OF *STHANIKA DHOOPANA* IN WISTAR RATS USING EXCISION WOUND MODEL- AN IN VIVO STUDY

[1]Geetanjali Sahu [2]Krishna Patel [3]Reetu Sharma [4]Gaurav Sharma

1. Assistant Professor, KJIAR, Vadodara
2. Assistant Professor, KJIAR, Vadodara
3. Associate Professor, NIA, Jaipur
4. Pharmacologist, DDDU NIA, Jaipur

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ABSTRACT

Background: Wound healing is a critical consideration in the field of surgery, and the primary goals are to prevent infection and expedite the healing process. Ancient Ayurvedic texts, such as those written by Acharya Sushruta, place significant emphasis on *Vraṇa* (wound) and its treatment. While many antiseptic agents are used to prevent infections, they often lack the ability to enhance the healing process and can even be harmful as they may have cytotoxic effects. In response to this, an innovative polyherbal formulation known as " *Ġaṇa dhūpa* "(Ayurvedic formulation fumigation) was developed for the practice of "*Sthanika Dhūpana karma* "(localized fumigation). The objective of this study aims to investigate the antimicrobial and wound healing effectiveness of *Dhūpana karma* using an excision wound model in Wistar rats. **Materials and method:** Eighteen male Wistar rats were acquired for the study and divided into three groups, each containing six rats, all of which were infected with pre-sub cultured *S. aureus* bacteria. The test group received *Sthānika Dhūpana*, the control group was treated with Povidone iodine ointment, and the standard group received normal saline. Various parameters were assessed over a ten-day period and statistical analysis was performed to evaluate differences between the three groups at each time point. **Results:** Significant results were observed in various parameters related to wound healing. The percentage of wound contraction rate was notably higher in the test group when compared to the other groups. **Conclusion:** *Dhūpana karma* using *Ġaṇa dhūpa* is safe as well as effective in anti-microbial and wound healing activity.

Key words: *Dhūpana karma*, *Ġaṇa dhūpa*, *Vraṇa,* Ayurveda, antimicrobial activity

1. INTRODUCTION

Wounds represent a significant global health challenge, with an alarming statistic showing that around 10,000 out of every million patients succumb to microbial infections arising from wounds[1]. Such wounds result in the disruption of normal anatomical structures and their functions, affecting millions of individuals annually. The importance of wound care is highly emphasized in Ayurveda, and this is vividly reflected in the extensive writings of Acharya Sushruta. He defines " *Vraṇa* " as injuries that not only disrupt body tissues but also leave behind scars after they heal, which persist throughout an individual's lifetime. Acharya Sushruta's works delve into the detailed

classification, prognosis, and management of these *Vraṇa*, underscoring the profound significance of wound care in the field of medicine[2]. Ayurvedic wound management aims to prevent infections and promote healing through topical methods like *Dhūpana karma*(fumigation). This ancient technique, detailed in the Kashyapa Samhita, utilizes medicated smoke to create an aseptic environment that eliminates harmful microbes. Ayurveda's broad perspective on "*krumi*"(microbes) covers a wide spectrum of infections, making it a holistic approach to wound care.

In recent decades, there has been a significant rise in microbial infections[3]. The overuse of antimicrobial drugs has given rise to drug-resistant microorganisms, a global health challenge. Ayurveda's *Dhūpana karma*, proven effective in reducing bacterial counts, offers a safe, cost-effective solution. Its external application is safer than internal medications. Validating this traditional Ayurvedic practice can enhance Ayurveda's global recognition as a scientifically grounded healthcare system, especially in regions with limited scientific understanding.

2. AIM & OBJECTIVE

This study aims to investigate the antimicrobial and wound healing effectiveness of *Dhūpana karma* using an excision wound model in Wistar rats.

3. MATERIALS AND METHODS

The present study was conducted in the Animal house of National Institute of Ayurveda, Deemed to Be University (De Novo), Jaipur (Rajasthan). This study was approved by the Animal Ethics Committee of the institution (IAEC no.- NIA/IAEC/2021/06 on 26/08/2021).

