"MANAGEMENT OF SADYOVRANA BY MADHUSARPI."

THESIS

Submitted for the degree of

Doctor of Philosophy(Ayurved)

By

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महाराष्ट्राचे आराध्य दैवत श्री विठ्ठल चरणी अर्पण.

न तु कामये राज्यं

न स्वर्गं नापुनर्भवम् ।

कामये दुःख तप्तानां

प्राणिनां अर्तिनाशनम् ॥

Certificate

This is to certify that ***Vd. R.H. Amilkanthwar*** has completed his research work under my guidance and submitting his thesis entitled *" Management of Sadyovrana By Madhusarpi "*, For Ph.D. Award in Shalya Tantra, Faculty of Ayurveda in Swami Ramanand Teerth Marathwada University, Nanded.

Dr. R.H. Amilkanthwar has done his research work independently and this thesis is not submitted for any other exam or to any other University.

This thesis is recommended to be submitted for Ph.D. Award in Shalya Tantra, faculty of Ayurveda of Swami Ramanand Teerth Marathwada University, Nanded.

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DECLARATION

I **Dr. Rajendra Haribhau Amilkanthwar**

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INTRODUCTION

Throughout the history man has had to contend with dermal wounds. In primitive societies, substances derived from animals, plants and minerals formed the basis of crude remedies[1] needed to staunch bleeding, reduce swelling, minimize pain, remove damaged tissue, treat infections, mask foul smells and promote healing. The earliest documented records of topical wound treatments were found in Mesopotamia; these inscriptions on clay tablets have been dated to approximately 2500 BCE. The development and dissemination of later wound treatments can be traced from the ancient Egyptians, via the Greeks to Roman medicine[1], but the history of progress in wound care during the Middle Ages to the present time is incomplete[2].

An injury is the adverse effect of a physical force upon a person. The force involved in most injuries is mechanical[3]. The incidence of wound trauma is very large globally. The number of patients with wounds presenting to the emergency department and to the general practitioners is more as compared to any other health problem. Occasionally, the patients with minor and superficial injuries turn into major complications. Complicated wounds are the major cause of absentees at the working places and hospitalizations. A large share of economy is spent annually, for the prevention of complications and management of the wounded patients.

"Healing...is not a science but the intuitive art of wooing nature".

(W.H. Auden, "The Art of Healing")

Wound healing is a natural restorative response to tissue injury. Healing is the interaction of a complex cascade of cellular events that generates resurfacing, reconstitution and restoration of the tensile strength of injured skin. Healing is a systematic process, traditionally explained in terms of three classic phases viz. *inflammation, proliferation, and maturation*. A clot forms and inflammatory cells debride injured tissue during the inflammatory phase. Epithelialization, fibroplasia, and angiogenesis occur during the proliferative phase. Meanwhile, granulation tissue forms and the wound begin to contract. Finally, during the maturation phase, collagen forms tight cross-links to other collagen and with protein molecules, increasing the tensile strength of the scar. For the sake of discussion and understanding, the process of wound healing may be considered a series of separate events. In actuality, the entire process is much more complicated, as cellular events that lead to scar formation occur in tandem. Many aspects of wound healing have yet to be elucidated[4].

The major aspect of the management of the fresh wound is prevention of the infection and speedy healing. Reducing pain, discharge and less discoloration after healing are the other important factors. The proper initial care of the fresh wound will definitely prevent the inadvertent use of the oral and systemic antibiotics.

Antibiotics are potent antimicrobial agents with high specificity[5]. With the invention of Penicillin by *Alexander Fleming* eighty years ago, it was

considered that there will be no threat of infection in future. But the dream shortly came to an end with the development of resistance to penicillin. Now, after eighty years of its discovery, penicillin is making its return by showing sensitivity to some organisms. Same is happening with the topical applications. Since then the never ending efforts started for newer and newer antimicrobial agents. Many types of antimicrobial agents were searched and used successfully but the success was only transient. The problem remained the same and many generations of antibiotics have failed to provide long lasting antimicrobial effect. The organism kept adapting to the new antimicrobial and evolutes as multi-resistant strains.

Only recently the medical professional and researchers have realized that spending time in search of newer antimicrobial is of no use. Thus relentless emergence of antibiotic-resistant strains of pathogens, together with the retarded discovery of novel antibiotics has led to the need to find alternative treatments[5]. Now the scenario is changing and the whole world is looking towards the traditional and herbal medicine for the management of infection. [5]The most frequently used topical antimicrobials in modern wound care practice include iodine and silver containing products. In the past, acetic acid, chlorhexidine, honey, hydrogen peroxide, sodium hypochlorite, potassium permanganate and proflavine have been used. Some of these products seem to be making a return and other alternatives are being investigated[5].

Though much information is available today about wound healing, we are still way behind the actual process. Many factors are responsible for the

healing purpose and that makes the whole event complicated. We have invented many antiseptic agents for local application to prevent the contamination of the wound along with many oral antibiotics. Oral, par-enteral and local antibiotics prevent infection but have no role to accelerate the natural healing process. Also there is no effective agent to prevent the discoloration of the skin. The resulting scar tissue is not predictable. The rate of healing also varies depending upon many factors. For each of above purposes we give different medication i.e. a single agent can not do all the actions.

It is interesting to know in this regard about the ancient methods of wound healing. As we all know, *Ayurveda* was the mainstream medical science of the ancient era. We are having written evidences of the glorious past of this science. The medicine as well as surgery was at the peak in that era. We were far ahead of rest of the world in the field of health. We find many master techniques of surgical practice in the classical text of *Sushruta*. Surgery is not without wounds and trauma. If it was so well developed in that period, they must have effective wound healing measures. These wound healing measures are found well described in the text. A complete chapter has been devoted for the treatment of traumatic wounds[6]**.** Also they have described separate measures for healing of the operative wounds after each operative procedure**.** These are time tested, well proven and effective remedies being in use since thousands of years. But in the current practice, due to the dominance of the western medicine these measures remained somewhat hidden behind the concepts of modernization. In this age of research it was thought necessary to explore them.

Sushruta, as we know, is the *Father of Surgery*. In his text "*Shashti Upakramas*" are described for the treatment of the wound (*Vrana*) [7]. These are the 60 different regimes for the purpose of the wound healing. They cover all the aspect of the wound healing viz. rate, discoloration, scar formation etc. These all 60 are effective. One of them which was taken for the present research work is *Madhusarpi* i.e. combination of honey and ghee. It has been also described in the chapter of fresh wounds *(Sadyo-vrana)* [6]. *Charaka*, the great physician of ancient Indian medical science has also described the surgery related portion in brief. He has described 36 types of management of *Vrana*. In these 36 *upkramas*, he has mentioned *Ghrita* as one of the healing agent[20].

Honey is an ancient remedy[8] which has been re-discovered for the treatment of wounds[9]. Many therapeutic properties have been attributed to honey including antibacterial activity and the ability to promote healing[10]. Evidence of antibacterial activity is extensive, with more than 70 microbial species reported to be susceptible [11]. Later, *in vitro* studies have shown that active *manuka* honey is bactericidal against strains of antibiotic resistant bacteria isolated from infected wounds [12, 13, 14].

The main aspect of wound healing is the rate. It must be fast, so that the patient can recover as early as possible. This combination works faster compared to the natural healing or the other agents available. Honey has been described to possess the properties of *Ropana.* It promotes healing. Several authors have commented on the rapidity of healing seen with honey dressings. *Descottes* [15] refers to wounds becoming closed in a spectacular fashion in 90%

of cases, sometimes within a few days. *Burlando* [16] refers to healing being surprisingly rapid, especially for first and second degree burns. *Blomfield*[17] is of the opinion that honey promotes healing of ulcers and burns, better than any other local application used before. *Bergman* [18] has observed clinically that healing in open wounds is faster with honey, as has *Hamdy* [19] who also found that it accelerated making wounds suitable for suture.

The other ingredient of the combination is *Ghrita (Ghee).* It has been described of having thousands of properties which are useful to man kind[21]. It has been used extensively in wounds of recent origin. *Vagbhata* has described it to have great healing and binding properties[22]. *Charaka* has advised its application on the extensively injured wounds[23]. In the text of *Bhav-prakasha* also the *Rakshoghna* property of *Ghrita* has been described[24].

Owing to the different useful properties of these two *(Ghee and Honey),* there combined use is found in greater extent. They work synergistically in the wounds. *Vagbhata* has described the use of these two after surgical procedure for the postoperative wounds[25].

The other point is *quality of healing*. It has been observed that this combination results in better quality of healing. It is being used by many traditional practitioners and as a home remedy since years. The results are well known. It leaves minimum scar and minimum discoloration. It was intended to know whether it is having any effect on the strength of the scar.

Another important factor is *pain*. Every trauma is associated with some degree of pain for which one has to give the pain killers in the form of oral or

par-enteral medication. It is not required with this combination. It also possesses analgesic action as described in the texts. The anti-inflammatory property has been proved in the isolated studies[26].

Any other antiseptic agents are not required. The given combination has antimicrobial action also. The researches done separately on honey and *ghee* have proved that they act against many microbial agents. Now it was interesting to know how the combination works.

The classical properties of an ideal medicine are:

'An ideal medicine should have many properties, the various forms of it can be used, it should be available easily and in large quantities and it should be an appropriate drug for the condition.' (Cha. Su. 9/7,)

The given drug possesses all these properties.

It has been well indicated in the text for the above said purpose:

'Madhu and Sarpi are very useful for fresh wounds. They alleviate the pain and burning sensation and aid healing of fresh wound.' (Su Chi. 1/130.)

To establish these facts on the modern parameters a thorough study is needed.

No adverse effects are known and none are described in the texts. It has shown no local or systemic toxicity. No references of the same are available in the ancient texts. But it was needed to prove these things on the modern parameters. One of the objectives of the research was to study the effects of the drug –local and systemic on the human body.

Animal study was planned to assess the efficacy. It was carried out at the animal house of *National Toxicological Centre at Pune*. The combination has proved to be very effective in the rats and mice by promoting healing and leaving better quality scar. No local or systemic adverse effects were observed in the animals.

In the year 2001 the global wound care market was estimated at US$ 13156 million with an annual growth rate of 15% [27]. This projection was for 40-45 million surgical procedures, and other chronic wounds like 8-10 million leg ulcers, 7-8 million pressure sores and an equal number of burn wounds. To treat these, large scale use of antiseptics was anticipated. Including this, the anticipated market share for wound care products in 2006 was reached approximately US $ 25000 million.

This work was planned to establish the efficacy of the combination on the basis of the modern parameters and also to find out the action mechanism on the modern grounds. It is aimed to establish this drug as a better option for the existing local applicants. It is easily available, cost effective and multipurpose. It can alone fulfill the need of antiseptic, cosmetic and healing enhancing agent. Both the ingredients being easily available in India, we can carry out the large scale production in the form of *ointments*, Tulles, and Patches etc. It will be available for instant use, will be economical and will prove to be a blessing for the mankind.

References :

1. Forrest RD. Early history of wound treatment. *J R Soc Med* 1982; **75**(3): 198-205.
2. Forrest RD. Development of wound therapy from the Dark Ages to the present. *J R Soc Med* 1982; **75**(4): 268-73.
3. Baily and Loves short textbook of surgery
4. Thomas Romo III, MD, FACS**,** Chief, Clinical Instructor, Department of Otolaryngology, Division of Facial Plastic and Reconstructive Surgery, New York, Wound Healing, Skin, article 2007, e-medicine.com
5. Rose Cooper BSc, PhD, PGCE, C Biol, MI Biol, Principal Lecturer in Microbiology, article, A review of the evidence for the use of topical antimicrobial agents in wound care, http://www.worldwidewounds.com/2004/february/Cooper/Topical-Antimicrobial-Agents.html
6. *Sushruta Samhita, Ambikadatta shstri, Choukhamba* publication, 2001, *Chikitsa sthana,Chp.2,Sadyovrana chikitsa adhyaya.*
7. *Sushruta Samhita, Ambikadatta shstri, Choukhamba* publication, 2001, *Chikitsa sthana, Chp.1, Vrana chikitsa adhyaya*.
8. Jones R. Honey and healing through the ages.. In: Munn P, Jones R, editors. *Honey and Healing*. Cardiff: IBRA, 2001.
9. Zumla A, Lulat A. Honey--a remedy rediscovered. *J R Soc Med* 1989; **82**(7): 384-5.

10. Molan PC. The role of honey in the management of wounds. *J Wound Care* 1999; **8**(8): 415-8.
11. Molan PC. The antibacterial activity of honey. Part 1. Its use in modern medicine. *Bee World* 1992; **80**(2): 5-28.
12. Cooper RA, Molan PC, Harding KG. The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds. *J Appl Microbiol* 2002; **93**(5): 857-63.
13. Cooper RA, Halas E, Molan PC. The efficacy of honey in inhibiting strains of Pseudomonas aeruginosa from infected burns. *J Burn Care Rehabil* 2002; **23**(6): 366-70.
14. Cooper RA, Wigley P, Burton NF. Susceptibility of multiresistant strains of Burkholderia cepacia to honey. *Lett Appl Microbiol* 2000; **31**(1): 20-4.
15. Descottes B. De la ruche a l'hospital ou l'utilisation du miel dans l'unité de soins. L'abeille de France et l'apiculture 1990(754):459-460.
16. Burlando F. Sull'azione terapeutica del miele nelle ustioni. *Minerva Dermatol* 1978; 113:699-706.
17. Blomfield R. Honey for decubitus ulcers. *J Am Med Assoc* 1973; 224(6):905.
18. Bergman A, Yanai J, Weiss J, Bell D, David MP. Acceleration of wound healing by topical application of honey. An animal model. *Am J Surg* 1983; 145:374-376.
19. Hamdy MH, El-Banby MA, Khakifa KI, Gad EM, Hassanein EM. The antimicrobial effect of honey in the management of septic wounds,

Fourth International Conference on Apiculture in Tropical Climates, Cairo, 1989. International Bee Research Association, London.

20. *Charaka Samhita, Naraindatta Shastri, Choukhamba* publication, 2001, *Chikitsa sthana,Chp.1,Vrana chikitsa adhyaya.*
21. *Ashtang Hridaya, Sutrasthana 5/39*
22. *Ashtang Hridaya, Sutrasthana 5/40*
23. *Charaka Chikitsasthana 25/65*
24. *Bhav Prakasha, Ghrita varga, 5*
25. *Ashtang hridaya, Sutrasthana 29/26*
26. MS Charde, SV Fulzele, PM Satturwar, SB Joshi, AV Kasture, Indian journal of pharmaceutical science, 2006 Vol: 68, Issue 1,Page 26-31.
27. Market Analysis, in Advanced Wound Care Biologics: World Market Analysis, Chapter 6, PJB Medical Publications Inc. 2002

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AIM AND OBJECTIVES

AIM

To study the role of *Madhusarpi* on *Sadyovrana.*

OBJECTIVES

1. To see the efficacy of the drug in the *sadyovrana.*
2. To see whether the drug has any antimicrobial properties.
3. To see the effect on the rate of healing of the wound.
4. To see the effect on the quality of healing of the wound.
5. To see the analgesic effect of the drug.
6. To see any adverse or toxic effects of the drug.

HISTORICAL REVIEW

History tells us about the past time, how the time begins, the development and evolution of the mankind occurs etc. It helps to reveal hidden facts and ideas of concerned subject. It also assists to pave the pathway for future. Large numbers of references pertaining to the wound and wound healing were found in ancient Indian literature.

- ***Rugveda*:**

In reference to transplant of the head of *Yagya (Madhuvidya and Kaksyaya –Vidya)*

- ***Samveda*:**

Vrana Ropana karma in the case of injured prince. (*Jaminiya Brahmana-3/94-95).*

- ***Atharvaveda*:**

References of some drugs for *Vrana ropana* and *Vrana Shodhana* are:

1. *Laksha* for *Vrana Rohana (Kaushikkasutra 28/5/14).*

2. *Mansa Rohini* for *Vrana Ropana (Atharvaveda 6/139/5)*

3. *Raksoghna Dravyas*.

4. *Gomutra in Vrana*

5. Exploration of ripped *vrana (Brahmana)*

6. Sault for unripe *vrana (Atharvaveda 7/10/1-2)*

- ***Ramayana*:**

The reference of *"Sanjivani Jaddibutti"* is described as *Vrana Sandhanakara* and *Vrana Svarnakara* by *Tulsidas.*

- ***Mahabharat*:**

The battle of *Kurukshetra* took lives from either side. Soldiers used to carry first aid kit along with them and in this period, drugs and other materials were stored by the authority for war period.

- ***Buddhakala*:**

Drugs for *vrana ropana* are:

1. Tila Kawth

2. Dhupana taila

3. Vranaropanarth taila

- ***Smriti and Purana*:** *(Agnipurana)*

For the haemostatic purpose *"Durva"* was applied on the wound.

- ***Mauryakala*:** *(Bhadant Nagsen)*

Management of *Vrana* by *"Malhara Ksara Proyaga".*

- ***Charaka Samhita*:**

Dvivraniya Adhaya in *Chikitsasthana* regarding *Vrana* and management of *Vrana*.

- ***Bhela Samhita*:**

Like *Charaka, Bhela* also explained management of *vrana*.

- ***Sushruta Samhita*:**

Detailed review of *Vrana* and its management has been discussed by *Sushruta*. During this time the knowledge of wound was at its peak. Being a surgeon *Acharya Sushruta* knew the importance of wound in the practice.

- ***Kasyapa Samhita*:**

  Nija and *Agantuja* types of *vrana* with management are described.

- ***Navnitakam*:**

  Different types of *Taila* and *Lepa* were described for management of *vrana.*

- ***Astanga sangraha*:**

  The knowledge of wound and its healing was edited and classified on the basis of different stages by *Vagbhata*. He advocated preparation and application of ghee based ointment for local use.

- ***Astanga hridaya*:**

  Acharya Vagbhata clearly described the types of *Vrana* and its management.

- ***Madhava chikitsa*:**

  Bhagna and *Sadhyo Vrana* were described in chapter *45– 46.*

- ***Vrinda Madhava*:**

Jatyadi Sarpi mentioned in the management of *Agantuja Vrana*.

- ***Bhaisjya Ratnavali*:**

  Description of *Karpoor Sarpi* and many other preparations for *Nija* and *Agantuja Vrana* are found in the management of *Sadhyo Vrana Chikitsa Adhyaya*

- ***Yoga ratna samucchaya*:**

  Vrana Ropak Taila and other preparations were described for wound healing.

- ***Madhava Nidana*:**

  Types, characters and classification of *Vrana* were described in chapter *41* and *Agantuja (Sadhyo Vrana)* in chapter *42*.

- ***Sarangdhara Samhita*:**

  Application of *Nimba Kalka* in the management of *Vrana* and *Dusta Vrana*. In *Purva Khand*, *Taila* for *Vrana* under *Taila Kalpana.*

- ***Bhavprakash*:**

  Complete chapter - *Chikitsasthana 47*, is devoted for *Vrana, Vrana-Shotha, Vrana Shodhana and Vrana Ropana*. Uses of different drugs in particular to healing are also dealt there.

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Modern medicine:

- **16th to 19th century:**

These centuries were well known for the French revolution and Russian revolution. French army surgeon *"Dr. Ambroise Pare"* re-discovered gentle method during the battle of violence. *Dr. pare* was forced to apply milder treatments to the amputation wounds. To his surprise these wound healed much faster and with lesser complication. From this was the beginning of modern era of gentle wound management evolved.

John Hunter, William Stewart, Alexin Carrel and many other great clinical biologists emphasized and demonstrated that minimizing the tissue injury produces rapid and effective healing. The ethics of wound healing is based on *"Minimal Interference"* concept. If a surgeon can remove all impediments, normal wound healing processes produce the best results.

In *18*th century *John Hunter* brought out that a wound heals fasters if suppuration after suturing due to ancients hands of surgeons was stopped, but

could not bring out the actual organism responsible for suppuration. This was later discovered by *Louis Pasteur (1822-95).*

- **20th century:**

Main event of this century was World War I and II. During these, the greatest difficulty faced was the curing of wounds and their protection from infection from outside sources like skin, clothing, missiles and dirty soil, which would have serious repercussions like tetanus, gangrene which would have spread and rapidly convert the muscles into a mass of putrefying flesh

distended with stink. It was the mobile casual cleaning stations equipped with every operative necessity which came to their rescue.

However it was not certain that the wound will heal even if it was treated surgically as chances of infection were still there. Once Penicillin was isolated, the above problem was demolished. It was used both for prophylaxis and cure of contamination. Scientific study towards wound healing was first done by *Alexis Correl et.al.* and evaluated the observations on the surface of wounds and watched the process of healing in laboratory animals and patients to demonstrated the effect of age, temperature, infection and other conduction of wound healing.

Parameter Evaluation	***Parameter Evaluation***
1) Computer aided planimetry:	Rate of open wound healing
2) Laser Doppler Imaging:	Wound perfusion
3) Tensiometry:	Wound strength
4) Histopathology/cytology:	Microscopic evaluation of wound tissues and fluids
5) Biochemistry (HPLC/RIA):	Various drugs and biochemical components of healing tissues
6) Electrodiagnostics:	Relationship of wound healing and innervations.
7) Scintigraphy:	Radionuclide imaging of wound tissues.
8) Kinetic pressure evaluation system:	Evaluation of pressures associated with various bandage and splint materials

- **21st century:**

Development and research reaches at high level in this century. Some of the research about wound healing as follows. Some wound healing program has capabilities to evaluate wound healing using several parameters.

These include:

- **Parameter Evaluation**
- **Areas of recently completed research include:**

1) Effects of Omega-3 fatty acid enriched diets on wound healing.
2) Effects of collagen wound dressings on wound healing.

3) Application of tissue adhesives in the treatment of wounds.
4) Use of intra-lesional injection of healing stimulants in healing of wounds in weight-bearing tissues.
5) Effects of magnetic field therapy on wound healing.

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AYURVEDIC LITERATURE REVIEW

Ayurveda has been practiced in this country from time immemorial and has stood the test of time. The study of *shalya* science brings out very clearly that *vrana – wound* is the most significant entity on which the whole science of surgery revolves. *Ayurveda* classic at various places have emphasized to take care of wounds which occurs either as a result of vitiated *Doshas (Humours)* or due to traumatic origin.

- **Etymon of the word *"Vrana":***

The words derived from the root *"Vriya"* having the meaning of "to recover", which is further suffixed by *"ach"* in the sense of *Bhava*. The *"Ch"* sound is elided and the form remains *"Vran" + "a"*, in the sense of *"Gatra Vichuranane". (Shabdakalpadruma, Su.Chi. 1/6)*

Definition:

Vrana Gatra Vichurnane, Vranayati iti Vranaha. (Su. Chi. 1/6)

"Gatra" means tissue (tissue or part of body)

"Vichurnane" means destruction, break, rupture and discontinuity (of the Body or tissue)

"The destruction / break / rupture / discontinuity of body tissue / part of body, is called *Vrana*."

In *Sutrasthana Chapter 21, Acharya* has clarified that as "the scars of a wound never disappear even after complete healing and its imprint persists lifelong, it (the lesion) is called *vrana* by the wise".

Sushruta has described 15 types of *Nija Vrana* according to *dosha*. It includes :

1. *Vataj*
2. *Pittaja*
3. *Kaphaja*
4. *Shonitaja*
5. *Vata pittaja*
6. *Pitta kaphaja*

7. *Vata kaphaja* 8. *Sannipataja* 9. *Vata Shonitaja*

10. *Pitta Shonitaja* 11. *Kapha Shonitaja* 12. *Vata pittaja shonitaja*

13. *Pitta kapha shonitaja* 14. *Vata kapha Shonitaja*

15. *Vata pitta kapha shonitaja*

The detailed description of these types regarding *lakshanas* and treatment has been described in the *Sushruta Samhita*.

✓ ***Sushruta Samhita*: Types and *Lakshanas of Agantuja Vrana:***

Sr. No.	***Type of Agantuja Vrana***	***Lakshanas***
1	***Chinna*** *(Su.Chi.2/10)(Ma.Ni.43/3)*	Extensive cut injury oblique or straight, separation of Parts of body.
2	***Bhinna*** *(Su.Chi.2/11)(Ma.Ni.43/4)*	Perforation of *Asaya* and mild discharge.
3	***Viddha*** *(Su.Chi.2/19)(Ma.Ni.3/11)*	Deep injury Without Perforation of *Asaya.*
4	***Kshata*** *(Su.Chi.2/20)(Ma.Ni.43/12)*	Neither a cut injury nor a perforation but exhibits the nature of both uneven shaped.
5	***Picchita*** *(Su.Chi 2/21)(Ma.Ni.43/13)*	Crushed injury extended filled with blood and Bone marrow.
6	***Ghrishta*** *(Su.Chi.2/22)(Ma.Ni.43/14)*	Rub injury skin gets peeled off, burning sensation and Discharge.

❖ ***Lakshana of Shuddha Vrana***

✓ ***According to Sushruta, (Su. Su.23/18, Su. Chi. 1/7)***

- Recent origin.

- Unaffected by the three *Doshas.*
- Edges with a slight blackish colour and having granulation tissue.
- Absence of pain.
- Absence of secretion.
- Even surface through out the wound area.
- Slimy surface.
- Regular surface

✓ ***According to Charaka (Ch. Chi. 25/86)***

- Color of wound is reddish black.
- Moderate pain.
- No any type of elevation and depression.

✓ ***According to Ashtang Sangraha, (As. San. Ut. 29/12)***

- No pain.
- No discharge.
- Color of wound is blackish.
- Even margins,
- Opposite character of *Dusta vrana.*
- Slight elevation in the middle.

✓ ***According to Ashtang Hridaya (As. Hr. Ut. 25/11)***

- Surface of wound is just like tongue.
- Soft.
- Wound is Un acute.
- Surface is smooth and normal.
- Absence of pain and secretion.

Similar description is found in *Madhava Nidana* also.

Shuddha Vrana only heals properly within due course of time.

❖ ***Lakshanas of Dusta Vrana***

✓ ***According to Sushruta, (Su. Su. 22/7)***

- Extremely narrow or wide mouthed.
- Too soft.
- Elevated or Depressed
- Black or red or white colored.
- Too cold or hot.
- Full of slough or pus or veins or flesh or ligaments or putrid pus.
- Upward or oblique course of suppuration.
- Pus runs in to cavity and fissures cadaverous smell.
- Burning sensation.
- Redness.
- Itching.
- Pustules crop up around secrete with blood.

✓ ***According to Charaka (Cha. Chi. 25/24-25) (Cha. Chi. 25/83)***

No specific *Lakshanas* mentioned by *Charaka*. He has described some features according to classification.

✓ ***According to Vagbhata (As. Hr. 25/2-4)***

- Too hard/Too soft.
- Too elevated/Too inverted.
- Too hot/Too cold.
- Colour of *Vrana* is red/pandu/black.
- Severe painful.
- Burning sensation.
- Inflamed.
- Redness and itching is present.
- Chronic in nature.

Similar description is found in *Madhava Nidana and Sharngdhara Samhita (Ma. Ni. 42/7,Sa. Pu. Kh. 7/71-74).*

❖ ***Ruhyamana Vrana Lakshana***

✓ ***According to Sushruta, (Su. Su. 23/19)***

- Absence of any type of discharge.
- Presence of healthy and new granulation tissues.
- Yellowish colored wound.
- Surrounding area of wound is
- Hard

Charaka has not given any description of *Ruhyamana vrana*.

✓ ***According to Vagbhata (As. Hr. Ut. 25/22)***

- Done colored without any type of mucoid secretion.
- Stable.
- Good granulation tissue.

✓ ***According to Madhava Nidana (Ma. Ni. 42/9)***

- Blackish white colored. *
- Moist less and dry.
- Immobile/stable with granulation tissue.

❖ ***Samyaka Rudha Vrana***

Charaka and Vagbhatta have not described the *samyaka rudha vrana.*

✓ ***According to Sushruta (Su. Su. 23/20)***

- Edges: Firmly adhere.
- Pain: No pain.
- Swelling: Not appears.
- Leaves cicatrices of the same line with the surrounding skin.

✓ ***According to Madhava Nidana (Ma. Ni. 42/10)***

- Edges: Even.
- Pain: No pain.
- Swelling: Not present.

Some other types of *Agantuja vrana* are as follows

Sr.no.	Types of *Agantuja vrana*	*Sushruta*	*As.sam.*	*As.hr.*	*Sha.sam.*
1	*Avakrta*	----	+c	+	----
2	*Anuviddha*	----	+v	----	----
3	*Atividdha*	----	+v	----	----
4	*Anubhinna*	----	+v	----	----
5	*Atibhinna*	----	+v	----	----
6	*Avrana*	----	+p	----	----
7	*Avikrta*	----	----	----	+
8	*Bhinna*	+	----	+	+
9	*Bhinnotundita*	----	+v	----	
10	*Chhinna*	+	+	----	+
11	*Ghrista*	+	+c	+	+
12	*Ksata*	+	----	----	----
13	*Nilambita*	----	+c	----	----
14	*Nividdha*	----	+v	----	----
15	*Nipatita*	----	----	----	+
16	*Pichhita*	+	+	----	----
17	*Patita*	----	+c	+	----
18	*Pravalambita*	----	----	+	----
19	*Prachalita*	----	----	----	+
20	*Savrana*	----	+p	----	----
21	*Uttundita*	----	+v	----	----
22	*Viddha*	+	+	+	+
23	*Vicchinna*	----	+c	+	----
24	*Vibhinna*	----	+v	----	----
25	*Vidalita*	----	----	+	----
26	*Vilambita*	----	----	----	+

(+C: This all type of *agantuja vrana* comes under *Chhinna* category.

+V: This all type of *agantuja vrana* comes under *Viddha* category.

+P: This all type of *agantuja vrana* comes under *Pichhita* category.)

Description of some other *Agantuja Vranas* in classical texts:

Name	***Lakshanas***
Avakrta (As. Sa.Ut. 31/3)	Injury in skin and little portion of muscle.
Anuviddha (As. Sa.Ut. 31/4)	Injury of muscular tissues.
Atividdha (As. Sa.Ut. 31/4)	Perforation of the part and peeping outside the skin of the other side.
Anubhinna (As. Sa.Ut. 31/4)	Perforation of the *Kostha*.
Atibhinna (As. Sa. Ut. 31/4)	Injury in *Kostha*.
Avrana (As. Sa. Ut. 31/5)	Injury without *vrana* with mild local temperature.
Avaklipta (Sa. Pu. Kh. 7/76)	Injury with cutting type of pain, breaking of irregular extremities, loss of strength.
Bhinnotundita (As.Sa.Ut. 31/4)	Injury to the *Kostha*.
Nirbhinna (As. Sa. Ut. 31/4)	Injury to the *Kostha* by a *shalya* and it pierces the opposite side.
Nirviddha (As. Sa. Ut. 31/4)	Perforation of part totally.
Nishalyo vrana (Ma. Ni. 43/15)	Mild tender, mild inflammation.
Nipatita (Sa. Pu. Kh. 7/76)	Bone brakes in to many places, abnormal deformities.
Patita (Sa. Pu. Kh. 7/76)	Complete cut off of an organ.
Pracchalita (Sa. Pu. Kh. 7/76)	Injury to *Asthi dhatu* and causes vitiation of *vayu*.

Pravilambita (As. Hr. Ut. 26/4)	Injury where the bone has not cut completely destroyed. Inactivity of sense organs Different type of pain, bloody discharge.
Savrana (As. Sa. Ut. 31/5)	Injury with *vrana*, painful and Oozing.
Sasalyasyavrana (Ma. Ni. 43/15)	Injury due to the *vrana*, inflammation, blackish in color.
Uttundita (As. Sa. Ut. 31/4)	Injury to the deep portion with protrusion to other side.
Vicchinna (As. Sa. Ut. 31/3)	Injury to the deep skin and greater portion of the muscle involved.
Vilambita (As. Sa. Ut. 31/3)	Injury up to the bone ligaments and muscles coming out from the *vrana mukha*.
Vidalita (As. Hr. Ut. 26/5)	Crushed injury along with bonemarrow damage, severe pain.

According to *Sushruta* "Every *vrana* will occur in certain type of tissues and that is *Vranavastu".* Eight types of *Vranavastu* have been described by *Sushruta and Charaka.*

- ✓ ***Sthana* of *vrana* according to *Sushruta* and *Charaka***

(Su. Su. 22/3, Ch. Chi. 25/26)

	Sushruta	**Charaka**
1	*Tvaka*	*Tvaka*
2	*Mansa*	*Mansa*
3	*Sira*	*Sira*
4	*Snayu*	*Snayu*
5	*Asthi*	*Asthi*

6	*Marma*	*Marma*
7	*Kostha*	*Antarasraya*
8	*Sandhi*	*Meda*

- ✓ ***Varna (Color) of vrana:***

Varna (Color) of *vrana* described by *Sushruta* according to *dosha.*

(Su. Su. 22/13)

- ✓ ***Gandha (Odor) of vrana:***

Acharya Charaka has described eight different types of *"Vrana Gandha"* (Odor)

(Cha. Chi. 25/27)

- ✓ ***Shape of "Vrana" (Su. Su. 22/5, As. Hr. Su. 28/18)***

Sushruta	***Hradayakar***
Elongated	It is recognized by the shape of *Shalya*/Foreign body inserted.
Elliptical	
Rectangular	
Circular	
Triangular	

- ✓ **"*Vrana srava*" according to *Vrana sthana. (Su. Su. 23/8)***

Sthana/Vranavastu	***Vrana srava***
Tvaka	Yellowish Watery
Mansa	Thick *Ghee* like

Sira	Excessive bleeding, after suppuration pus discharge.
Snayu	Thick Mucoid and blood stain discharge.
Asthi	Mix discharge with blood and bone marrow.
Shandhi	Discharge less in rest condition but mixed with pus and blood and pus exudates come out on movement.
Kostha	Blood, Urine, Stool, Pus, and watery or serous discharge.

- ✓ **"*Vrana srava*" according to *dosha involved (Su. Su. 23/9)***

Vrana Dosha	***Vrana srava***
Vata	Rough, Blackish, Like frost, Yoghurt, Alkaline water, Washing of meat, Rice water.
Pitta	*Gomeda* gem, *Cow's* urine, Ash powder of conchshell, Astringent water, *Madhavika* oil like.
Kapha	Like Butter, *Kasis*, Bone marrow, Rice cake, water of Coconut, Fat of pig.
Rakta	Like *Pitta* but more bloody discharge
Sannipataj	Water of Coconut, vinegar, liver, juice of *Mudga.*

Charaka has described fourteen different types of *Srava.* *(Ch. Chi. 25/28)*

Vranitagar:

According to *Acharya Sushruta* for a wound affected man must live in *Vranitagar*. *(Su. Su.19.)* The concept of *Vranitagar in Ayurveda* is resembles to the surgical ward (Inpatient Department) seen in Modern Hospitals today.

■■■■■■

MODERN LITERATURE REVIEW

The problem of wound healing has been dealt with at various levels by mankind ever since the advent of humanity. The mission of the wound healing is to increase our basic understanding of the molecular and cellular events of the cellular repair and wound healing processes, and to use this information as the basis for developing new therapies that minimize the adverse consequences of injuries. However, not many wound healing agents have been in use in the modern allopathic medicine, either for internal or external injuries.

It has been estimated in America that 3-5% of all hospitalized patients suffer from pressure ulcers and if the patients have spinal cord injuries, the percentage of pressure ulcers patients is between 25-85%. In America the cost of institutional care is supposed to be $ 1000 per day. While no such estimation are available for Indian institutions, the same demographic study has projected market expenditure of over $7 billion worldwide for provisions of wound healing therapies.

Wounds, whether caused by accidental injury or a surgical scalpel, heal in three ways:

(1) Primary intention (wound edges are brought together, as in a clean surgical wound),

(2) Secondary intention (the wound is left open and heals by epithelization),

(3) Third intention, or delayed closure (the wound is identified as potentially infected, is left open until contamination is minimized, and is then closed).

Choosing which method is best, depends on whether excessive bacterial contamination is present, whether all necrotic material and foreign bodies can be identified and removed, and whether bleeding can be adequately controlled. Normal healing can occur only if the wound edges are clean and can be closely opposed without undue stress on the tissue. An adequate blood supply to the wound is essential. If the tissue is tight and the edges cannot be closed without tension, the blood supply will be compromised. Cutting under the skin to free it from the underlying subcutaneous tissue may allow the edges to be brought together without tension. If direct approximation is still not possible, then skin grafts or flaps are used for closure. In this chapter some aspect of the physiology and pathology of wound and its healing will be discussed.

Definition of wound:

"The term Wound is break in the continuity of soft parts of body structures caused by violence of trauma to tissues".

- *Taber's Medical Cyclopedia*

"The Disruption of normal anatomical relationships as a result of injury or more specifically of trauma".

- Blakiston's New Gold Medical Dictionary

Definition of ulcer:

"Ulcer word is derived from the Latin word "ulcus". It means an open sore or lesion of the skin or mucous membrane accompanied by sloughing of inflamed necrosis tissue". - *Taber's Medical Cyclopedia*

Types of Wounds:

Although all wounds follow roughly the same healing process, there are many different causes of wounds. One medical term for a wound is an ulcer. Partial-thickness wounds penetrate the outer layers of the skin, the epidermis and the superficial dermis, and heal by regeneration of epithelial tissue (skin). Full-thickness wounds involve a loss of dermis (deeper layers of skin and fat) and of deep tissue, as well as disruption of the blood vessels; they heal by producing a scar. Wounds are classified by stage.

- ***Stage 1:*** wounds are characterized by redness or discoloration, warmth,

  and swelling or hardness.
- ***Stage 2:*** wounds partially penetrate the skin.
- ***Stage 3:*** describes full-thickness wounds that do not penetrate the tough

white membrane (fascia) separating the skin and fat from the deeper tissues.

- ***Stage 4 :*** wounds involve damage to muscle or bone and undermining of adjacent tissue. They may also involve sinus tracts (red streaks indicating infected lymph vessels).

One more school of thoughts has described wound as follows

1) Superficial wounds:

Only the epidermis is damaged. The true skin – corium – is intact. Thus the tensile strength of the tissue remains unchanged. Continuity is restored by growth of epithelium from the wound edges and/or from hair follicles, sebaceous or sweat glands. Healing occurs without scar formation. However, changes in skin pigmentation may appear.

2) Deep wounds:

In deep wounds healing will differ depending on whether there is loss of tissue or not.

a) Deep wounds without loss of tissue:

In these wounds the wound edges can be adapted by sutures and/or surgical tape. Both continuity and strength must be restored. Continuity is restored in the deeper layers by formation of connective tissue and on the

surface by epithelial overgrowth. During the inflammatory phase there is redness and swelling in the wound area and the patient may initially have pain. The wound feels stiff. At the end of proliferative phase the scar is a narrow red line.

During maturation the scar turns white but often also becomes border. Uncomplicated healing of a wound of this type is called healing by first intention.

b) Deep wounds with loss of tissue:

As in other wounds the inflammatory phase lasts a few days. During the proliferative phase the wound gradually fills with granulation tissue when vessels and fibroblasts invade the coagulum.

Replacement of lost tissue and restoration of continuity requires formation of a fair amount of new tissue. This will take time. In the previously described deep wound without loss of tissue the granulation tissue is rapidly covered and protected by epithelium. In wounds with loss of tissue the granulation tissue is only slowly covered by epithelium advancing from the wound edges. Even under favorable conditions the epithelial border advances only about one millimeter a day. The area to be covered by epithelium diminishes by wound contraction caused by certain cells within the granulation tissue-myofibroblasts. The unprotected granulation tissue is associated with increased risk of complications, i.e. infection during healing.