Chemicals & consumables: Inj. Ketamine, Inj. Xylazine, Ethanol, Halothane, Picric acid, 10% formalin solution, Povidone-iodine solution, Normal saline, Agar media, glycerin, eosin, benzene, Paraffin wax, xylene, safranin, acetone

Equipment & apparatus: Polypropylene cages, Digital Vernier Caliper, Surgical instruments, Surgical gloves, Surgical mask, Tissue processor, Microtome, Cotton, Anesthesia chamber, Microscope, Glass slides, Cover-slips, Macintosh sheets, Syringes (1ml), Dropper

- **Test animal strain:** Wistar albino rats (*Rattus novergicus*)
- **Gender:** Male
- **Number of animals in the study:** 18
- **Body weight:** 150-200 gm
- **Housed cages:** Polypropylene cages, 1 rat in 1 cage
- **Ethical approval:** Approval of Institutional Animal Ethical Committee (IAEC)
- **Protocol ID:** DDDU/2022/10/02
- **IAEC Approval No:** NIA/IAEC/2021/06
- **Housing:** Animals were housed in polypropylene cages & kept there for seven-days allowed for acclimatization with the laboratory conditions. All rats were housed in individual cages with dry wheat waste (Post hulled) placed as bedding material which was changed every morning.
- **Temperature:** Temperature range was maintained between 18°-29°C (64.4 to 84.2 F)
- **Humidity:** 35-70 RH

- **Light cycle:** 12/12 hr. light/dark cycle (the animals were exposed to 12 hours light and 12 hours dark cycle)
- **Feeding material:** Standard feed
- **Drinking water:** R.O. Water, which was given in polypropylene bottles with stainless steel sipper tube
- For identification of the rats, marking was done using pieric acid on each rat of every group as **H, B, T, HB, BT & HT** where H stands for Head of the rat, B stands for Back of the rat, T stands for Tail of the rat, HB stands for Head-Back of the rat, BT stands for Back-Tail of the rat, HT stands for Head-Tail of the rat.

2.1. Source of raw drugs and its preparation

Ingredients of *Ġaṇa dhūpa*[4] i.e., *Ghrita* (Butyrum departum*), Akshata* (Oryza sativa L.), *Jātiipuspa (*Jasminum officinale*), Madhu* (Honey)*, Sarsapa* (Brassica campestris) and *Vacā* (Acorus calamus)100gm each were procured from the from the GMP certified pharmacy of N.I.A., Deemed to be University, Jaipur.

2.2. Interventions

Group A (Control): Wounds were treated with Povidone-iodine ointment.

Group B (Trial): Wounds were subjected to *Ġaṇa dhūpa Dhūpana*.

Group C (Standard/Negative control): No intervention was applied (only normal saline wash).

2.3. Excised Wound Healing Activity[5]

- All experimental animals were weighted & as per protocol, only those animals having weight more than 150 gm & less than 200 gm were taken for trial. Weight of all animals were noted for further steps.
- Then, as per weight, anaesthesia Inj. Ketamine was given intra-peritoneal. Then by observing the absence of deep pedal reflexes, anaesthesia effect was confirmed.
- Part preparation at operative site i.e., from neck to complete back was done & with the help of depilatory cream, all the furs were removed from selected operative field.
- Now, animals were taken to the operative table and an impression was made on the depilated dorsal thoracic surface 2 cms behind the ears and 1-1.5 cm away from the vertebral column. Then with the help of iris scissor, excision of full thickness of impressed area in circular shape was made & wound of approximately 2 sq.cms was created.
- Same steps were taken on each animal, wounds of approx. 2 sq.cms. were created & measurement of wound thickness were taken by surgical digital vernier calliper. All the three measurements along X, Y & Z axis were noted & then average diameters of wounds were calculated.
- All 18 Wistar rats in three groups were infected with Staphylococcus aureus bacteria by applying pre-sub cultured bacteria on the wound with a sterile cotton swab, followed by 24 hours of containment in their respective polypropylene cages.
- After 24 hours, sample for microbial culture was taken from the open wound of all 18 rats and observation of microbial growth were made with the help of culture analysis on agar media plate, individually.