During the proliferative phase the wound is weeping and often covered by light yellow fibrinous coagulum. Beneath this fibrin the granulation tissue appears as a grainy, easily bleeding, red surface. Advancing thin epithelium may be seen as a light grey – red brim at wound edges.

When finally the surface is covered by epithelium there is a continuous maturation of the granulation tissue with remolding of the collagen structure and reduction of the number of blood vessels. The newly formed epithelium is thin and has low resistance to trauma of any kind.

This type of healing, is called healing by second intention, takes longer and gives a proper result in appearance and strength than healing by first intention.

Other types of wounds:

1) Excoriations (Abrasion):

An excoriation is loss of the surface epithelium but with preserved continuity in the skin. The wound does not gape and subcutaneous fat is not seen. Epithelium remains undamaged in hair follicles, sweat and sebaceous glands.

2) Incisions and cuts (vulnus incisum):

Tissue injury is small, loss of tissue minimal and contamination usually limited. Injuries on tendons, vessels and nerves may occur and must be diagnosed.

3) Stab wounds (vulnus punctum):

These wounds are caused by pointed objects. Tissue injury is usually small. The stab wound is however, often irregular due to displacement of the tissue layers at the injury. The possibility of a foreign body remaining within the wound must always be considered. It is important, therefore, to know what the pointed object was made of, and whether it was broken at the time of injury. Foreign bodies may be a part of the object or pieces of cloth or leather which were carried into the wound. Remaining pieces of metal can be identified and localized by x-ray using indicators.

4) Laceration:

Wound in which the tissues are torn with ragged confused edges provides many avenues for infection underlying soft tissue is pulped blood vessels being torn. Twisted little bleeding is apt to result.

5) Amputation:

Any sharp cut where tissues are not served may be either aseptic or infected, depending on circumstances that caused it. The bleeding can be readily controlled and the underlying nerves, vessels and tendons may be cut partially or completely.

6) Avulsion:

Wound in which the tissues are scrap of from its particular place due to rubbing the surface epithelium by fractional violence. In this type of wound proper cleaning of wound is must and after that suturing is done by experts.

7) Contusion injury (vulnus contusum):

These injuries are caused by blunt trauma. The skin wound is often irregular with rather marked tissue injury. The damage to deeper tissues may be more extensive than that of skin and may include fractures.

The degree of contamination varies with the cause of the injury. Treatment of concomitant fractures requires special considerations and should be left to experienced orthopedic surgeon.

8) Missile wounds (vulnus sclopetarium):

Missile wounds are always associated with major tissue injury and marked contamination. Wide debridement should be performed by an experienced surgeon.

9) Bites (vulnus morsum):

Bites are a combination of puncture wounds and contusion injuries. The degree of tissue injury varies but the wound is always contaminated. Bacteria from the mouth are said to penetrate into surrounding tissues especially rapidly. Bites that only cause excoriation are cleansed and handled asexcoriations in general. Bites penetrating the skin should be completely excised and closed by wide spaced sutures. Bites from dogs and cats are most common. These animals have a gram-negative rod pasteurella multocida in their mouths. This rod causes typical infections with only slight inflammatory

reaction but marked tendency to tissue necrosis and yellow – grey odorless pus. Excision should be followed by parenteral antibiotics.

10) Injection injuries:

Injection injuries have lately become more frequent. There are two different types.

- **Type one:** Caused by high pressure injection of substances, usually into the hand, e.g. when performing anti-rust treatment or pressure lubrication.
- **Type two:** Caused by extra-vascular injection or infusion. The extent of injury depends on the amount deposited.

11) Burns.

Most burns occur in the home. They can be caused by scalding hot liquids, grease fires, car accidents, chemical explosions, frayed electrical cords, house fires, hot objects (stoves, irons, tailpipes), or even the sun.

12) Arterial ulcers:

The arteries supply blood, which carries the oxygen that cells need to live. If arterial circulation is partially or completely blocked, the tissue will begin to die, resulting in a painful wound. Treatment of arterial ulcers has two goals: re-establishing circulation with medical treatment and healing the wound(s).

- ***Ischemic leg ulcer:*** An ischemic leg ulcer is usually localized to the foot or the outer side of the lower leg.

13) Venous ulcers:

Venous ulcers are the most common wounds affecting the legs, and are frequently found on the ankles. They are shallow, not too painful, and may have a weeping discharge. Although venous valves cannot be repaired, the return of blood through the veins can be improved by physical activity and by compression, which can be supplied by compression stockings, dressings, or mechanical pumping devices.

- ***Venous leg ulcer:***

Uncomplicated varicose veins never cause leg ulcers, while there is a considerable risk of leg ulcers when there are incompetent perforating veins.

14) Diabetic ulcers:

Diabetics have impaired wound healing and impaired resistance to infection. Diabetes results in a narrowing of the small arteries, which can cause ulcers. This narrowing cannot be resolved, but can be prevented by careful glucose control. Diabetes also causes peripheral neuropathy and the loss of sensation, especially sharp-dull discrimination, in the legs and feet. For this reason, injuries to the feet may go unnoticed and can progress into serious wounds.

15) Pressure ulcers:

Also known as bedsores, pressure ulcers are very common in older and immobile persons. When too much pressure is placed on them, cells do not get enough oxygen.

16) Cold injuries:

When body temperature decreases to 32°-34°C consciousness is lowered. At still lower temperatures there is complete loss of consciousness but before this happens the patient may experience a paradoxical feeling of heat. This explains why people dying from exposure to cold are sometimes found partly undressed.

17) Chemical injuries:

The most common cause of chemical skin injuries is careless handling of acid or alkali. The degree of tissue injury will depend on the concentration of the substance and the time of exposure.

Patho physiology of wound healing:

The mission of the wound healing is to increase our basic understanding of the molecular and cellular events of the cellular repair and wound healing processes. In order to describe the complex cascade of events that follows injury, it is convenient to look at this process as a number of overlapping phases: i.e. Inflammation, formation of granulation tissue with angiogenesis, and scar formation (extracellular matrix remodeling). Injury to tissue leads to loss of structural integrity, instigating the coagulation cascade

to prevent localized hemorrhage. In skin, mucosa, and gut especially, injury is also complicated by the invasion of microorganisms. These events play an important role in initiating the defense and repair mechanisms by sealing off served vessels and transferring blood constituents, circulating cells, and bioactive substances to the site of the wound. This transfer and the ensuring defense processes constitute the early aspect of wound healing, commonly referred to as the inflammatory phase. The inflammatory reaction in soft tissue, which begins literally second after injury, is the same whether caused by a surgeon's sterile blade or by invading bacteria after a street injury. Qualitatively, inflammation is the same, but it is likely to be more prolonged in the latter case. More specifically, the mechanism of leukocyte adhesion to the vascular wall after injury followed by diapedesis, a major part of the inflammatory response, is essentially the same in all wounds whether resulting from surgery or trauma.

- **Phases of wound healing**

Inflammatory phase:

A) Sequence in inflammation:

The inflammatory phase is triggered by two classes of mediators (soluble signal factors): those controlling vessel permeability and those attracting or trapping cells. The clinical signs of inflammation are caused by

changes in blood vessels – with dilatation leading to erythema, and endothelial cell separation allowing plasma extravasations, producing localized swelling. There are overlapping stages but, in general, the order of arrival at the wound site from an intravascular space is thought to occur in the following sequence: plasma with soluble components and cellular constituents, first platelets, then neutrophils, followed by monocytes and lymphocytes. The migration of epithelial cells to resurface the injured tissue begins during this phase.

Alterations in micro vascular permeability after injury allow both fluid and plasma components to pass to the tissue. Vasoactive amines and peptides (including histamine from mast cells, serotonin from platelets, and bradykinin from neutrophils) cause the reversible opening of junctions between endothelial cells and allow the passage of neutrophils and monocytes.

Hageman factor (factor XII), a plasma glycoprotein, is activated by adsorption on to fibrillar collagen, leading to the generation of bradykinin and initiation of the complement cascade. The complement system is composed of 20 interacting soluble proteins in the serum and extracellular fluid, which can be activated by IgM and IgG antibodies bound to antigens on the surface of micro – organisms or by bacteria lipopolysaccharides. Large quantities of IgM or IgG lead to complement fixation by the classical pathway, whereas endotoxin released from bacteria and small quantities of IgG antibody enhance the activation process by the alternate pathway.

These proteins are the substances responsible for the acute inflammatory reaction. IgM can lyse Gram-negative bacteria and neutralize viruses. The C5 to C9 factors of complement combine to form a large protein complex that mediates lysis of bacteria cell walls. Complement factors also opsonize invaders (coat their antigen with antibody), making them recognizable to phagocytic cells. The factor C5a is also chemo-tactic, attracting polymorphonuclear cells, neutrophils to the site. The complement component C3b binds to specific receptor proteins on phagocytic cells and to microbial cell walls and enhances the ability of the phgocytes to bind, ingest, and destroy micro-organisms.

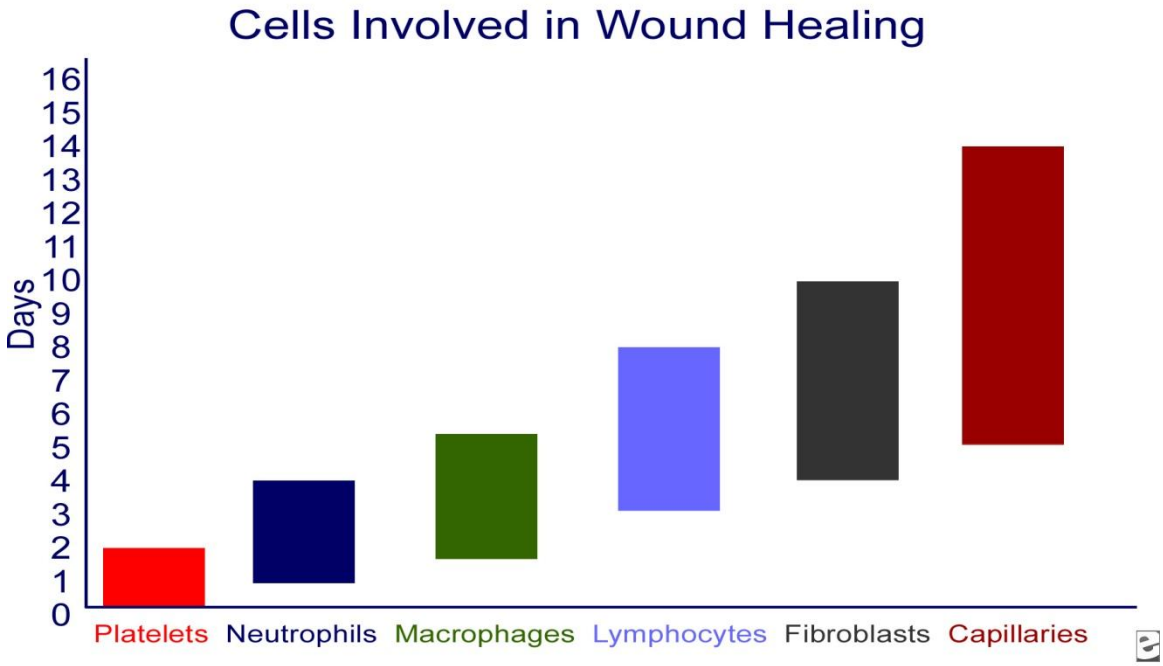
B) Platelets:

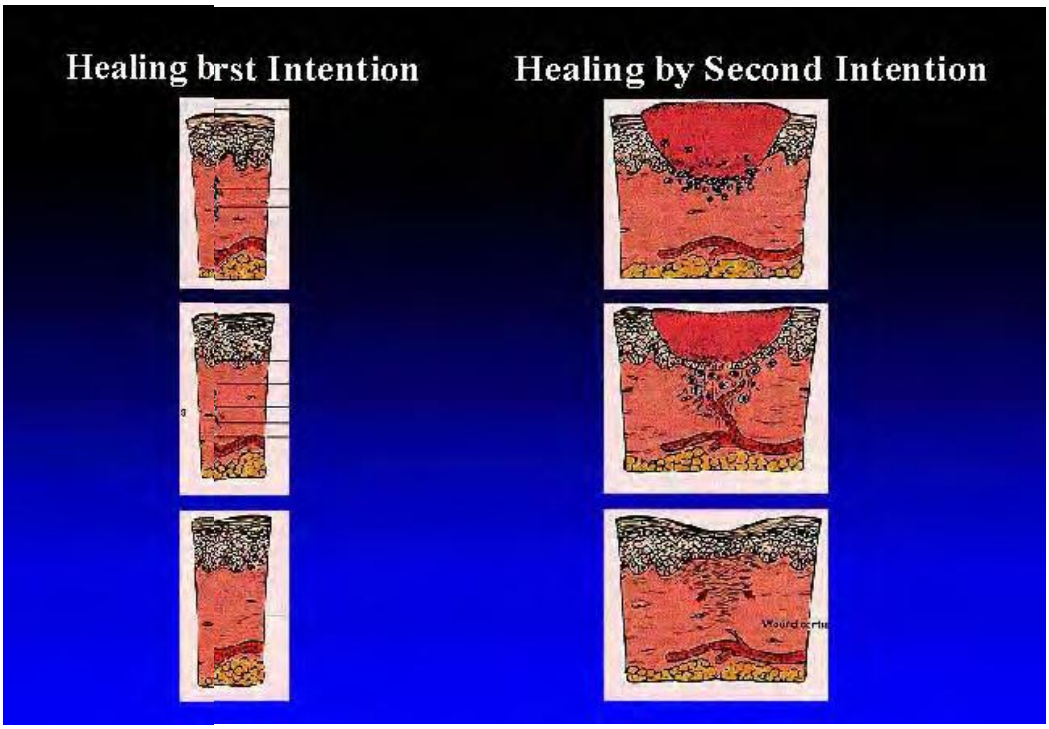
The earliest circulating cell or cell fragment detected in the injury site is the platelet. Platelets contain three types of organelles involved in haemostatic and initiation of the inflammatory phase.

1. α-Granules, which contain adhesive glycoproteins such as fibrinogen, von *Willebrand* factor, fibronectin, thrombospondin, and also growth factors – platelet- derived growth factor (PDGF), transformating growth factors α and β (TGF- α and TGF- β), and platelet factor 4.

2. The ‘dense body‘, the main storage site of serotonin, also contains adenine, nucleotide, calcium, and pyrophosphates.

3. Lysosomes, containing neutral and acid hydrolysases, elastase, collagenase, antitrypsin, and α2 macroglobulin. The above substances are released when the platelets are activated by various factors. When injury occurs, contact is made between platelets and insoluble components of the subendothelial matrix, particularly collagen, promoting the release of the α-granule contents which then trigger the coagulation process. The activation of platelets is enhanced by some of the complement factors and by bacterial lipo-polysaccharides. The latter produce a 50-fold increase in the amount of serotonin released. Activated platelets become sticky and aggregate to form a plug that temporarily occludes small vessels. Both damaged platelets and tissues release thrombokinase, which converts prothrombin to thrombin, and this in turn ensures the conversion of soluble fibrinogen to insoluble fibrin. The release of serotonin and adenine nucleotides contained in the dense bodies of the platelets induces the aggregation of platelets, which interact with the fibrin network to form a clot which is stronger and more durable than the initial platelet plug. If the clot is allowed to dehydrate, it transforms to a dry eschar covering the wound.


Cells Involved in Wound Healing
Days
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Platelets
Neutrophils
Macrophages
Lymphocytes
Fibroblasts
Capillaries


Healing brst Intention
Healing by Second Intention

Other substances released by the α-granules, such as platelet derived growth factor (PDGF), and by the dense body, such as cyclic adenosine monophosphate (cAMP) are motactic for neutrophils; both transformating growth factor- β (TGF- β) and PDGF are chemotactic for macrophages, while TGF- α and TGF- β are angiogenic factors.

The role of platelet-derived growth factors in enhancing both experimental and clinical wound healing has been highlighted recently in a number of publications, and is elaborated in more detail below.

C) Accumulation of neutrophils:

1. Adhesion:

Interaction between damaged tissue and serum release the complement factor C3, and the C3e fragment of this provokes the release of neutrophils from the bone marrow. At the same time, circulating leucocytes near the wound site, particularly neutrophils, cease to flow and adhere to the endothelium. It has been shown in vitro that adherence is enhanced by inflammatory mediators, such as C5a (the fifth component of complement), platelet activating factor, and leukotriene. There is a very fast initial response, with onset of adherence as early as thirty sec. after injury and with a maximum response at two min.

The binding of leucocytes to endothelium results from the interaction of complementary receptors in both cell types. Their expression is enhanced

by cytokines and bacterial lipo polysaccharide. Physical factors, such as haemodynamic shear stress, also influence adherence. This first stage of adherence is critical. While there is some evidence that some wounds can heal without the presence of neutrophils, patients with leucocyte adhesion deficiency, lacking an essential glycoprotein, are unable to mobilize neutrophils or monocytes, and exhibit decreased pus formation and impaired wound healing.

2. Diapedesis:

Vasopermeability factors act on actin microfilaments inside the endothelial cell and the reversible opening of junctions so that neutrophils are able to pass between the endothelial cells to the extravascular space. It is suggested that the secretion of elastase and other enzymes by the neutrophils enables them to degrade elastin and components of the endothelial basement membrane.

3. Migration:

Molecules released by platelets following disruption of the blood vessels, e.g. kallikrein (an enzyme that leads to the formation of vasodilating peptides) and fibrinopeptides, diffuse to the site of the wound and set up a concentration gradient of chemotactic factors which attract the neutrophils that have traversed the endothelium through the extracellular space to the injury site.

4. Phagocytosis:

The neutrophils - form the first line of defense against the invading micro-organisms. The neutrophils phagocytose bacteria, and then kill the ingested cells by the production of microbiocidal substances – oxygen metabolites such as hydroxyl radicals, hydrogen peroxide, and the superoxide ion. Release of some of these substances to the outside of the cell may also lead to tissue damage and prolong the inflammatory phase.

Some bacteria may be killed by non – oxidative mechanisms, but these are not defined in vivo. If bacterial contamination is low, the density of neutrophils declines, but if numbers of micro – organisms persist, the bacterial lipopolysacharides continue to arrival of further neutrophils. The neutrophils are unable to regenerate their enzymes and so they decay after phagocytosis.

D) Accumulation of macrophages:

The macrophage is indispensable in the degradation of injured tissue debris and in the reparative phase of wound healing. If the macrophages are inhibited, wound healing is radically impaired.

Normal tissues contain very few macrophages, but, in response to chemotactic factor released after injury, circulating monocytes are attracted to the site of injury several hours after the first neutrophils arrive. Endothelial cells in wounded tissue also play a role in this process, and have been shown to regulate the preferential adhesion of monocytes and lymphocytes to

endothelium. At the injury site, monocytes differentiate into macrophages. One of the signals prmoting this differentiation is the binding of fibrionectine to surface receptors for pahgocytosis.

Macrophages develop functional complement receptors and undertake similar operation to the neutrophils. However, further interactions with the interferons, and subsequently with bacterial or viral products, induce further differentiation in to a fully activated phenotype. Interferons enhance endocytosis and phagocytosis and modulate the surface receptors functions of newly migrated macrophages. Injection of bacteria by endocytosis triggers the primary oxigenase which converts molecular oxygen to the superoxide, which then reacts to produce hydrogen peroxide and hydroxyl radicals required for microbiocidal activaty.

Oxygen is essential. If the partial pressure or oxygen falls below 30 mm of Hg, macrophages are inactivated; their phagocytosing potential is reduced. The relationship between oxygen pressure and healing has been shown to be linear, explaining the beneficial role of oxygen pressure in repair. The activated macrophage is the major effector cell for degrading and removing damaged connective tissue components, collagen, elastin, and proteoglycans. Initial degeneration takes place extra-cellularly—up to several millimeters from the macrophage. Collagen and other fragments are then ingested and degraded by the cathepsin enzymes and other peptides. In contrast to neutrophils, macrophages can continue to synthesize the necessary

enzymes, thus persisting for a longer time. They also phagocytose the decaying neutrophils.