- Now, wounds of all the animals were intervened with their respective drugs which was considered as 0^{th} day of the study.
- For observing the growth of bacterial colonies on wound, culture analysis was done on agar media plate on 0^{th}, 2^{nd}, 4^{th}, 6^{th}, 8^{th} and 10^{th} day[6,7].

INTERVENTION METHOD

• At the end of the stipulated 10 days, feeds were withdrawn, the rats were subjected to a 12 hour fast but had access to water. Wound was isolated from ketamine anesthetized rat. The harvested wound area was carefully dissected out, trimmed all fat and connective tissue and blotted dry to remove any blood.

• The tissues were fixed in 10% formal saline and then transferred to a graded series of ethanol. On day 1, they were placed in 70% alcohol for 7 hours, then transferred to 90% alcohol and left overnight. On day 2, the tissues were passed through three changes of absolute alcohol for an hour each then cleared in xylene. Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58°C.

• Vertical sections of 5 um thick were obtained from a solid block of tissue, fixed on clean albuminized slides to prevent sections coming of the slides and later stained with Haematoxylin and Eosin staining techniques, after which they were passed through ascending grade of alcohol, cleared in xylene and mount in DPX mountant, allowed to dry at room temperature and observed histopathologically under digital light microscope.

Image no. 1- Sample collection from infected wound

Procedure for *dhūpana*: For this study, an improvised instrument was made to create localized medicinal smoke. Medicine was heated on a hot plate to make smoke, which was then collected within a funnel shaped covering and sent through a tube to a specific area for treatment.

Image no. 2- *Dhūpana* instrument

4. RESULTS

All the values were expressed as mean ± standard error of the mean (S.E.M) of six animals each across the groups. Statistical analysis of data was carried out using two-way ANOVA analysis of variance with the help of Graph Pad Prism software. Dunnett's multiple comparisons test was used to compare the results in between the groups, where P value < 0.05 is considered to be statistically significant.

4.1. Antimicrobial study

Microbial growth on wounds was observed with the help of culture analysis on agar plate on 0^{th}, 2^{nd}, 4^{th}, 6^{th}, 8^{th}, and 10^{th} day. To find the total viable cell count, the number of colonies were counted, multiplied by the appropriate dilution factor. Then the average total microbial count of all the three groups was calculated and recorded in Mean±SEM value of all the observation days as per protocol. Calculated data were found as mentioned below:

Table no. 1- Microbial count data of all the three groups

Group A (No. of Colony) (x 10^7)						
Marking	**0 day**	**2nd day**	**4th day**	**6th day**	**8th day**	**10th day**
H	3.58	1.35	0.96	0.34	0.065	0.0025
B	3.45	2.32	1.65	0.97	0.012	0.0016
T	5.25	3.65	2.19	1.08	0.23	0.00
HB	5.47	1.65	0.85	0.34	0.015	0.0079
BT	4.21	2.85	1.29	0.84	0.15	0.0065
HT	3.85	1.69	0.95	0.12	0.0016	0.00
Group B (No. of Colony) (x 10^7)						
Marking	**0 day**	**2nd day**	**4th day**	**6th day**	**8th day**	**10th day**
H	5.15	4.32	2.74	1.85	0.0018	0.00
B	4.25	2.65	1.84	0.54	0.041	0.0051
T	4.65	2.49	1.48	0.68	0.0015	0.00
HB	2.56	1.95	1.06	0.14	0.0058	0.00
BT	3.28	1.95	0.96	0.49	0.014	0.0054
HT	3.54	2.46	1.68	0.98	0.015	0.0016
Group C (No. of Colony) (x 10^7)						
Marking	**0 day**	**2nd day**	**4th day**	**6th day**	**8th day**	**10th day**
H	3.55	4.65	2.91	3.94	4.95	3.28
B	3.94	4.26	4.59	5.14	5.95	4.26
T	4.74	3.45	3.65	4.67	6.12	2.84
HB	4.66	2.95	5.85	4.25	3.45	3.85
BT	4.17	3.65	4.71	3.95	4.23	4.69
HT	4.91	4.88	5.26	3.48	3.68	2.58

Table no. 2- Average microbial count of all the three groups

Observation Days	Group A (Mean±SEM)	Group B (Mean±SEM)	Group C (Mean±SEM)
0 day	4.302±0.352	3.905±0.390	4.33±0.216
2nd day	2.252±0.356	2.637±0.357	3.97±0.305
4th day	1.315±0.212	1.627±0.263	4.50±0.436
6th day	0.615±0.162	0.780±0.241	4.24±0.241
8th day	0.079±0.038	0.013±0.006	4.73±0.464
10th day	0.003±0.001	0.002±0.001	3.58±0.337

Graph.1Graphical presentation of average microbial count of all the three groups

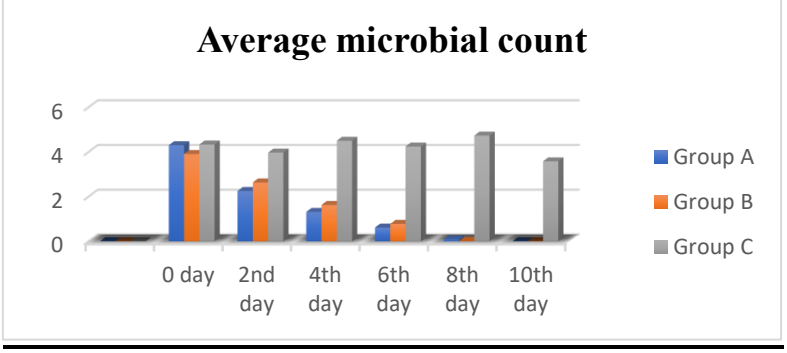
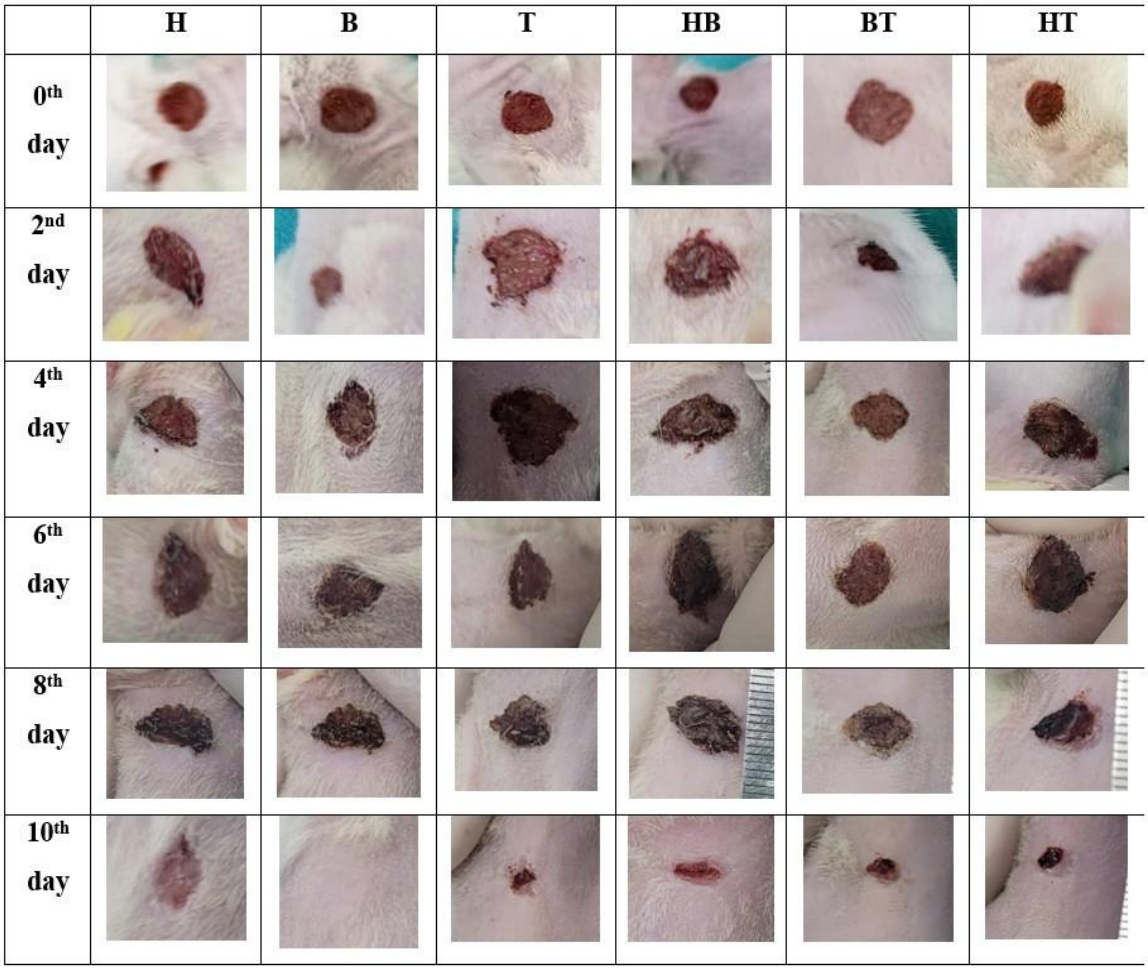