Apart from their role in debridement, macrophages secrete chemotactic factors which bring additional inflammatory cells to the wound site. Macrophages also produce prostaglandins, which are strongly vaso-dilatory and affect the permeability properties of micro-vessels. The macrophages act after the amines and kinins, and are produced on demand, prolonging the inflammatory phase. Prostaglandins also augment the adenyl cyclase activity in T lymphocytes, which accelerates the mitosis of other cells.

The angiogenesis stimulated in the early phase of wound healing has been shown to be related to the presence of macrophages. Increased levels of lactate production, up to 15-fold, have been found in wounded tissue, and have caused macrophages to produce and release angiogenic substances. The macrophages also produce growth factors, such as platelet – derived growth factor (PDGF), transforming – growth factor – β (TGF- β), and fibroblast growth factor (FGF), which are necessary for the initiation and propagation of granulation tissue. In this way the macrophages mediate the transition from the initial inflammatory response to the early repair phase of wound healing.

E) Lymphocytes:

B lymphocytes may be absent from the wound site. However, helper T cells are activated following injury, when they recognize any foreign antigen on the surface of antigen – presenting cell, e.g. Langerhans cell in skin, and

certain types of macrophage. The T lymphocytes migrate into the wound along with the macrophages. Advances in the past 5 years have helped to elucidate the role of the T cell in wound healing. Monoclonal antibody staining has permitted the identification of sets and subsets of lymphocytes, and cell culture and biochemical studies have identified and characterized some of the lymphokines, molecular messengers secreted by lymphocytes, which influence other cells, particularly macrophages and fibroblasts.

Thus, lymphocytes can produce macrophage chemotactic factor (MIF), regulating movement, macrophage inhibiting factor (MAF), and interleukin – 2 (IL-2) which enables the T cells to proliferate by an autocrine mechanisms. TGF- β, produced by the α-granules of platelets, is chemotactic for both fibroblasts and macrophages, and γ-interferon (γ-IFN) modulates the surface receptor function of newly migrated macrophages and enhances their phagocytic activity, and also activates macrophage oxidative metabolism and antimicrobial activity. T lymphocytes also produce colony stimulating factors (CSF). These are glycoproteins that act on neutrophils and macrophages through specific reseptors which have recently been identified—granulocyte – CSF, macrophage – CSF, granulocyte/macrophage – CSF, and interlukin – 3 (IL-3). The colony stimulating factors are very potent, being effective at very low concentrations (pg/ml). They are involved in the stimulation of proliferation, and of the commitment of the monocyte to differentiation and maturation. They stimulate the function of phagocytosis, and the production by macrophages of substances such as prostaglandins, tumor necrosis factor

(TNF), γ-IFN, and further colony stimulating factors. As quantities are very small, it is not known whether all cells are able to produce colony stimulating factors. They are induced in vivo by the presence of micro-organisms. Colony stimulating factors are currently in clinical use for the treatment of neutropenia, both congenital and induced by cancer therapy. It has been suggested that there could be a prophylactic role for them in abdominal and genitourinary surgery, where infections are common.

Macrophages and lymphocytes have been shown to be present from day *1* in wounds, although lymphocytes are fewer in number than macrophages. In study on human wounds, macrophages peaked between *3* and *6* days and lymphocytes between *8* and *14* days. Thus they persist into the early repair phase of wound healing. Both macrophages and lymphocytes disappear from mature wound by an unknown mechanism, but in abnormal scar both persist long afterwards. In hypertrophic scar, macrophages and lymphocyte levels have been found to be very high *4* to *5* months after wounding, and lymphocytes were still present at *40* per cent of the high level after *2* years. It has been suggested that control of lymphocytes might be a useful approach to control of scaring.

F) Epithelial cells:

Epithelial cells are important in the inflammatory phase as well as in the later repair aspect of wound healing. In partial thickness wounds, epithelial

cells migrate from the edges of the wound and from the epithelial linings of hair follicles, sebaceous glands, and sweat glands and begin to proliferate. In full thickness wounds, only the epithelial cells at the edges of the wound are available to migrate, because of the destruction of dermal appendages, and closure takes longer. In sutured surgical wounds epithelial migration begins within the first 24 h. of injury and may be completed as early as 72 h. in healthy individuals. Closure of the wound is not the only function of epithelial cells in the inflammatory phase. The development of techniques in molecular biology has led to unequivocal identification of many cytokines. Keratinocytes have been shown to produce the granulocyte/macrophage colony stimulating factor and interleukin-3 (IL-3) or multicolony stimulating factor (GM-CSF), as well as the growth factors TGF-α, TGF-β, and TNF-α. Keratinocytes also produce interleukin-1 (IL-1) which stimulates fibroblast proliferation and enhances the production of type I and III collagen mRNA and of an angiogenic factor. Thus they help to prepare and promote the next phase of wound healing. They also produce IL- 6, whichinduces in the liver the synthesis of proteins, some of which act to terminate the inflammatory phase.By definition, chronic ulcers have a deficit in epithlialization. This could arise through reduced cell proliferation, or excess cell loss. Early studies of mitotic frequency at the edge of superficial ulcer failed to show any difference between those which healed expeditiously with treatment and those which did not. It is therefore probable that the surface extracellular matrix of such wounds governs the process of wound closure by forming an

environment which may be either permissive of, or prohibitive for, epithelial cell adhesion and migration. The nature of these interactions remains relatively unexplored.

FORMATION OF GRANULATION TISSUE:

Various chemotactic, growth, and activating factors produced in the inflammatory phase are concerned in the initiation and development of granulation tissue which lasts from about day *4* to day *21* after wounding. Granulation tissue comprises a loose matrix of fibrin, fibronectin, collagen, and glycosaminoglycans, particularly hyaluronic acid, containing macrophages, fibroblasts, and ingrowing blood vessels. In deep wounds, granulation tissue serves as a scaffold for new tissue in-growth. In incisional wounds during this phase the wound begins to gain tensile strength, although it is during this early period that wound dehiscence and evisceration most frequently occur.

F. Fibroblasts:

In the initial phase after wounding, fibroblasts migrate into the wound site *24* hrs after injury. During this phase of healing (*4* to *21* days) the fibroblasts are activated and undergo a burst of proliferative and synthetic activity, initially producing high amounts of fibronectin, and then synthesizing the other protein components of the extracellular matrix, including collagen and elastin, and glycosamino glycans. The fibroblast aligns them along the wound axis and form cell to cell links, which contribute to the contraction of

the wound. There has been much discussion about the type and origin of fibroblast that appear in the wound. These fibroblasts have characteristics in between those of normal resting fibroblasts and smooth muscle cells.

This altered phenotype, which has been called the 'myofibroblast' is more mobile and more contractile than the inactivated fibroblast, and disappears on the completion of wound healing. Early distinctions between fibroblasts and myofibroblasts were based on ultrastructural criteria, but immunochemical analyses have, more recently, led to identification of subspecies of myofibroblasts based on permutations of expression of vimentin, desmin, and a smooth muscle actin. It has now been shown that smooth muscle cells in culture can reversibly modulate from contractile to synthetic cells, i.e. the reverse of the myofibroblast development, and this may reflect changes occurring in vivo. In addition, it has been demonstrated that smooth muscle genes can be switched on transiently in certain circumstances by other non-muscle cells, including macrophages and some epithelial cells. It is still not known what controls the change in phenotype.

Angiogenesis:

Research into factors influencing angiogenesis has been directed at means of inhibiting new vessel growth in regard to tumor metastasis or, in the case of wound repair, means of stimulating angiogenesis to enhance healing. Hypoxia following injury, if not so severe as to lead to tissue death associated with ischaemia, acts as a major stimulus for angiogenesis, which is required

for restoration of blood flow. Along with fibroblast proliferation, neovascularization is a common feature of granulation tissue in the early phase of healing.

One stimulus for new vessel growth in fibroblast growth factor, while other angiogenic factors, such as those secreted by macrophages and other cell, also contribute to the neovacularization. The growth of vessels in surgical wounds starts from capillary loops a few days after surgery, and vascularization may be complete in *6 to 7* days. In burns, development is later and may be com complete in *12 to 16* days. The secondary wound (reopened and re-sutured) revascularization at a significantly faster rate than a control wound which has not been re-opended and re-sutured. This aspect of the importance of angiogenesis in wound healing has been observed in other type of wound where differences in regional vascularity and healing are directly related.

The endothelial migration seen in granulation tissue is supported by the increased fibronectinin this tissue. Mitotic activity leads to the formation of capillary buds which sprout from blood vessels adjacent to the wound and extend into the wound space. There is a gradual establishment of flow. Endothelial cell proliferation is stimulated by a low wound *Po2* in the early stages, but growth of vessels is later enhanced by a high wound *Po2* which is also essential for the synthesis of collagen necessary for the complete formation of the vessels. The pattern of vascular growth is probably the same in the healing of skin, muscle, and intestinal wounds. In fractured bones,

vessel growth can be stimulated by repeated muscle contraction which increases bone blood flow, while vascularization is reduced by immobilization.

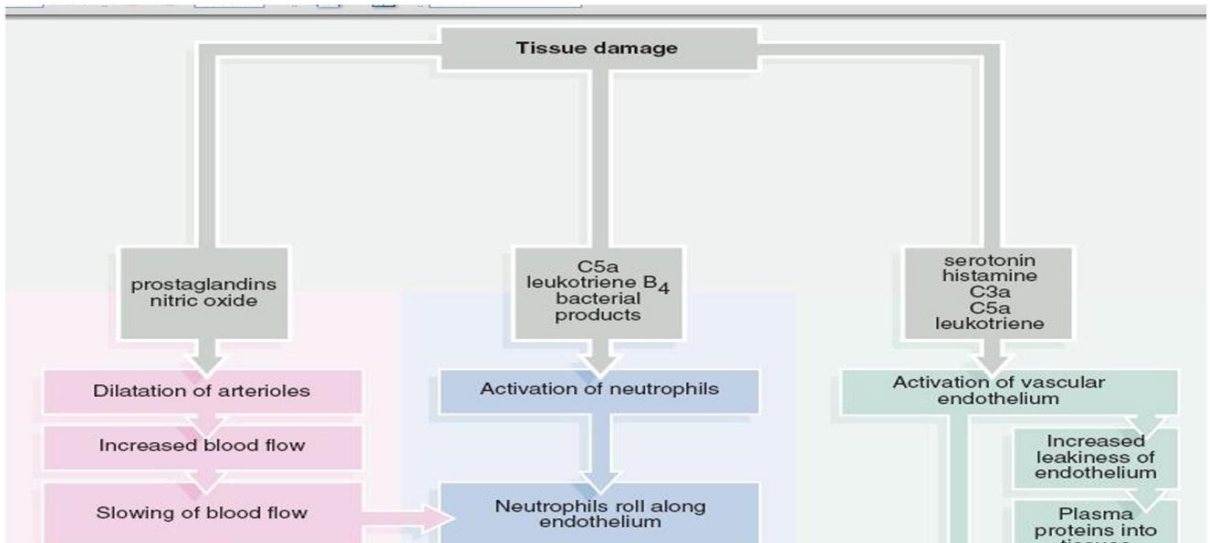
Contraction:

Wound closure by contraction, the inward movement of the edges of the injured tissue, is a normal part of the healing process. However, in some wounds, such as full thickness freeze injury, contraction does not occur. Wound contraction begins between days *8 and 10* after injury. It is controlled both by the fibroblasts and by the extracellular matrix, and is due to the fibroblasts applying tension to the surrounding tissue matrix.

COLLAGEN—MATRIX FORMULATION AND REMODELLING

Collagen synthesis plays an important role in the early stages of healing and the formation of the granulation matrix. Production of collagen remains a major process in wound repair for several weeks after wound

WOUND HEALING MECHANISM



closure, and the collagen continues to undergo remodeling for *2* years or more until the injured tissue is finally restored. Collagen is the major component by weight of the extracellular matrix of the skin, accounting for about *60* to *80* percent of the dry weight of the tissue. There are known to be at least *13* different genetically distinct collagen types, six of which occur in human skin.

Collagen type	*Chain composition*	*Tissue distribution*
I	**[α1(I)]2α2(I)**	Ubiquitous in most connective tissues, including skin, bones, tendons, etc.
II	**[α1(III)]3**	Skin, blood vessels, predominant in fetal tissue and in early wounds.
III	**[α1(IV)]2α2(IV)**	Basement membranes, anchoring Plaques
IV	**[α1(V)]2α2(V) [α1(V)]3**	Ubiquitous
V	**α1(VI)α2(VI) 3(VI)**	Extracellular microfibrils
VI	**[α1(VII)]3**	Skin, fetal membranes

Extracellular matrix:

The extracellular matrix of tissues is composed of various polysaccharides and proteins and their complexes. These are secreted by cells *in situ* and different amount and types are assembled to form diversely organized structures related to the functions of the particular tissue. The matrix not only serves as a support, but has a role influencing the behavior of the cells in contact with it, affecting their development, migration, proliferation, shape, and metabolism, all of which are important with regard to wound healing. The polysaccharides are glycosaminoglycans—long, unbranched chains of disaccharide repeating units. They fold with wide curvature in a random fashion and absorb large amounts of water, filling much of the extracellular space. Proteoglycans are formed by the combination of a number of glycosaminoglycan chains with a protein, and may contain up to *95* percent

(w/w) carbohydrate. Glycoproteins, on the other hand, are composed of short, behind oligosaccharide chains, containing from *1 to 60* percent carbohydrate. The proteins of the extracellular matrix are principally structural proteins such as collagen and elastin, and adhesion proteins such as fibronectin and laminin. All collagen molecules are composed of three polypeptide α - chains with a left-handed triple-helix configuration. The chains have about *1000* amino acids and have a distinctive amino acid composition of *33* percent glycin and *20* percent of the imino acids, praline and hydroxyproline, with a particular repeating trimeric sequence of glycin—X— Y, where either X or Y is often praline.

Synthesis of collagen:

The synthesis of collagen involves a progression in the combination of amino acids to form chains which associate to form molecules, and then association to form fibrils which aggregate into fibres or bundles. Fibroblasts are the major cell type to synthesize collagen. The first stages of synthesis take place intracellularly, to produce procollagen molecules which undergo activation stages in the extracellular space.

A. Intracellular synthesis:

In the nucleus the genes are activated and there is translation of mRNAs, specific for single polypeptide chains. The mRNAs pass into the

cytoplasm and are translated on the ribosomes of the endoplasmic reticulum, the three polypeptide chains being synthesized simultaneously.

The three α-chains may be identical (as in type III collagen), or a hybrid molecule consisting of two identical chains and a different third chain (as in type I), or three different chains (as in VI). The molecule is a triple chain molecule by the time it is detached from the ribosomes. The small, regularly spaced glycine residues situated in the central area of the chains allow them to pack tightly together to form the triple helix. This is the preprocollagen molecule. The molecules then undergo post-translation modifications, principally the hydroxylation of a large number of the praline and lysine residues by the enzymes lysyl hydroxylase and 3-and 4-prolyl hydroxylase. Hydroxylation is a rate – limiting step for collagen secretion, and appears to be tightly regulatedby tissue levels oxygen and lactate.

The non – enzymatic glycosylation of some of the hydroxylysine residues also takes place at this stage. The hydroxyl groups of hydroxyproline residues from interchain hydrogen bonds, which contribute to the stabilization of the triple-stranded helix. The 4-hydroxyproline moieties stabilize the collagen triple helix at physiological temperature (if not hydroxylated it unwinds above *24°C*). The hydroxylysine-saccharide units are also factors in the proper subunit alignment and the subsequent assembly of fibers. This precursor procollagen molecule has extension non-healical peptides of *15 to 20* amino acids in non-collagenous sequences at both ends of the chains. These propeptides contain intra- and intermolecular disulphide bounds, giving a

globular form and probably serving as the starting point for the rapid triple-helix formation. They also prevent intra cellular formation of large collagen fibers. These post-translational modifications result in the formation of the procollagen molecule, which is then transported to the Golgi apparatus, enclosed in vesicles, and taken via the microtubules to the cell surface.

B. Extracellular synthesis:

The processing of the procollagen to collagen fibers takes places in the extracellular space. The first step is the activation of the molecule by the cleavage of amino- and carboxy-peptide ends by aminoand carboxyl-propeptidases . Lack of, or defects in, one of these enzymes results in defective fibres, e.g. type VII Ehlers-Danlos disease, dermatosporaxis in calves lacking the aminoprotease. The sequence of charge and hydrophilic amino acids in the collagen molecules is such that it allows self-assembly of collagen into fibrils *in-vitro*, possibly because the helical portions allow electrostatic interaction with adjacent collagen molecules, but the *in-vivo* process is considered to be more complex. The ε-amino group of certain lysine and hydroxylysine residues is converted to aldehyde by the extracellular enzyme lysyl oxidase. The aldehydes react to form covalent bonds between the short, nonhelical end of the collagen molecules, thus cementing the overlaps. The polymerization of many molecules in a staggered arrangement gives rise to the typical periodicity of *60* nm seen in electron microscope sections and this arrangement maximizes the tensile strength of the structure. At this stage type III fibrils have diameters of *40 to 60* nm and type I *100 to*

500 nm. The size of the collagen fibrils may depend on the order of cleavage of the non-helical domains, cleavage of the carboxy-terminal first resulting in thin fibrils and of the amino-terminal first leading to thick fibrils. The build-up of the propeptides released in the transformation of procollagen to collagen inhibits collagen synthesis and thus provides a feedback system may be a contributory factor in excessive scarring.

Cross – links:

The aggregates of collagen molecules formed in the extracellular space then undergo cross-linking. The extent and types of cross-links, or ratio of types, vary from tissue to tissue, with age, and in disease. In skin, the collagen produced after injury is initially stabilized by cross-links derived from hydroxyallysine. In normal wound healing these changes to cross-links derived from the modified amino acid allysine, but this change does not occur in hypertrophic scar. With time, these cross-links change to 'mature', more stable cross-links, which have not been completely characterized.

In skin the major mature cross-link may be hydroxyaldohistidine. In most other connective tissue a hydroxypyridinium cross-link is predominant. The greater the number of cross-links, the stiffer a tissue will be, although stiffness may also be influenced by the type of cross-link. In normal bone, for example, the hydroxyl pyridinium cross-link occurs with a frequency of *0.24* moles per mole of collagen. The organization of the collagen in tissue is also influenced by the kinds and amounts of non-collagenous macromolecules that

the cells secrete along with the collagen. In addition, the fibroblasts have a mechanical role in the assembly, crawling over the collagen, pulling and compacting it.

The final architecture of the collagen network is related to its function. Thus collagen fibers in the papillary dermis are aligned in thin bundles almost perpendicular to the basement membrane and they hold up the dermal papillae. Some of the fibers glide into the loops of the anchoring fibrils attached to the basement membrane. In the reticular dermis the thick, undulating bundles are nearly parallel to the epidermal plane, and are connected by interlacing fibers, allowing the tissue to resist stress in all directions. In the hypodermis interlacing collagen fibers surround the adipocytes.

Collagenolysis:

In the extracellular space, procollagenase is transformed to collagenase which cleaves the helical portion of the collagen molecule, causing the fragments to unwind. The single-stranded polypeptides are then susceptible to degradation by extracellular proteinase and peptidase, or undergo endocytosis and are degraded by intracellular enzymes. Some procollagen may be retained and degraded in the cell rather than secreted. There is some evidence for this, but the mechanisms are not understood. Mature collagen may also be absorbed by phagocytosis. Mature banded collagen found in vacuoles in the cytoplasm of fibroblasts must have come from the extracellular space, because it is only there that such collagen is formed. If it were from the intracellular synthetic

pathway, it would be in the form of pro collagen. The peak levels of collagen pahgocytic have been shown to correspond with the period when a change of configuration and fiber orientation was occurring.