Table no. 3- Comparison of Anti-microbial activity on statistical parameters using Two way ANOVA test

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Group A					
0 day vs. 2nd day	2.050	1.024 to 3.076	Yes	****	**<0.0001**
0 day vs. 4th day	2.987	1.961 to 4.013	Yes	****	**<0.0001**
0 day vs. 6th day	3.687	2.661 to 4.713	Yes	****	**<0.0001**
0 day vs. 8th day	4.223	3.197 to 5.249	Yes	****	**<0.0001**
0 day vs. 10th day	4.299	3.272 to 5.325	Yes	****	**<0.0001**
Group B					
0 day vs. 2nd day	1.268	0.2422 to 2.294	Yes	**	**0.0095**
0 day vs. 4th day	2.278	1.252 to 3.304	Yes	****	**<0.0001**
0 day vs. 6th day	3.125	2.099 to 4.151	Yes	****	**<0.0001**
0 day vs. 8th day	3.892	2.866 to 4.918	Yes	****	**<0.0001**
0 day vs. 10th day	3.903	2.877 to 4.929	Yes	****	**<0.0001**
Group C					
0 day vs. 2nd day	0.3550	-0.6711 to 1.381	No	ns	**0.8450**
0 day vs. 4th day	-0.1667	-1.193 to	No	ns	**0.9927**

		0.8595			
0 day vs. 6th day	0.09000	-0.9361 to 1.116	No	ns	**0.9997**
0 day vs. 8tht day	-0.4017	-1.428 to 0.6245	No	ns	**0.7731**
0 day vs. 10h day	0.7450	-0.2811 to 1.771	No	ns	**0.2325**

Table no. 4- Pictorial comparison of average wound contraction in Trial Group



5. DISCUSSION

4.1 Probable mode of action of *Dhūpana karma*

4.1.1 On the basis of *Prakṛti vighāta*:

As far as the ayurvedic concept of *Kṛimi*(microbes) is concerned, *Kapha(phlegm)* and *purīṣa*(stool) both are conducive to the growth of *kṛimi*. And therefore, the *Dravya(drug)* which are opposite to the properties of *kapha* perform the function of *prakṛti vighāta*(altering the host environment).

4.1.2 On the basis of Ethnopharmacological aspect:

- The *Dhūpana* drugs have been well-established for their antimicrobial activity when used in extract form, as demonstrated in previous research. However, there has been relatively less research from phytochemical and

ethnopharmacological perspectives regarding the practice of *Dhūpana*.

- During *Dhūpana*, as the herbal drugs reach their boiling points, their volatile constituents vaporize, and these gaseous forms diffuse into the surrounding environment. Additionally, the combustion of cellulose and other carbohydrates produces steam in significant quantities due to the combination of hydrogen from decomposed organic molecules with oxygen.