The failure of regulation also causes a problem in the formation of keloids, when the synthesis and breakdown are both stimulated, but the former to a greater extent, thus leading to imbalance and overgrowth. The remodeling of scar tissue also requires the degradation and synthesis of collagen.

Collagen and wound healing:

In the adult, the normal repair of wounds occurs by the formation of granulation tissue and its organization to a scar. Scar is a dynamic, metabolically active tissue. Precise regulation of collagen metabolism during the repair process is exerted by cytokines and by the interactions of the cellular matrix with fibroblasts.

Fetal wound healing:

In contrast to the healing by scar formation in the adult, the healing of fetal wounds up to the early third trimester of gestation proceeds without scar formation. Collagen is deposited more quickly in the fetal wound than in the adult, but is rapidly organized and is not excessive. It is thought that this might be due to glycosaminoglycans, which are also deposited. The nature and ratio of the glycosaminoglycans, which affect the cross-linking of collagen fibrils and the migration of fibroblasts, vary in different stages of wound healing.

Hyaluronic acid, in particular, the content of which is high in the fetal wound matrix and which is found wherever there is tissue regeneration or repair, has been shown in vitro to facilitate the movement of fibroblasts. While hyluronic acid is present only in the early stages in adult wounds, it is present throughout the process in fetal healing and the wound is closed by mesenchymal ingrowth on to the

hyaluronate-enriched matrix.

Wound Care

Wound management includes topical agent as well as dressing. A topical agent is that which is applied to a wound. A dressing is a covering on a wound which is intended to promote healing and provide protection from further injury. There are four basic steps to follow in caring for any wound. Perhaps the most important factor in wound healing is compliance, in other words, caring for the wound consistently and correctly.

Dressings:

The dressing over a wound should

1. Prevent traumatization and soiling of the wound

2. Immobilize the wound area

3. Counteract oedema

4. Protect clothes and linen from blood and discharge

General principles:

Operative wounds are usually made in such a way that tension and traumatization are minimized. Routine for dressings can therefore easily be developed. The operative wound is adequately protected against traumatization and soiling by porous tape which also helps to keep the wound edges together. During the first post-operative hours there is often some discharge from the wound. Therefore, an absorbing pad can be suitably applied over the tape. The pad can be removed after the first day. The tape then offer sufficient protection and is left in place until the sutures are removed. After removal of sutures the wound is protected with new tape.

Porous tape has good adhesiveness to clean and dry skin. The adhesion can be further important if plastic glue is applied on the skin around the wound. If spray-solutions are used it is important to make sure that neither the plastic material nor the propulsive gas comes into contact with the wound. Spray-plast solution should never be used in the face. It is not proven that a longer period of relief of tension results in a narrower scar. However, the combination of relief of tension and pressure by tape may decrease the incidence of scar hypertrophy, for example over the deltoid and over the sternum. Tension must then be relieved for 2-3 months, i.e. until the scar tissue has reached a certain degree of maturation.

Edema in operative wounds is usually small except in areas with very loose skin. In such location oedema can be counteracted by a pressure

dressing. Movements of a joint means continued traumatization of nearby wounds. Immobilization will therefore provide better conditions for healing. Sometimes this can be achieved by an elastic bandage but splinting is often preferable. Bleeding from a sutured wound is hardly influenced by addressing.

Debridement

Debridement means the removal of dead tissue. It can be accomplished in an autolytic manner, in which the wound itself is encouraged to do this task by the use of dressings. A medical professional may also use biochemical enzymes, wet-to-dry dressings (in which a wet dressing is allowed to dry, trapping material in it, and is then carefully removed), or mechanical implements such as scalpel or scissors to remove dead tissue from more serious wounds. Cleansing refers to the removal from the wound of any foreign debris (such as residuum from previous dressings) and any bacteria. Cleansing is usually accomplished by irrigating the wound with fluid from a disposable syringe. Many previously accepted wound-cleansing solutions have been found to be toxic to fibroblasts and lymphocytes, the cells required to heal wounds. These solutions include povidone-iodine, acetic acid, iodophor, hydrogen peroxide, and Dakin's solution (sodium hypochlorite). Commercially prepared solutions are not regulated by the FDA, and many have been found to be cytotoxic.

The only acceptable wound-cleansing solution is normal saline solution (0.9% sodium chloride [salt] in water). Normal saline solution effectively removes contaminants and has the same salt concentration as the fluid in cells, so it doesn't damage cells by pulling water out of them. It is also inexpensive and readily available.

Maintain a moist environment:

During wound healing, cells and fluid are slowly exuded, or discharged. The exudate provides an environment that stimulates healing because it contains white blood cells, growth factors, and other special enzymes and hormones. A moist environment preserves this exudate, speeding wound healing and promoting skin growth. It also prevents dressings from adhering to the wound and damaging the fragile tissue when they are removed.

A moist environment can easily be maintained using gauze moistened with normal saline solution. The solution will support autolytic debridement, absorb discharge, and trap bacteria.

Infection

Infection of a wound with a large number of bacteria, a process known as colonization, will slow the healing process. All wounds, however, contain some bacteria. This is called contamination and does not affect the healing

process. The difference between contamination and colonization is the concentration of bacteria. Signs of infection include red skin around the wound, discharge containing pus, swelling, warmth, foul odor, and fever. Health care providers can also conduct laboratory tests to investigate for signs of infection. Since all wounds are contaminated, sterile materials and technique are not necessary.

The best way to prevent infection is to carefully wash your hands. Antibiotic creams should be used only if signs of infection are present, and then only sparingly to prevent bacterial resistance (bacteria develop the ability to live in the presence of the medication). If a wound is infected and does not respond immediately to over-the-counter antibacterial creams, it must be evaluated by a health care professional, who may prescribe antibiotics.

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MADHU [HONEY]

Honey is *madhura by rasa*, *kashaya by anurasa*, *ruksha* (dry), *sheeta virya*, and good for normal complexion, causes cleaning and healing of the wound. It penetrates deep in the tissue.

(Su.su 45/132)

It is having *yogavahi* action i.e. synergistic to other medicines, here *ghee*.

(Su.Su.45/142)

Honey is a useful agent for debriding i.e. cleaning, contraction and healing of the wound.

(Va. Su. 5/53)

Honey has been used to treat infections in a wide range of wound types. These include burns, venous leg ulcers, leg ulcers of mixed etiology, diabetic foot ulcers, pressure ulcers, unhealed graft donor sites, abscesses, boils, pilonidal sinuses, and infected wounds from lower limb surgery, necrotizing fasciitis and neonatal postoperative wound infection. In many of these and other cases, honey has been used to heal wounds not responding to treatment with conventional antibiotics and antiseptics[1].

Honey is useful in superficial and partial-thickness burns[1]

Topical application of honey is beneficial in the treatment of wounds and burns, according to a review article in the Journal of Wound, Ostomy, and Continence Nursing[1].

The pH of honey is commonly between *3.2* and *4.5*. This relatively acidic pH level prevents the growth of many bacteria.

Some studies suggest that the use of honey may reduce odors, swelling, and scarring; it may also prevent the dressing from sticking to the healing wound.

Some studies suggest that the use of honey may reduce odors, swelling, and scarring; it may also prevent the dressing from sticking to the healing wound[2].

Hydrogen peroxide in honey is activated by dilution. However, unlike medical hydrogen peroxide, commonly *3%* by volume, it is present in a concentration of only *1 mmol /l* in honey.

Iron in honey oxidizes the oxygen free radicals released by the hydrogen peroxide.

$$\textbf{Glucose} + H_2O + O_2 \rightarrow \textbf{gluconic acid} + H_2O_2$$

When used topically (as, for example, a wound dressing), hydrogen peroxide is produced by dilution with body fluids. As a result, hydrogen peroxide is released slowly and acts as an antiseptic[3].

The analysis of the sugar content of honey is used for detecting adulteration.

Composition of honey is as follow[4]:

Content	***Percentage***
Fructose	38%
Glucose	31%
Sucrose	1%
Water	17%
Other sugars (maltose, melezitose)	9%
Ash	0.17%

Other contents

Sr. No.	***Contents***	
1	**Vitamins**[5]	vitamin B6, vitamin C, thiamin, niacin, riboflavin, pantothenic acid
2	**Minerals**	calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, zinc
3	**Amino acids**	
4	**Antioxidants**[6]	chrysin, pinobanksin, vitamin C, catalase, pinocembrin

The specific composition of any batch of honey will depend largely on the mix of flowers available to the bees that produced the honey[7].

Honey has a density of about *1.4* kg/liter (*40%* denser than water)[8].

References

1. *Waikato honey research unit..*
2. *Honey as an Antimicrobial Agent, Waikato Honey Research Unit,* November 16, 2006. *Retrieved on* June 2, 2007
3. Typical honey analysis (Ref: *Questions Most Frequently Asked About Sugar, American Sugar Alliance*)
4. National Honey Board. "Carbohydrates and the Sweetness of Honey". http://www.honey.com/downloads/carb.pdf.
5. NutritionData. "Nutritional Summary for Honey." Last accessed April 14, 2007. http://www.nutritiondata.com/facts-C00001-01c21Ro.html
6. (*Martos I, Ferreres F, Tomás-Barberán F (2000). "Identification of flavonoid markers for the botanical origin of Eucalyptus honey". J Agric Food Chem* ***48*** *(5): 1498-502.* PMID 10820049.
7. *Gheldof N, Wang X, Engeseth N (2002). "Identification and quantification of antioxidant components of honeys from various floral sources". J Agric Food Chem* ***50*** *(21): 5870-7.* PMID 12358452.)

8 Rainer Krell,. Value-Added Products from Beekeeping (Fao Agricultural Services Bulletin). Food & Agriculture Organization of the UN (FA. ISBN 92-5-103819-8.)

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SARPI [GHEE]

Gau Sarpi (Clarified Butter)

In *Ayurveda* there is description of four types of *'Sneha' viz.Sarpi, Taila, Vasa, Majja.* According to *Samhitas- Sarpi* is considered as superior among all due to its property of *"Sanskaaranuvarti*". It means that if it is processed with other drug, it accepts all qualities of that drug without changing its own. Also among all types of ghee, *Gau Sarpi i*s described as superior due to its properties.

(As. Hr. Su. 16/2,3)

Properties *(Cha. Su. 27/231,32)*

- *Name* -- *Clarified butter.*
- *Rasa* -- *Madhur*
- *Virya* -- *Sheeta*
- *Vipak* -- *Madhur*
- *Guna* -- *Mrudu, Shlakshna, Guru.*
- *Doshghnata* -- *Vataghna & Pittaghna*

According to *Sushruta*:

Sarpi is sweet in taste, soothing, soft, *sheetvirya,* slightly slimy,alleviates *udavarta,* insanity, epilepsy. It also alleviates colic, fever, hardness in bowel & *vata pitta*[1,2]. It lubricates the connective tissues. *Ghee* makes the body flexible. It also acts as *snehanakara, shoolaghna, kshobhanashaka, shukrala, dahashamak, varnya.*

Properties of *GoSarpi:*

It is *madhur in rasa, madhur in vipaka & sheetvirya.* It alleviates *vata, pitta & visha.* *(Su. Su. 45/97)*

Sarpi has *Rakshoghna* property. *(Su. Su. 45/66)*

Here *Rakshoghna* means antimicrobial. The ancient scholars were having an idea of microbes and therefore they also described the antimicrobial measures for it.

Sarpi, Taila, Vasa, and Majja are the best *sneha dravyas* of all. Amongst them *Sarpi* is the *sneha dravya* par–excellence because of its power to assimilate effectively the properties of the substances. The medicated *ghee* or oils of our pharmacopoeia, which are prepared by successively boiling or cooking them with drug decoction etc. we know how potent and efficacious they prove in the hands of our *vaidyas.*

Sarpi is obtained from the class Mammalian of the animal kingdom *(jangama)* especially cow, she-buffalo, goat, sheep, she-camel, and mare. Out of these experts the last two, the rest are the main sources of milk and milk products in the area of their habitat. Though the *Sarpi* of these animals posses many common features, *Ayurveda* discriminates their particular features also and recommends the *G*au *Sarpi* (cows-ghee) as best and the *Sarpi* of choice for both, food and medicinal purposes. So that in the *Ayurvedic* classics and

tradition, if not specified, the epithet *Sarpi* always applies to *Gau Sarpi* (cow-ghee).

In *Bhava Prakash*, it is mentioned that *Sarpi (ghee)* is a *Rasayana*, tasty, good for the eye, stimulant for digestion, supports glow and beauty, enhances memory and stamina, promotes longevity and protects body from various diseases.

Modern view[1]

Clarified milk fat or butter fat is known as *Sarpi (ghee).* It is prepared by heating butter or cream to just over 100ºC to remove water content by evaporation. The residue is filtered out as pure ghee.

The composition of ghee residue obtained from Indian cow is as follows:

Moisture	14.4%
Fat	32.4%
Protein	36.0%
Lactose	12.0%
Ash	05.2%

The color of *ghee* is yellow to white depending upon the carotene content. Ghee contains approximately *8%* lower saturated fatty acid which makes it easily digestible. These lower saturated fatty acids are the most edible fat and which are not found in any other edible oil or fat.

Ghee also contains Vitamin A, D, E and K. Vitamins A and E are anti-oxidant and are helpful in preventing oxidative injury to the body. No other edible fat or oil contains Vitamin A except fish oil. Vitamin A keeps epithelial tissue of the body intact; keeps the outer lining of the eyeball moist and prevents blindness. *Ghee* also contains *4-5%* linoleic acid, an essential fatty acid, which promotes proper growth of human body. Lipophilic action of ghee, they easily facilitate transportation to a target organ and final delivery, inside the cell, because cell membrane also contains lipid. This lipophilic nature of ghee facilitates entry of the formulation into the cell and its delivery to the mitochondria, microsome and nuclear membrane.

Constituents	***Percentage (%)***
Triglycerides	97-98
Diglycerides	0.25-0.4
Monoglycerides	0.016-0.038
Keto Acid	0.011-0.018
Glycerylesters	0.011-0.015
Free Fatty Acids	0.1-0.44
Phospholipids	0.2-1.0
Sterols	0.22-0.41
Vitamin A	2500 I.U. per 100gms
Vitamin D -7	8.5×10 gm per 100gms
Vitamin E - 3	24×10 gm per 100gms
Vitamin K - 4	1×10 gm per 100gms

Composition of Cow Milk *Ghee*[1]

In the process of evaluating the activities of natural compounds, it has been found by means of sophisticated research that when herbs are mixed with ghee, their activity and utility is potentiated, many times.

Fatty Acids	***Percentage (%)***
Butyric acid	4.5-6.0
Caproic acid	1.0-1.36
Caprylic acid	0.9-1.0
Capric acid	1.5-1.8
Lauric acid	6.0-7.0
Myristic acid	21.0-23.0
Palmitic acid	19.0-19.5
Stearic acid	11.0-11.5
Arachidic acid	0.5-0.8
Oleic acid	27.0-27.5
Linoleic acid	4.0-5.0

Composition of Cow Milk Ghee glycerides

Ghee contains beta-carotene and Vitamin E and both are known anti oxidants. It is estimated that *80% to 90%* of degenerative disease related to excessive production of free radicals of re – active Oxygen species. When free radicals are in excess, they try to latch on to whatever is available in their surrounding area, and this is how the lipids in the blood and cell membranes are oxidized. The oxidized lipids or the lipid peroxides are injurious to the

system. As we know, they trigger the process of atherosclerosis. The reactive oxygen species also cause damage to the DNA in the cells. Excessive free radicals have been associated with inflammatory diseases like lupus, D.M., ageing, atherosclerosis, cancer, skin pigmentation, wrinkling and skin tumors in sun exposed areas. The effectiveness of many *Ayurvedic* compounds is due to potent anti-oxidant properties of removing of scavenging free radicals. *Sarpi* alleviates *pitta* and *vata.* This is beneficial for *Rasa Dhatu, Rakta Dhatus, Sukra Dhatus* and *Ojas*. It has the qualities of *Shita Guna* (cooling), *Mrudukarma* (sofetening) *Svara prasadanam* (improves voice) and *vrana ropana*.

References

1. Dr.H.M.sharma, Indian Journal of Clinical Practice, Vol.1No.2 July 1990.

MATERIALS

The *'Madhu and sarpi (Goghrita-cow ghee)'* combination was studied in the animal house. The animal trial was carried out for the effect of the combination on the recent wounds. The drug was tried on rats as well as on mice. Excellent results were observed in both types of animals. Hence the drug was taken for clinical trial. The combination was used in equal proportion as a local applicant on the recent wounds in humans.

- ***Madhu (Honey) (Bhavprakash)***

  Honey available in local market was used for the study. The floral honey was collected from the local Market of *Akkalkot, Dist Solapur (Maharashtra)*

- ***Sarpi (Ghee) (Bhavprakash)***

  It was prepared by author himself using the method described in *Ayurvedic* Classical text. Milk of Cow was used for making curd and butter milk from it. The butter was isolated form butter milk by centrifugation. The isolated butter was given heat until it is converted into Ghee.

Both the drugs were taken according to the reference of *Bhavprakasha.* These drugs were standardized in the laboratory before application. The lab reports are attached herewith.

✓ ***Table 1: Honey Sample 1 (In vitro antibacterial activity)***

Sample	**Anti bacterial activity against**	**Zone of inhibition(*ZOI*)**
Honey(1)	E.Coli	No ZOI
	Psedomonas	No ZOI
	Salmonella	NoZOI? Probiotic
	S.aureus	No ZOI

✓ ***Table 2: Honey Sample 2 (In vitro antibacterial activity)***

Sample	**Anti bacterial activity against**	**Zone of inhibition**
Honey(2)	E.Coli	No ZOI
	Psedomonas	No ZOI
	Salmonella	No MZI
	S.aureus	No ZOI

✓ ***Table 3: Sodium, Potassium and calcium estimation of samples of Honey (24/09/2006)***

S.No	***Code of Sample***	***Na*** Meq/L	***K*** Meq/L	***Ca*** Meq/L
1	***Honey (1)***	355	965	3.5
2	***Honey (2)***	430	580	6.5

✓ ***Table 4: Spectral study of Honey (24/09/2006)***

	Honey (1)	Absorbance
1	Wave length 214nm	0.885
2	Wave length 278 nm	0.405
	Honey (2)	
1	Wave length 219	0.839
2	Wave length 281	1.165

✓ ***Table 5: Other parameters of Honey Sample (1)***

Honey 1	
PARAMETERS	**SPECIFICATIONS**
Free acidity	33.2 meq/kg
pH	4.12
Moisture	17.1 %
Reducing sugar	79 gm%

✓ ***Table 6: Other parameters of Honey Sample (2)***

Sample	Anti bacterial activity against	Zone of inhibition(*ZOI*)
Ghee	*E.Coli*	2mm ZOI
	Psedomonas	minimal ZOI
	Salmonella	No ZOI
	S.aureus	1mm ZOI

✓ ***Table 7: Antibacterial activity of Ghee***

✓ ***Table 8: Spectral study of Ghee***

S.No	Ghee	Absorbance
1	Wave length 253nm	7.0
2	Wave length 266 nm	1.72

PARAMETERS	**SPECIFICATIONS**
Saponification value	274
Acid value	7.62
Free acidity	32.4 meq/kg
pH	4.0
Moisture	16.7%
Reducing sugar	83.8 gm%

Honey 2

PARAMETERS **SPECIFICATIONS**

✓ ***Table 9: Other Parameters of Ghee***

✓ ***Table 10:*** **Sodium, Potassium and calcium estimation osf *Ghee***

PARAMETERS	SPECIFICATIONS
Sodium	1939 Meq/L
Potassium	1135 Meq/L
Calcium	100 Meq/L

- ***Animal trial***

The animal study was done prior to the clinical trial. 6 animals used were rats and mice.

- **Methodology**

The animals were given Inj. Ketamine *0.4mg* IM.

After anesthetizing, wounds were induced using sterile knife on the back of the animal. Each wound measuring *2* centimeters in length, *2* centimeters in width and the depth was immediately above the first layer of muscle. i.e. *2* ×*2* × *0.5* centimeters. Three wounds were induced in each animal.

Photographs were taken. The wounds were dressed in the following manner:

1. With betadine soaked gauze.
2. With a gauze soaked in *Madhu sarpi* .
3. With sterile gauze.

Dressings were performed daily under all aseptic precautions. Observations were noted and photographs were maintained at each dressing. Histopathological study was done.

All the animals were sacrificed after 15 days.

- **Observations**

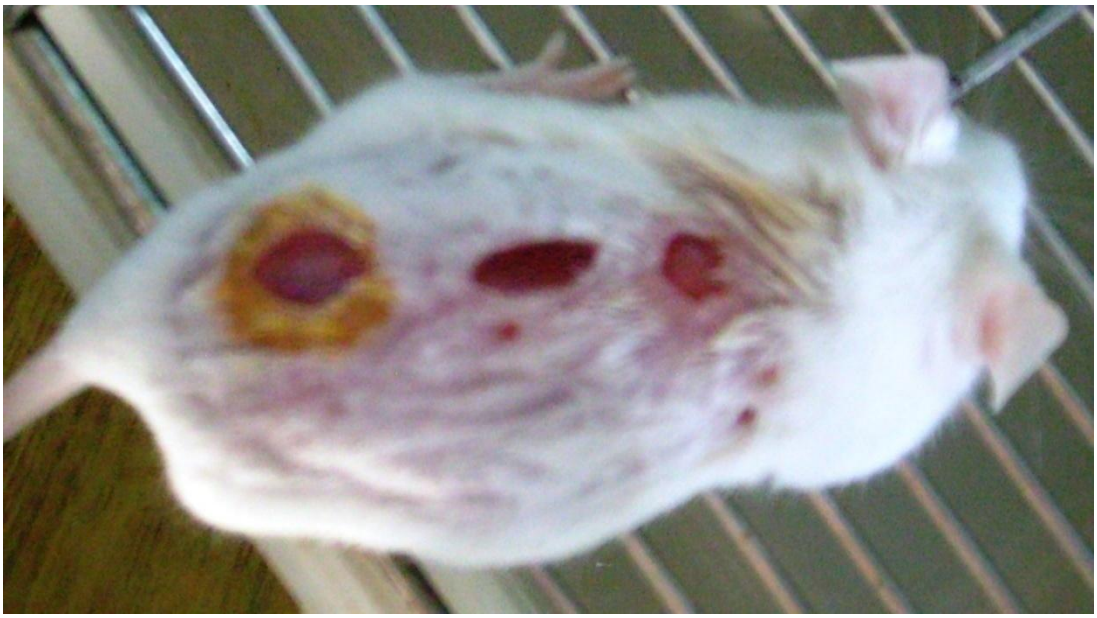
Depth and quality of granulation tissue was determined microscopically and the degree of epithelisation measured as the distance from the skin border to the wound centre. The *Madhu-sarpi* treated tissue underwent more rapid and more extensive epithelisation than did the betadine –treated and saline- treated control. After *3* days, the *Madhu-sarpi* treated tissue had *58%* more skin growth *(P<0.001),* after *6* days it had *114%* more *(P<0.001)* and after *9* days, *12%* more *(P<0.01)* than the controls. *Madhu-sarpi* -treated mice had a greater thickness of granulation tissue in the centre of the wounds *(P<0.001)* compared to the control mice. No bacterial infections were detected in any of the wounds, which may reflect hygienic standards in the original surgical procedure.

All the wounds were healed within the period of *15* days leaving no scar. Hair could be seen growing again at the wound site.

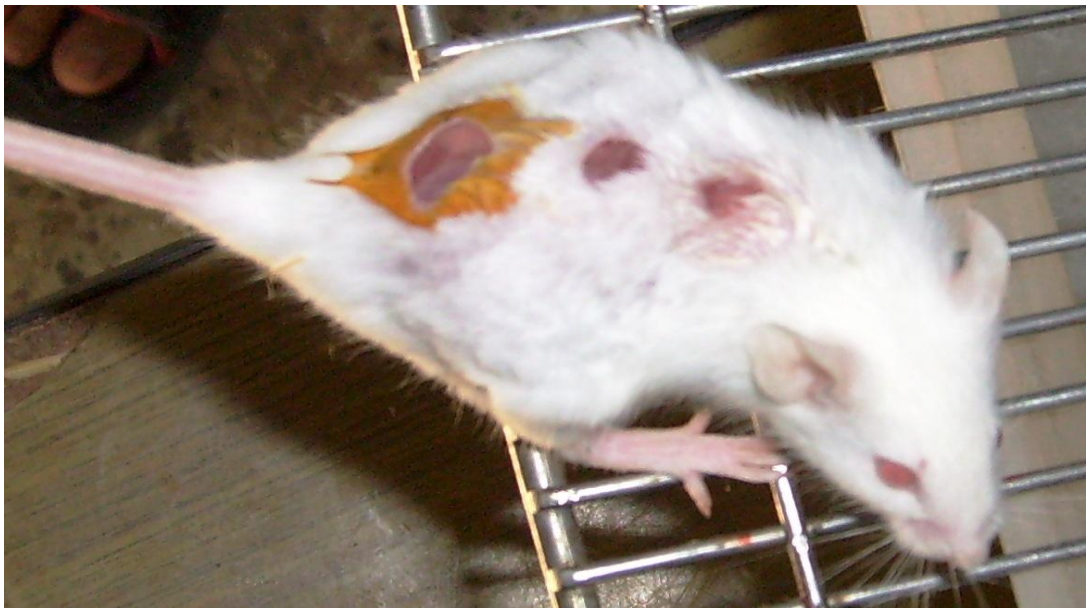
The rate of healing was more in the group E wounds as compared to the group B wounds and C wounds. The group E wounds healed within the period of *6 – 8* days whereas the group B wounds were healed in *9-12* days. The group C wounds were healed in *12-14* days. This clearly indicates that the wounds in group E healed faster

ANIMAL TRIAL

Day-10



Day- 15



METHOD

1. In all 60 male & female patients were selected for this study from Govt. Ayurved College, Nanded and Govt. Ayurved College,Osmanabad.

The study performed in two different groups. 30 patients were treated in the each group.

- ***Group E (Experimental):*** Application of *Madhusarpi* gauze.
- ***Group C (Control):*** Dressing with Polyvinyl pyrrolidone iodine (PVP 1) gauze.

✓ **CRITERIA FOR THE SELECTION OF PATIENT**

2. Patient having *sadyovrana* of duration less than 6hrs.
3. Patient having *sadyovrana* related to the *twaka* and *mamsa* only and having size :

   Maximum Length: ***3 cms (L)***

   Maximum Width: ***3 cms (W)***

   Maximum Depth: ***1cms (D)*** and having no 'full thickness' skin loss.

4. Patients of age 12yrs to 75yrs.
5. Either sex.
6. Who can attain hospital for a regular follow-up.
7. Patients who give written informed consent for the clinical trial.

✓ **CRITERIA FOR THE REJECTION OF PATIENT**

1. Patients with *sadyovrana* of duration more than 6 hrs.
2. Patients with *sadyovrana* and having full thickness skin loss.
3. Known Diabetic patients.
4. The patients having *sadyovrana* with other complications like fractures, Marmaghat etc.
5. *Sadyovrana* having medico-legal problems.
6. *Sadyovrana* of paediatric & pregnant patients, and of patients above the age of 75 yrs.
7. Patients who are not willing to participate in the trial.

- **Protocol:**

History: Patients were interrogated to find out the cause of the injury.

Detailed history was taken regarding the pain, bleeding, and other present complaints related to the wound.

History of past illness was taken to rule out DM, chronic infection like Tuberculosis, leprosy, HIV etc.

History regarding the bleeding disorders, atherosclerosis, and other peripheral vascular diseases was taken.

History of any treatment with anticoagulants, antiplatelets was taken.

General survey was done. Symptoms regarding anaemia, jaundice were ruled out clinically.

General systemic examination was done of Respiratory system, cardiovascular system and central nervous system.

Finally local examination was done in a systemic way to properly assess the extent of injury. Injury assessment was done:

A. Inspection:

- ✓ *Site*
- ✓ *Size*
- ✓ *Bleeding*
- ✓ *Discoloration*
- ✓ *Contamination by dust or other foreign particles*
- ✓ *Floor*

B. Palpation:

- ✓ *Base*
- ✓ *Tenderness*

C. Assessment of wound size with square measuring technique.

Sterile transparent sheet with printed graph lines of size *1mm* × *1mm* was used for this procedure. The sheet was kept on the wound and the margins of the wound were traced on the sheet with the help of the marker. Then the sheet was removed. The number of small squares from the marked area was count.

In Experimental group the sadyovrana was cleaned by normal saline and sterile mixture of *Madhu and sarpi (1:1)* was used for application. The gauze soaked in *Madhu-sarpi* was used. The gauzes were prepared by soaking them in the *Madhu-Sarpi* combination and then the soaked gauzes were sterilized by autoclaving. Dressing was performed in each patient.

In second group the *sadyovrana* was cleaned by normal saline. Wound was dressed with *betadine* soaked gauze and dressing was performed.

This procedure was followed every day regularly upto healing or maximum for *15* days. The cases were taken for this study from OPD and IPD of *Govt. Ayurved College, Nanded and Govt. Ayurved College, Osmanabad*. The CRF was prepared and observations were recorded for statistical analysis. Through systemic examination of each patient was done and recorded in the respective CRFs.

The wound was assessed as per the criteria described in the synopsis. The size of the wound was assessed using square method. The assessment was done before treatment i.e. on the 1^{st} day, on 7^{th} day and on 15^{th} day. After obtaining

informed consent from patients, wound size was assessed on the days described above or until the wound healed.

Mathematical Calculation from Length (L) and Width (W) of the Wound

Dimensions were measured using a disposable millimeter ruler by recording L as the wound's longest axis and W as the longest dimension perpendicular to L, as recommended;

The assessment of pain was done using internationally accepted Visual Analogue Scale (VAS). The patients were asked to mark on the VAS on each follow up.

- ✓ **Pain was graded as follows**:

0 - No pain

1-3 - Mild pain.

4-7 - Moderate pain.

8-10 - Severe pain.

- ✓ **Discharge was graded as follows:**

-- : No discharge.

\+ : Mild serous/seropurulent/purulent discharge.

++ : Moderate serous/seropurulent/purulent discharge.

+++ : Severe serous/seropurulent/purulent discharge.

✓ **Inflammation was graded as:**

-- : No inflammation.

\+ : Mild inflammation.

++ : Moderate inflammation.

+++ : Severe inflammation.

The data was collected and analyzed using appropriate statistical methods. The analysis of the data has been described further.

OBSERVATION AND RESULTS

The 60 patients were divided in two different groups

- **E (Experimental)**-- 30 patients.
- **C (Control)** -- 30 patients.

✓ **Table 1: Age wise distribution of the patients:**

	E	***C***
13 to 30 yrs	16	21
30 to 50 yrs	07	07
50 to 75 yrs	07	02
Total	***30***	***30***

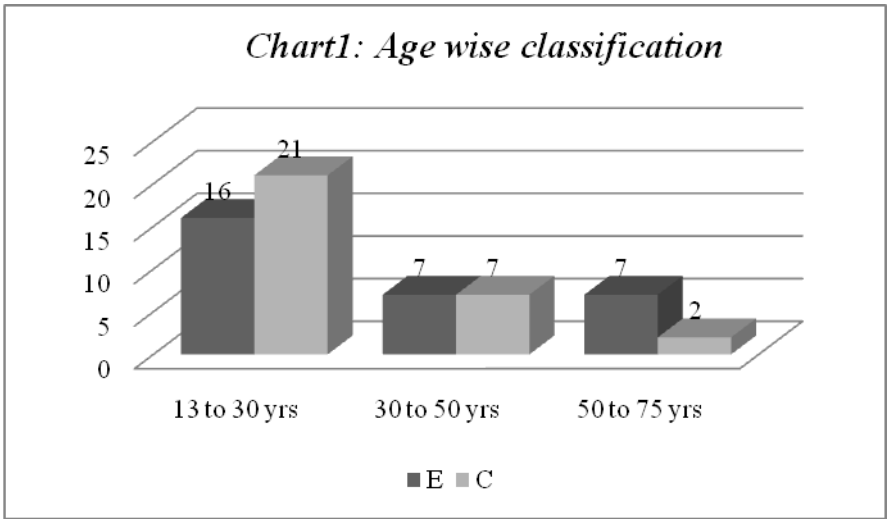


Chart1: Age wise classification
25
20
15
10
5
0
16
21
7
7
7
2
13 to 30 yrs
30 to 50 yrs
50 to 75 yrs
E
C

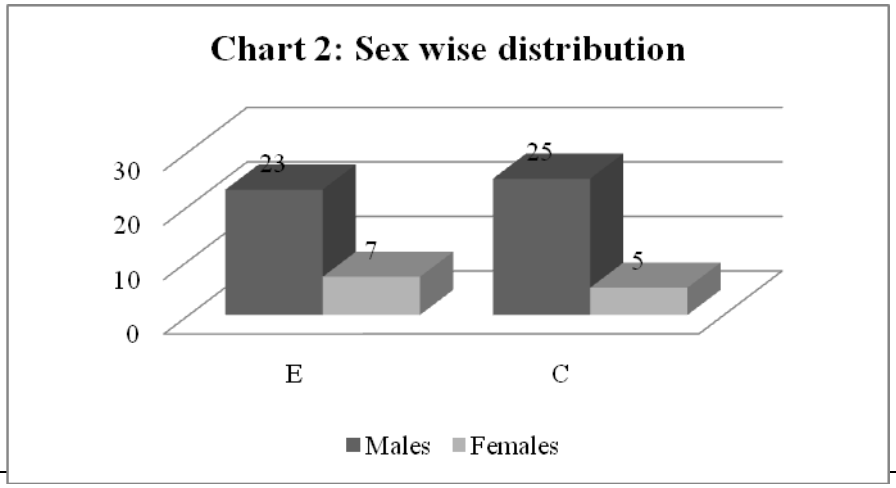
Chi square value	3.34
Df.	2
Sign.	***Insignificant***

Application of Chi square test to Age wise classification of patients

The chi square test when applied to the age wise distribution the difference is statistically insignificant. The groups were comparable and from the same population. Number of patients was more in physically active age group.

- ✓ **Table 2: Sex wise distribution of the patients:**

	E	***C***
Males	23	25
Females	7	5
Total	***30***	***30***



Chi square	0.41
Df.	2
Sign.	***Insignificant***

Application of Chi square test to Sex wise classification of patients

Sex wise distribution shows no significant difference in two groups. Men being involved more in outdoor activities, dominated in both groups.

Pain: ***(from the observations of VAS)***

- ✓ **Table 3: Pain assessment in Experimental Group**

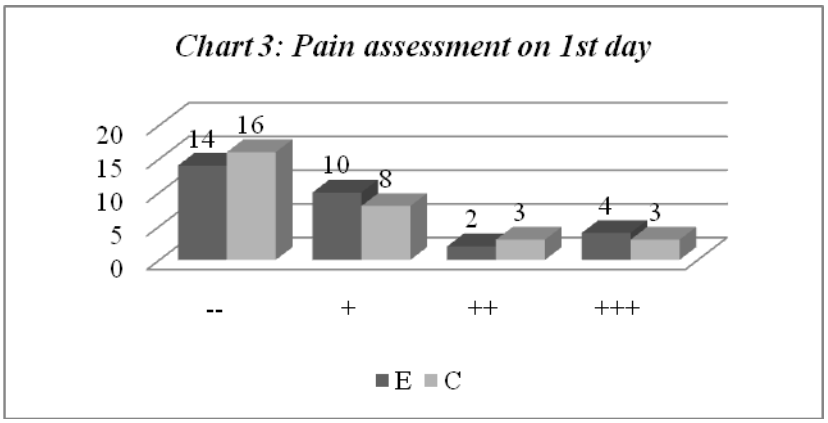
Degree of pain	***1st***	***3rd***	***7th***	***15th***
--	14	26	30	30
+	10	02	00	00
++	02	02	00	00
+++	04	00	00	00
Total	***30***	***30***	***30***	***30***

- ✓ **Table 4: Pain assessment in Control Group**

Degree of pain	***1st***	***3rd***	***7th***	***15th***
--	16	20	22	26
+	08	05	04	04
++	03	05	04	00
+++	03	00	00	00
Total	***30***	***30***	***30***	***30***

Pain assessment was done using VAS.

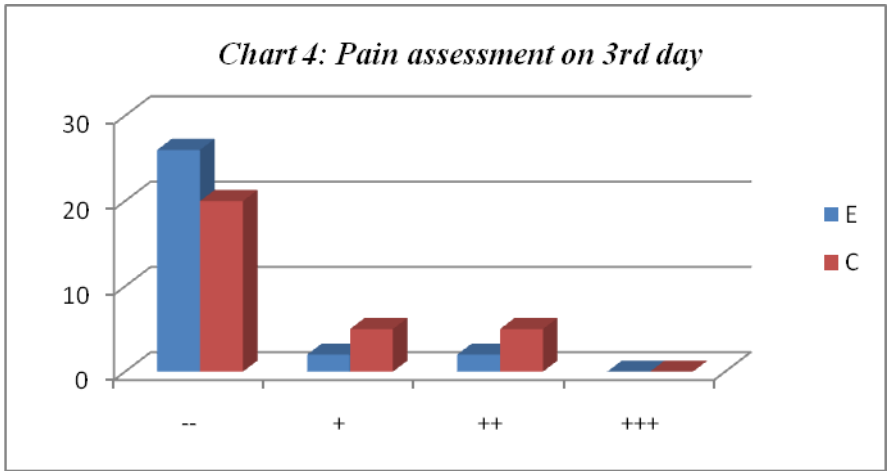
On the first day i.e. before treatment,



Chi square value	0.696
Df.	3
Significance	***Insignificant***

Application of Chi square test to Pain assessment on 1st day

So on the first day, there is no significant difference between two groups. Chi square value is not significant at *P<0.05.*

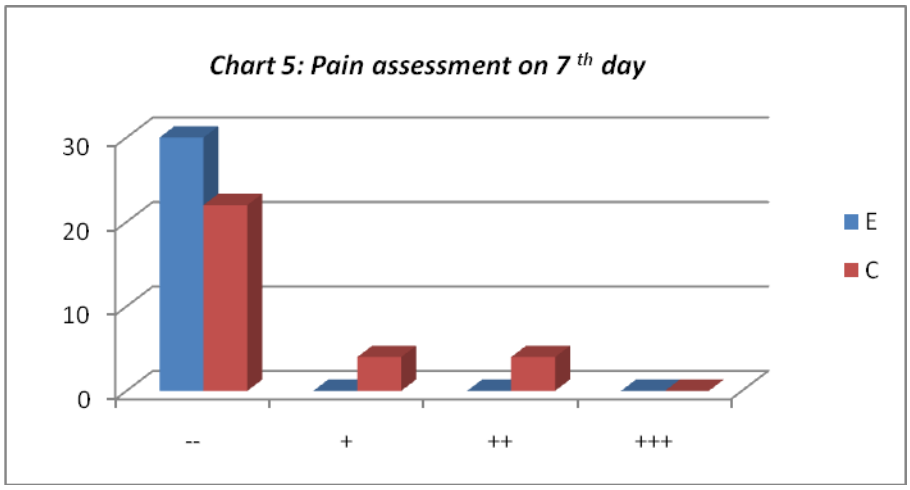


On the *3rd* day,

Chi square value	1.68
Df.	3
Sign.	***Insignificant***

Application of Chi square test to Pain assessment on 3rd day

On the *3rd* day also there was no significant difference seen between the two groups. Chi square value is again insignificant.