4.1.3 On the basis of *Paṁcamahābhūta* concept[8]:

According to Ayurveda, every entity in the world is believed to have a constitution based on the five fundamental elements, known as *pāṁcabhautika*, which are Earth, Water, Fire, Air, and Space. The potential mode of action of *Dhūpana* drugs can be explained based on the Ayurvedic concept of the *mahābhūtas*, specifically *vāyu, ākāśa*, and *agni*:

- *Vāyu Mahābhūta*: *Vāyu*, due to its property of *rukṣaṇa karma* (drying effect), might play a role in dehydrating the intracellular fluid of bacteria. This dehydration can disrupt the bacteria's metabolism, ultimately leading to bacterial death.
- *Ākāśa Mahābhūta*: The *Ākāśa* element may act by creating porosity and softness in the bacterial cell wall. This alteration can reduce the rigidity of their cellular structure, which is often responsible for their resistance to antimicrobial drugs. Therefore, it can aid in weakening the bacterial cell wall, potentially contributing to bacterial cell death
- *Agni Mahābhūta*: *Agni* (fire) may work by modifying the temperature requirements for various enzymatic and chemical reactions within the bacterial cell. This temperature alteration can disrupt the proper metabolism and growth of the bacteria, interfering with their vital processes and ultimately contributing to bacterial cell death.

4.1.4 On the basis of *Guṇa*:

In this study, the trial drug primarily possesses the qualities of *laghu* (light) and *rukṣa* (dry) *guṇa*, along with the *kaṭu* (pungent), *tikta* (bitter), and *kaṣāya* (astringent) tastes, as well as the *uṣṇa* (hot) potency.

- *Laghu guṇa*: The quality of *laghu* facilitates *lekhana karma*, which means it has scraping or cleansing properties.
- *Rukṣa guṇa*: The *rukṣa* quality results in *kledaśoṣaṇa*, leading to dryness. This dryness can lead to the absorption of moisture from both inside and outside of the bacterial cell. Consequently, it hampers the biochemical reactions within the cell, ultimately resulting in bacterial cell death.
- *Kaṣāya Rasa*: The *kaṣāya* taste may induce *stambhana* or constriction of the bacterial cell, affecting its growth and survival.

4.1.5 On the basis of *ġaṇa dhūpa* ingredients

Table no. 5- Analysis of *rasa paṁcaka* of trial drug of the present study

Dravya	***Rasa***	***Guṇa***	***Vīrya***	***Vipāka***	***Prabhāva***
Ghṛta	*Madhura*	*Snigdha, guru, śīta*	*śīta*	*madhura*	*Vātapittśāmaka*

Akshata	*Madhura*	*Laghu,Snigdha, Mridu, grahi*	*śīta*	*Madhura*	*tridoṣaghna*
Jātī	*Tikta, kaṣāya*	*Laghu,Snigdha, Mridu*	*Uṣṇa*	*Kaṭu*	*Kaphapittaśāmaka*
Madhu	*Madhura, kaṣāya*	*sukshma Laghu (su.), ruksha*	*Uṣṇa*	*Madhura*	*tridoṣaghna*
Siddhartaka	*Kaṭu,Tikta,*	*Tīkṣṇa, Rukṣa*	*Uṣṇa*	*Kaṭu*	*Kaphavātaśāmaka*
Vacā	*Kaṭu, Tikta*	*Laghu,Tīkṣṇa*	*Uṣṇa*	*Kaṭu*	*Medhya*

Most drugs used in this study have "sharp" and "hot" qualities—such as *Ushna* (hot), *Tikshna* (sharp), *sukshma* (penetrating) and *Katu* (pungent)—that are exactly opposite to the nature of microbes (*Kṛumi*). To enhance this effect, *Ghṛta* (ghee) is often added because it helps the herbs burn better and releases natural antimicrobial compounds. When these medicinal fumes are released, they can effectively target microbes while creating a sterile, healing atmosphere

These characteristics of the trial drug illustrate how the specific qualities and tastes attributed to it can impact the behavior of bacterial cells and contribute to their demise.

4.2 Discussion on anti-microbial action in animal study

- Throughout the study, samples were collected at regular intervals, specifically on the 2nd, 4th, 6th, 8th, and 10th days. These samples were analyzed to assess changes in microbial load, providing insights into the antimicrobial effects of *Ġaṇa dhūpa* over the course of the trial.
- Following the collection of samples, microscopical examinations were conducted, and the total microbial count was calculated and observed. The bacteria identified on the wounds at the 0th day were primarily *Staphylococcus aureus*, appearing in colonies.
- Before the treatment, the microbial load on the wounds at the 0th day in Group A (Control Group), Group B (Trial Group), and Group C (Negative Control) were found to be approximately 4.302±0.352, 3.905±0.390, and 4.33±0.216, respectively.
- After the treatment, at the 10th day, the microbial load on the wounds in Group A (Control Group), Group B (Trial Group), and Group C (Negative Control) were found to be 0.003±0.001, 0.002±0.001, and 3.58±0.337, respectively.
- Comparative analysis indicated that Group B (Trial Group), which was subjected to *Dhūpana Karma* using *Ġaṇa dhūpa*, showed a statistically significant reduction in total microbial count compared to Group A (Control Group), which was treated with Povidone-iodine ointment.
- Group C (Negative Control), with no specific intervention, was not able to achieve a significant reduction in total microbial count compared to Group A (Control Group).

These findings demonstrate the effectiveness of *Dhūpana Karma* using *Ġaṇa dhūpa* in reducing the microbial load

on wounds when compared to other interventions.

CONCLUSION

The experimental study aimed to evaluate the antimicrobial effect of *Ġaṇa dhūpa*. When comparing the total microbial load in the control group with the test and standard groups, the test group demonstrated a statistically significant reduction in total microbial count ($P < 0.0001$) compared to the control group. This indicates that the test group possesses *Vraṇa śodhana* properties, suggesting its effectiveness in wound cleansing.

However, it's worth noting that performing fumigation on rats presented challenges, and there is a need for a standardized methodology to further refine this approach. Standardization would help ensure consistent and reliable results in future research and applications of *Dhūpana* in wound management.

REFERENCES

1. Pruitt Jr BA, McManus AT, Kim SH, Goodwin CW. Burn wound infections: current status. World J Surg 1998; 22:135-45. doi:10.1007/s002689900361.

2. Shastri Kaviraja Ambika Dutta, editor. Sushruta samhita of acharya sushruta, chikitsa sthana, 1st chapter, verse 8. 1st ed. Reprint; Varanasi: Chowkhamba Sanskrit Sansthan; 2007. p. 397.

3. Tanwar J, Das S, Fatima Z, Hameed S. Multidrug resistance: an emerging crisis. Interdiscip Perspect Infect Dis. 2014; 2014:541340. doi: 10.1155/2014/541340.

4. Vriddhajeevak. Kashyap Samhita. Sharma H, editor. Vidyotini Hindi Vyakhya. Varanasi: Choukhambha Publications; 2013. Kalpasthan 1; p. 172.

5. Morton Jjp MH. Evaluation of vulnary activity an open wound procedure in rats. Arch Int Pharmacodyn Ther 1972 Mar;196(1):117-26. PMID: 5059357.

6. Levine NS, Lindberg RB, Mason AD, Pruitt Jr BA. The quantitative swab culture and smear: a quick, simple method for determining the number of viable aerobic bacteria on open wounds. J Trauma 1976; 16:89-94 doi:10.1097/00005373-197602000-00002.

7. Gardner SE, Frantz RA, Saltzman CL, et al., Gardner SE, Frantz RA, Saltzman CL, Hillis SL, Park H, Scherubel M. Diagnositic validity of three swab techniques in identifying chronic wound infection. Wound Repair Regen 2006; 14:548-57 doi: 10.1111/j.1743-6109.2006.00162. x.

8. Mhaske Rajesh Harishchandra et al, Study of Krimighna Effect of Nimb (Azadirachta Indica A.Juss.) Patra As Rakshoghna Dhoopan by Culture and Sensitivity Method W.S.R. To Pyogenic Bacteria, IRJP, 2012, 3 (6), 142-146.

Corresponding Author:
Dr. Geetanjali Sahu, Assistant Professor, KJIAR, Vadodara
Email id- drgeetanjali1995@gmail.com

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