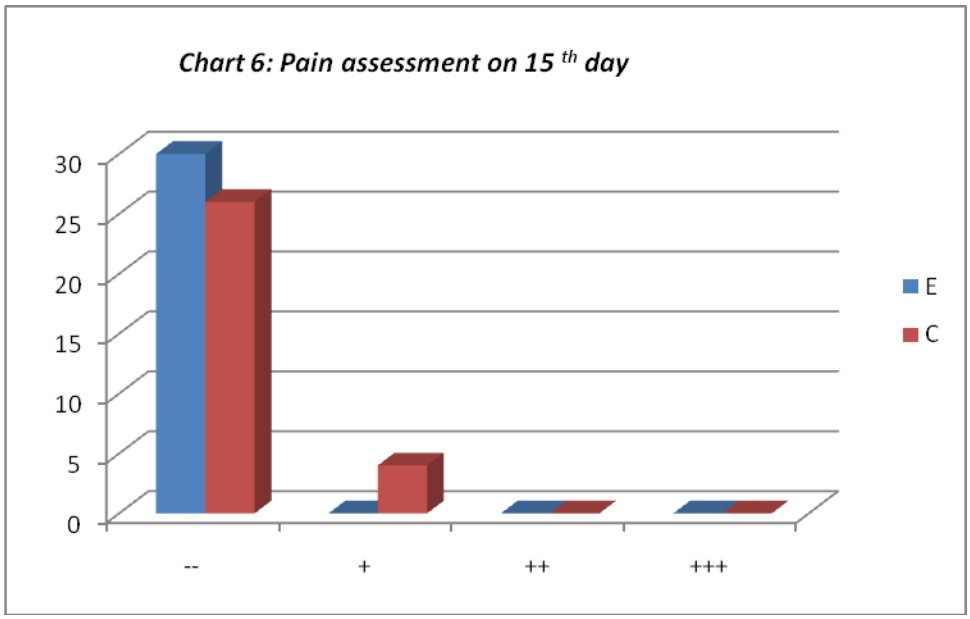


On the 7rd day,

Chi square value	8.61
Df.	3
Sign.	***Significant***

Application of Chi square test to Pain assessment on 7th day

The difference in pain sensation was significant on day 7. Chi square test is significant at P<0.05 on the 7th day.



0n 15th day,

Chi square	4.24
Df.	3
Sign.	***Insignificant***

Application of Chi square test to Pain assessment on 15th day

Again on the 15th day the difference in pain sensation was statistically significant. There was still some pain in patients of control group but in the patients of experimental group.

- ✓ **Table 5: Analysis according to the changes in the wound area:**

On the first day (Before treatment):

Area	***E***	***C***
0 to 3 sq.cms	06	08
4 to 6 sq.cms	16	14
7 to 9 sq.cms	08	08
Total	***30***	***30***

Chi square	0.41
Df.	2
Sign.	***Insignificant***

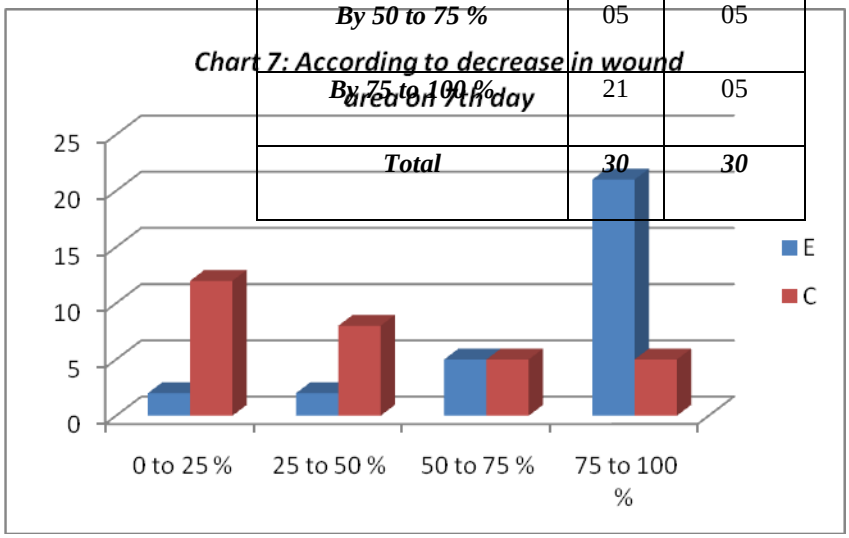
Application of Chi square test to decrease in wound area on *1st day*

When classified area-wise maximum patients had the wound of area *4 to 6 cm*^{2}. When the chi square test was applied to data the groups were found comparable.

✓ **Table 6:** ***According to decrease in wound area on 7th day***

Decrease in size percentage	***E***	***C***
By 0 to 25 %	02	12
By 25 to 50 %	02	08
By 50 to 75 %	05	05
By 75 to 100 %	21	05
Total	***30***	***30***

Chart 7: According to decrease in wound area on 7th day



Chi square	20.58
Df.	3
Sign.	***Significant***

Application of Chi square test to decrease in wound area on 7th day

The subsequent changes in wound area were determined in percentage. As per the classification data arranged and analyzed statistically. The decrease only up to *25 %* at the end of the study period was seen in *2 and 12* patients from experimental and

control group respectively. So the number of patients who didn't show significant healing was more in the control group.

The number of patients who showed the decrease between *25 to 50 %* was *2 and 8* in experimental and control group respectively. So in all *20* patients from control and *4* patients from the experimental group did not show healing more than *50 %* on the seventh day.

The number of patients who showed the decrease between *50 to 75 %* was *5* in both experimental and control group. *21* patients from experimental group and *5* patients from control group showed healing between *75 to 100 %* on the seventh day. The number was more in experimental group.

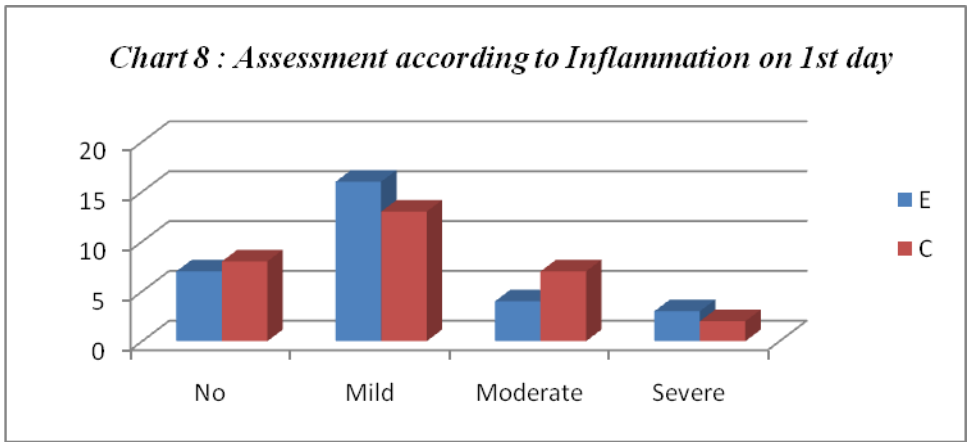
When statistical test applied to all this data the difference found was significant and the experimental group had better results.

✓ **Table 7: Assessment according to Inflammation before treatment:**

	E	***C***
No	*07*	*08*
Mild	*16*	*13*
Moderate	*04*	*07*
Severe	*03*	*02*
Total	***30***	***30***

Chi square	2.04
Df.	3
Sign.	***Insignificant***

Application of Chi square test to Assessment according to Inflammation on 1st day



The two groups were comparable as concerned to the inflammation observed in patients. Chi square test was not significant and the groups were from the same population.

- ***Experimental***

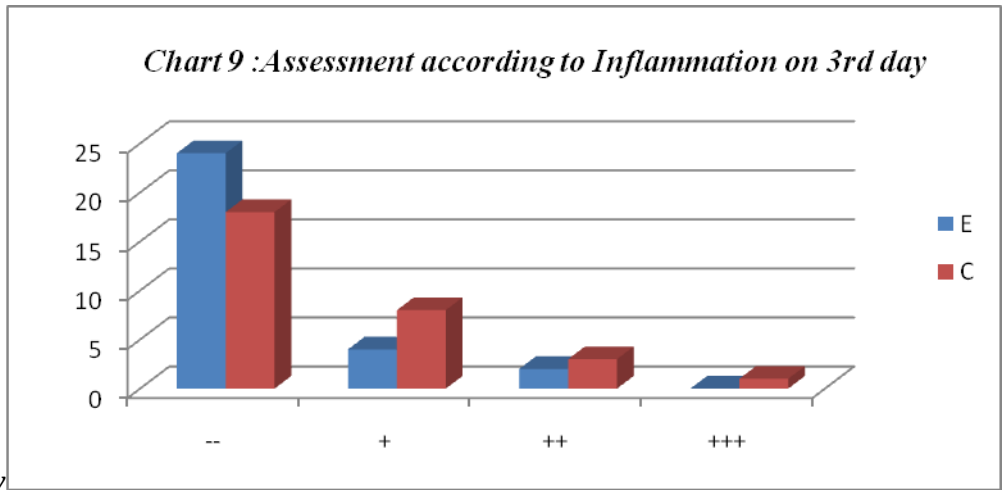
	1st	***3rd***	***7th***	***15th***
--	07	24	30	30
+	16	04	00	00
++	04	02	00	00
+++	03	00	00	00

Total	***30***	***30***	***30***	***30***

➢ ***Control***

	1st	***3rd***	***7th***	***15th***
--	08	18	22	26
+	13	08	04	04
++	07	03	04	00
+++	02	01	00	00
Total	***30***	***30***	***30***	***30***

On the third



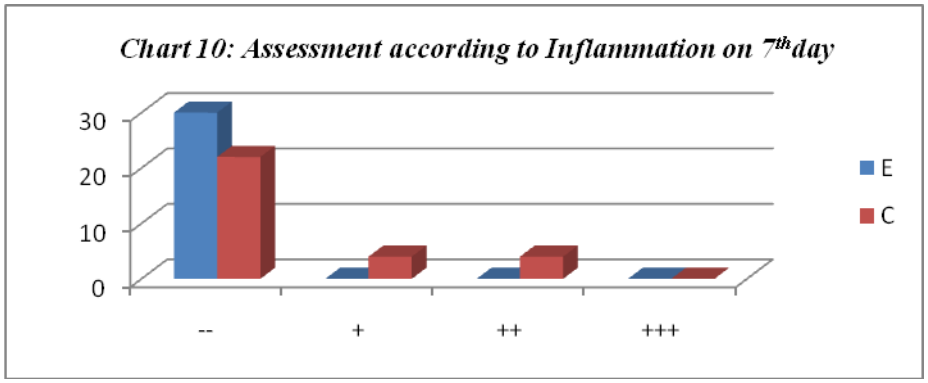
day

Chi square value	3.38
Df.	3
Sign.	***Insignificant***

Application of Chi square test to Assessment according to Inflammation on 3rd day

The difference was not significant statistically. On the 3rd day the both groups showed equal decrease in inflammation.

On the 7th day,

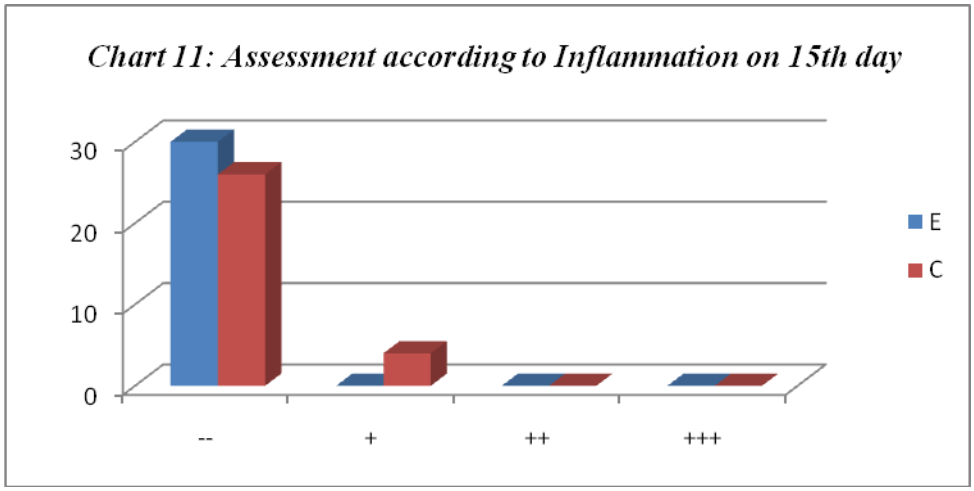


Chi square value	9.23
Df.	3
Sign.	***Significant***

Application of Chi square test to Assessment according to Inflammation on 7th day

More patients in control group showed signs of inflammation than in the experimental group. The difference was statistically significant when chi square test was applied.

On the 15th day,



Chi square value	4.28
Df.	3
Sign.	***Insignificant***

Application of Chi square test to Assessment according to Inflammation on 15th day

The difference was significant at the end of the treatment.

The experimental group showed better results than the control group.

Discharge:

The classification and observations of patients according to discharge were as following:

- ***Experimental:***

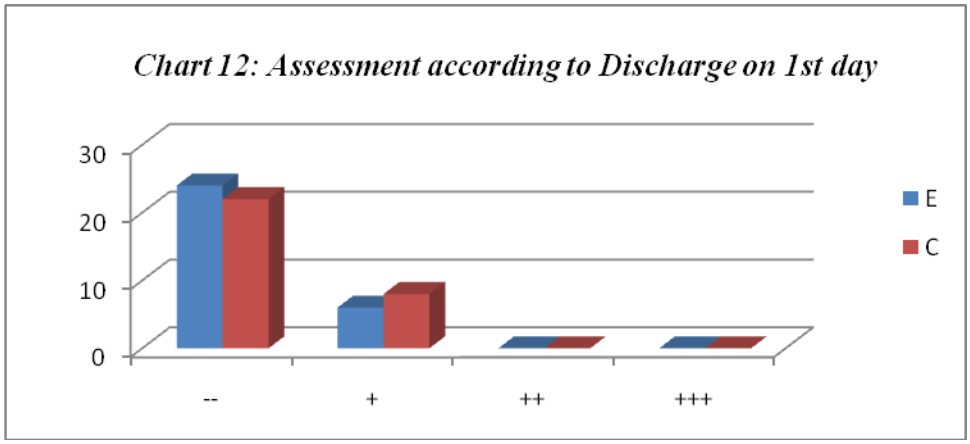
	1st	***3rd***	***7th***	***15th***
--	24	26	30	30
+	06	04	00	00
++	00	00	00	00
+++	00	00	00	00
Total	***30***	***30***	***30***	***30***

- ***Control:***

	1st	***3rd***	***7th***	***15th***
--	22	18	20	30
+	08	08	10	00
++	00	04	00	00
+++	00	00	00	00
Total	***30***	***30***	***30***	***30***

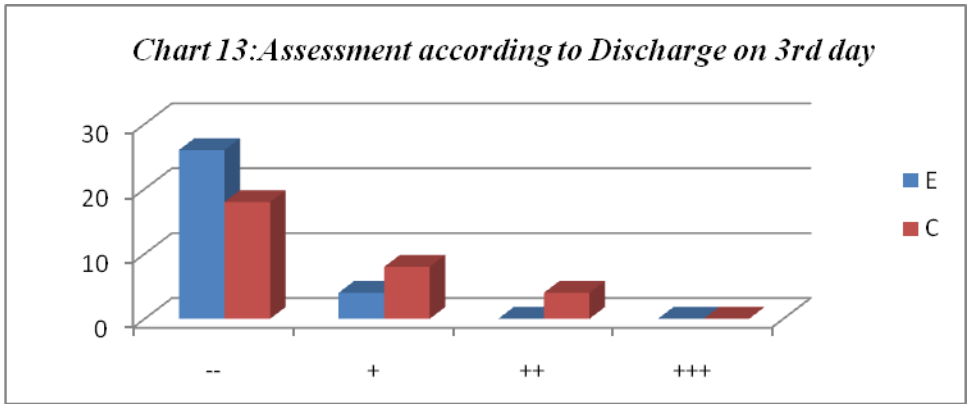
Chi square value	0.114
Df.	3
Sign.	***Insignificant***

Application of Chi square test to Assessment according to Discharge on 1st day



Before treatment both groups when analyzed for discharge it was seen that there was no significant difference in both groups. Statistically chi square showed insignificant difference at $P<0.05$.

On the 3rd day,

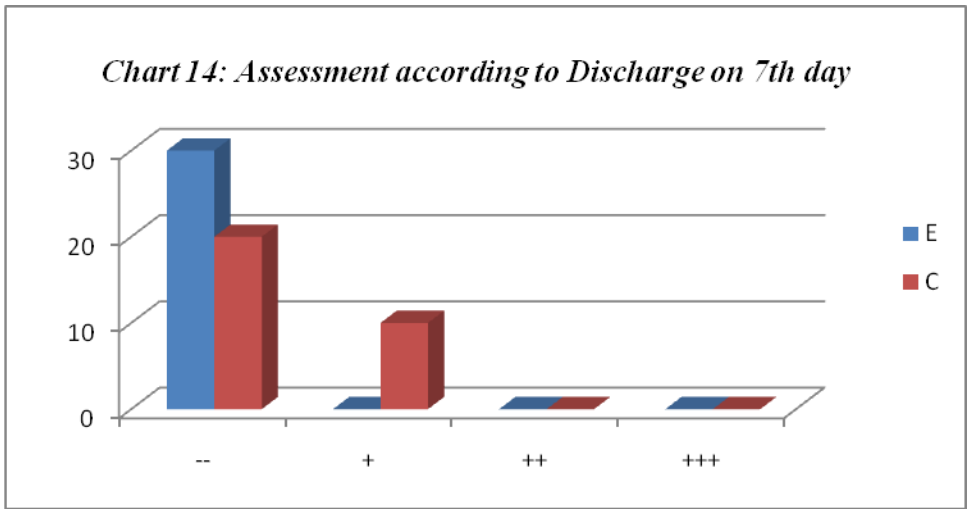


Chi square value	7.02
Df.	3
Sign.	***Significant***

Application of Chi square test to Assessment according to Discharge on 3rd day

On the third day 4 patients showed mild and 8 showed moderate discharge in control group, which was more than the experimental group. This data was analyzed statistically and the difference was significant.

On the 7th day,



Chi square value	12.00
Df.	3
Sign.	***Significant***

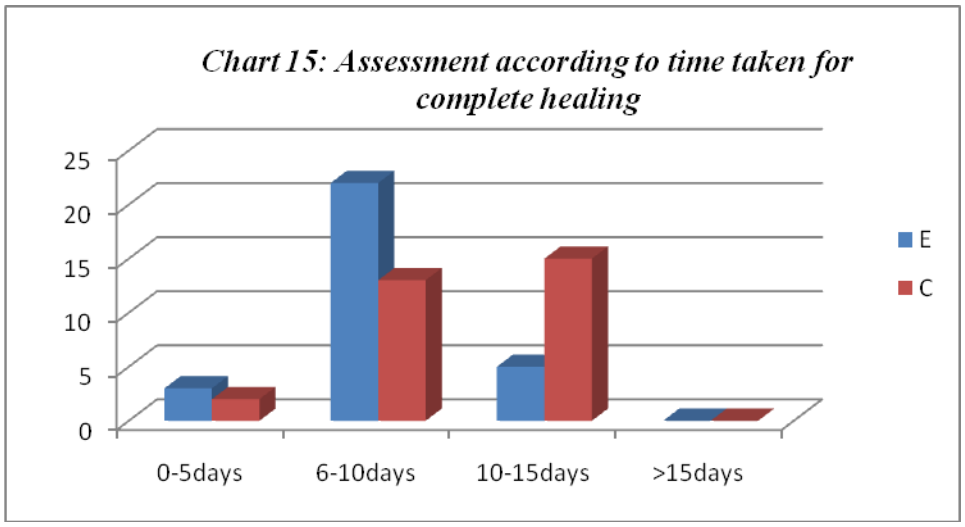
Application of Chi square test to Assessment according to Discharge on 7th day

On the *7th* day no patient from experimental group showed discharge but *10* patients from control group showed mild discharge. This difference was highly significant statistically.

- ***Time taken for complete healing***

	E	***C***
0-5days	03	02

6-10days	22	13
10-15days	05	15
>15days	00	00
Total	***30***	***30***



Chi square value	7.81
Df.	3
Sign.	***Significant***

Application of Chi square test to assessment of time taken for complete healing.

The data when classified according to the time taken for complete healing, maximum patients showed healing in 6 to 10 days in both groups. But the number was more in experimental group. The number of patients who took more time for healing was more in the control group. Thus relatively fast healing was seen in experimental group.

When the statistical test was applied to the data the difference seen was significant.

Data of healing was analyzed using Student's T test. This could give the information about the rate of healing in first three days , between three to seven days and between first to seven days. The findings were as follows:

Mean area of the wound on the first day was calculated in both the groups. Similarly mean area was calculated on day three and day seven for both the groups. Standard deviations were calculated for groups, for day one, three and seven. Difference in mean area of wounds was analyzed using t test. First the difference in mean area on day one and day three was analyzed.

t-test for decrease in area from 0 to 3 days

Group	N	Mean	Std Dev	SEM
C	30	1.905	0.95	0.1734
E	30	2.82	1.017	0.1857
Dif		-0.915	0.2541	

95% confidence interval for difference: -1.424 to -0.4064

t = -3.601 with 58 degrees of freedom (P<0.05)

The difference is significant in first three days. As the t value is more than 2 at 58 degrees of freedom the decrease in area in experimental group is more than control and is due to the application of test medication.

t-test for decrease in area from 4 to 7 days

Group	N	Mean	Std Dev	SEM

C	30	1.74	0.873	0.1594
E	30	1.55	0.787	0.1437
Dif		0.19	0.2146	

95% confidence interval for difference: -0.2396 to 0.6196

t = 0.885 with 58 degrees of freedom.

t value for the decrease in area between 4th to seventh day is 0.885 which is far less than the maximum value for 58 degrees of freedom. This indicates the decrease in areas of wounds does not differ much between both groups. Healing is comparable as t value is not significant.

t-test for decrease in area from 0 to 7 days

Group	**N**	**Mean**	**Std Dev**	**SEM**
C	30	3.62	1.61	0.2939
E	30	4.44	1.48	0.2702
Dif		-0.82	0.3993	

95% confidence interval for difference: -1.619 to -0.02077

t = -2.054 with 58 degrees of freedom.

t value for decrease in area from first day to seventh day is significant for P<0.05. This indicates better healing in the test group in seven days of treatment. The area decreased in test group is more compared to control group.

	Mean X	**Std. Dev**	**SE**		

	C	E	C	E	C	E	t	Interpretation
0-3 days	1.905	2.82	0.95	1.017	0.1734	0.1857	-3.601	Highly significant
3-7 days	1.74	1.55	0.873	0.787	0.1594	0.1437	0.885	Not significant
0-7 days	3.62	4.44	1.61	1.48	0.2939	0.2702	-2.054	Significant

(C: control group; E: test group; X: mean of difference in area between days mentioned in rows; Std. Dev: standard deviation; SE: standard error; t: value of Students t test)

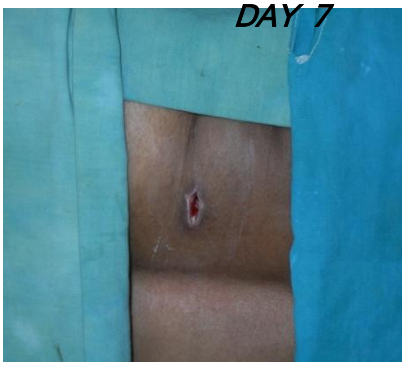
From above analysis it can be concluded that the healing is better with the drug in first three days. This can be attributed to rapid debriding action of the drug and additional antimicrobial activity of the drug. From day four to day seven healing is of similar rate in both the groups. Rapid action of the drug is discussed further in the chapter of discussion.

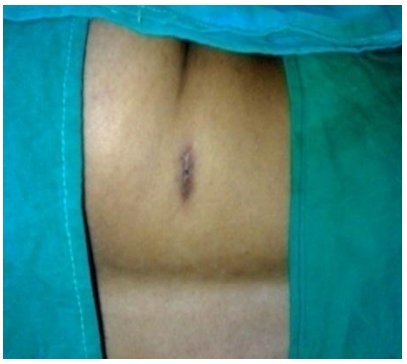
PRE AND POST TREATMENT PHTOGRAPHS OF PATIENTS

DAY 1

DAY 7

DAY 15

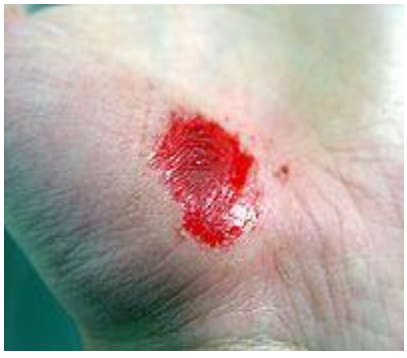


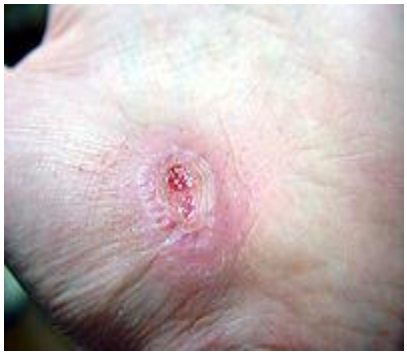


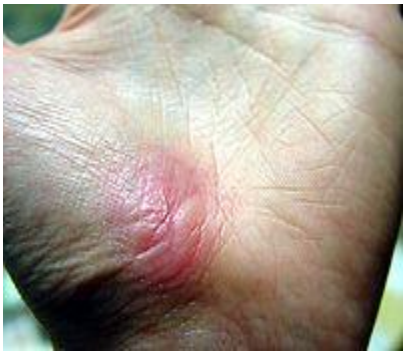
DAY 1

DAY 7

DAY 15



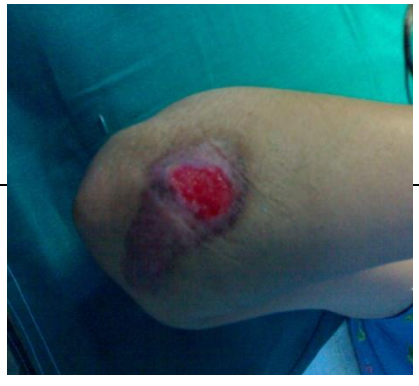


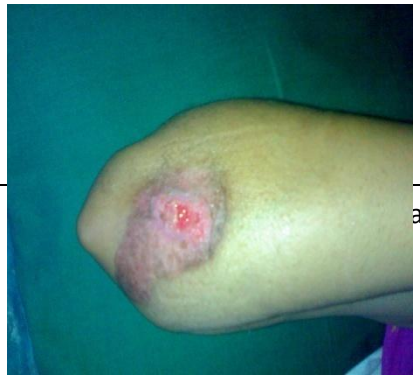


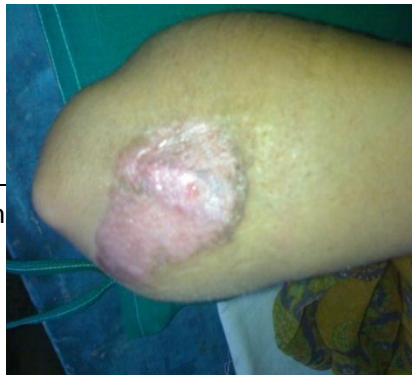
DAY 1

DAY 7

DAY 10







DISCUSSION

Wound healing is a complex and dynamic process, with the wound environment changing with the changing health status of the individual. The knowledge of the physiology of the normal wound healing trajectory through the phases of hemostasis, inflammation, granulation and maturation provides a framework for the understanding of basic principles of wound healing. Through this understanding, the health care professional can develop skills required to care for a wound and the body can be assisted in the complex task of tissue repair. Though we can treat the wounds considering the knowledge of healing with the help of modern technology, the wounds were treated in the similar fashion in ancient era. This complex procedure of healing has been described in an excellent way in the ancient classics of *Ayurveda*. They studied the healing through their keen observation, thorough understanding and divine power, in the absence of advanced modern techniques.

Maharshi Sushruta, the Father of Surgery, has described the management of wounds in his treatise. It is the best description ever, in the history of medical sciences in case of wound management. We don't find such a detailed and complete management in any stream of medicine. The work has been summarized in *Shashti Upakramas* i.e. Sixty measures (sixty different aspects) for wound management[1].

The wounds were basically divided as traumatic *(Agantuja)* and non traumatic *(Nija)* types[2]. The management was different for these two types. *Madhu Sarpi* has been described as the best medicament for the local management of traumatic *(Agantuja)* wounds[3]. This is *57th* measure of wound management among the *Shashti Upakramas*[1]. Basically described by *Sushruta* in detail, it has been recommended by other scholars also, including *Charaka* and *Vagbhata*. The drugs are from natural resources which would help enhance healing. The Mode of Action can be summarized as 'Anti-inflammatory healing promoting'. We will discuss it in detail considering the *Ayurvedic* as well as modern aspects.

Application of honey and ghee is indicated in traumatic wounds in order to control heat of the wound and to promote union[3].

In traumatic wounds local *Pitta* gets aggravated. It causes increase in local *Ushma.* This ushma causes local burning as well as *Shopha.* Being *Sheeta virya*[4] *Ghrita* acts as *Pittashmana*[4]. *Ghrita* counters the *Ushma* caused by *Pitta* i.e. local heat of the wound. Also local *Vata* gets aggravated due to loss of *dhatu (Dhatukshaya).* It results in pains. *Ghrita* being of *snigdha*[4] property acts as *Vatashamaka.* Thus *Ghrita* being *Pitta-Anilharam*[4] alleviates both *Vata* and *Pitta.* Because of this counter actions pain as well as *shopha* immediately subsides.

Also *Ghrita* by its *Agnideepaka*[4] property (the property of improving metabolism) causes improvement in local *dhatwagni*. This increases rapid replacement of lost *dhatu* which in turn results in rapid healing. It also aids

healing by its *Shodhana* and *Ropana* properties. Here *Shodhana* refers to cleansing and *Ropana* refers to healing i.e. replacement of lost tissue. *Varnya* property retains the natural skin color even after healing. The other important property described in *Ayurveda* is *Vishahara*[4]. Here in case of wounds it can be correlated to the toxins liberated by infecting micro organisms at wound site. These toxins are neutralized by the *Vishahara* acton of *Ghrita*.

In *Ayurvedic* era due to lack of technological aids the actual nature of micro organisms was not known. But the Ayurvedic scholars were well acquainted to the infection and its hazards. They observed the pus formation in the wound and septic manifestations of the infected wound. They named the factors responsible as *Rakshas*. The process of killing those *Rakshas* was coined as *Rakshoghna*. The *Rakshoghna*[4] property of *Ghrita* thus can be correlated to the anti microbial activity of *Ghrita*.

Madhu is *Madhur* and *Kashay* in *rasa*[5]. The *vipaka* of the drug is *Katu*. The *kashaya rasa* and *katu vipaka* help in *Shodhana*[5] (debriding) of the wound. Here in case of wounds with recent trauma *shodhana* is necessary to remove any contamination and to prevent any possible infection. Also *shodhana* refers to removal of devitalized tissue.

Madhu has been explained as *Raktapaha and Pittapaha*[6]. By virtue of these properties it alleviates the *ushma* caused by *pitta* as does the *ghrita*. The *chhedana* property of *Madhu* is nothing but the separation of dead tissue from the

surrounding healthy tissue. There are various discharges from the wound as per the humours involved and whether the wound is traumatic or atraumatic. These discharges delay healing. It is necessary to get rid of discharge. *Madhu* helps to decrease discharge by its *Ruksha guna*[5]. Also, after proper *Shodhana* of the wound, it helps in healing and bringing together the cut edges by its *Sandhana*[5] property. *Madhu* also possesses the property of *Agnideepana*,[5] which works in the same way as that of *Ghrita* i.e. acts on local *Dhatwagni*. The *Varnya*[5] property contributes in healing by preserving the natural skin color i.e. *Savarnikaranam*.

These functions are performed only after penetration of the drug deep into the tissue and at cellular level. This is achieved by *Sukshma Marga Anusari*[5] property of *Madhu*.

From above discussion, it looks like that both the drugs are having the same properties then why should one use both the drugs?

It is not so exactly. There is some difference in quality of the functions of these two drugs. When seen practically the *pittashamaka* property of *Ghrita* is more marked than *Madhu*. *Madhura rasa and Madhura vipaka* is more marked in *Ghrita*. So *Ghrita* is more useful for healing and new tissue formation. There is

additional *Kashaya rasa and Katu vipaka in Madhu* which indicates better *Shodhana* quality of *Madhu* than *Ghrita*. While *Ghrita* helps in *Ropana* by formation of new tissue, *Madhu* helps *ropana* by union of this newly formed tissue i.e. *Sandhanakar*. The *sukshma marga anusari* property necessary for the

drug to penetrate deep into the tissue is basically of *Madhu*. It is acquired by *Ghrita* by its *Sanskara anuvarti*[7] property. The *sneha guna* also penetrates deep into the tissue. When *sneha guna* combined with *sukshma anusari* property, the penetrating power increases and drug gives better results. This explains the *Kshato ushmano nigraha*[3] action of the combination on the basis of *Ayurvedic* principles. That may be the reason behind the combined use of both drugs.

The combined use of these two has been indicated in many traumatic conditions. In *Savrana Bhagna*[8] the drug is indicated for local application along with other herbs. Apart from *Sushruta, Charaka and Vagbhata* indicated the drug in different conditions. *Charaka* explains its use in wounds where the tissue loss is more[9]. *Vagbhata* has indicated it in post operative wounds[10].

If the modern literature is considered, the combined use of honey and clarified butter i.e. ghee has not been described in the management of wounds. In contrast, in *Ayurveda* this combination has been used invariably in the management of wounds. *Ayurvedic* scholars never considered honey or ghee as a single agent for the purpose. The work of modern science was initially directed to

the antimicrobial activity only. Not much attention was given to the other aspects of wound healing like rapidity, strong scar formation and less discoloration. In last few decades the view has changed. The agents which enhance healing of wound are being searched. Also, the agents which offer better quality healing are being searched. Many agents were tried experimentally in animals as well as in humans. But not a single agent became popular in medical community.

Much work has been carried out on the use of honey as a topical agent in wound healing. In spite of the thorough research work and proven efficacy, honey has not been introduced as a dressing agent in humans. A few products are available in the market for veterinarian use. In case of ghee, no research has yet been done regarding the topical action. Even *Ayurvedic* scholars never considered it as a sole agent for topical application. This is a small effort to re-search and re establish the drug which was most commonly used in Ancient era. This study is intended to prove the efficacy on the basis of modern parameters.

MADHU [HONEY]:

Honey is produced by honeybees that collect nectar from flowers and concentrate the sugars until it is honey. Honey consists mainly of sugars, water and very small amounts of organic compounds and enzymes.

- **Debridement:**

It has been noted that dirt is removed with the bandage when honey is used as a dressing, leaving a clean wound. It has been reported that sloughs, gangrenous tissue and necrotic tissue are rapidly replaced with granulation tissue and advancing epithelialization when honey is used in dressing, thus a minimum of surgical debridement is required. It has been observed that under honey dressings sloughs, necrotic and gangrenous tissue separated so that they could be lifted off painlessly, and others have noted quick and easy separation of sloughs and removal of crust from a wound. During this study also, no evidence of infection was observed[11].

- **Antimicrobial activity[12]**

The enzyme glucose-oxidase is added to the honey by the bees and is, together with other compounds, responsible for the honey's antibacterial activity. The antibacterial activity can be further explained as:

• ***Hydrogen peroxide*[12]:**

When honey comes in contact with the wound and mixes with wound fluids, the enzyme glucose-oxidase is activated. The enzyme glucose-oxidase is responsible for slow release production of low concentrations of hydrogen peroxide. The concentration of peroxide is high enough to kill pathogenic bacteria, but does not harm the healing tissue.

- ***Slow release*[12]:**

The hydrogen peroxide is produced gradually, and continues as long as there is honey present in the wound. The amount of time that the honey is effective depends on the level of exudation of the wound.

- ***Low pH*[12]:**

The activity of glucose-oxidase also causes production of gluconic acid. This acid is the major organic acid in honey and regulates that acidic environment

(low pH). The amount of acid in honey is an important measure for the anti-bacterial functionality. *(Bogdanov, 1997)*

In this study, as the fresh wounds are considered, the role of honey as an antimicrobial agent is prophylactic. As a few wounds were contaminated, it was necessary to have thorough cleansing of wound.

During the study no wound was found to be infected in the test group. The drug might have inhibited the growth of organisms in these contaminated wounds. After dressing of the wound, the slow release of hydrogen peroxide and low pH (acidic environment) at the wound site were responsible for the inhibition in test group.

Antibiotic resistant *Staphylococcus Aureus* (MRSA) are shown to be evenly vulnerable to medicinal honey as antibiotic sensitive staphylococcus. Comparable results were obtained with *Pseudomonas Aerigunosa*[12].

The low water activity, Osmolarity and Phytochemical components are the other probable modes of antimicrobial action of honey[12]. The clinical observations in this study are in agreement with the previous studies performed in animals.

- **Granulation tissue formation, Epithelialisation**

. Honey stimulates angiogenesis, granulation and epithelialisation by stimulating the growth of fibroblasts, and thus it helps skin regeneration[13].

It was observed in the follow up of the patients during study that wounds healed rapidly in the test group compared to the control. The mean period of formation of fresh granulation tissue was less in test group than the control group.

A few studies on humans have concluded the improvement of nutrition of wounds and also increased blood flow with free flow of lymph. The findings in this study are consistent with the findings of the previous studies.

Honey seems to cause more rapid epithelialisation presumably because of antibacterial properties as compared to control. The enzyme *catalase* present in honey has an anti oxidant property[14].

Another effect of honey on wounds that has been noted is that it reduces inflammation and hastens subsidence of passive hyperaemia. It also reduces edema and exudation, absorbing fluid from the wound[14].

- **Collagen**

Honey causes fibroblasts to act fast at wound site. It activates the fibroblasts and induces density of fibroblasts. This leads to rapid laying down of collagen fibres. It helps in improving keratinization of the surface wounds as observed in previous studies. The effect was observed in this study also. After epithelialisation the basement membrane thickness is an important factor in healing wounds. Honey is known for increasing the thickness of basement membrane and epidermis. It results in strong scar and rapid healing. The factor in better healing is collagen fibers. It plays a major role in wound healing. Honey increases the thickness of collagen fibers[15].

- **Direct nutrition effect** [11]

Honey can be expected to have a direct nutrient effect on regenerating tissue because it contains a wide range of amino acids, vitamins and trace elements in addition to large quantities of readily assimilable sugars. (The vitamin C content of honey, which is typically more than three times higher than that in serum, and may be many times higher, could be of particular importance because it plays an essential role in collagen synthesis.) In addition, the high osmolarity of honey causes an outflow of lymph which serves to provide nutrition for regenerating tissue which otherwise can only grow around points of angiogenesis

(seen as granulation): healing is delayed if the circulation to an area is poor, or if a patient is poorly nourished. Also it has been suggested that the decreased turgor resulting from the application of honey may increase oxygenation of tissues.

- **Analgesic and deodorizing action[11]:**

Analgesic action of honey is attributed to the anti-inflammatory action. It was well observed in the study as less pain was observed in patients in test group.

Also no odor was observed in the patients treated in test group. This may be due to known de odorizing action of honey. This de odorizing effect is attributed to the antibacterial action of honey[16].

Honey has been found to cause no pain on dressing or to cause only momentary stinging, to be non-irritating, to cause no allergic reaction, and to have no harmful effects on tissues. It has been noted that honey dressings are easy to apply and remove. There is no adhesion to cause damage to the granulating surface of wounds, no difficulty removing dressings, and no bleeding when removing dressings. Any residual honey is easily removed by simple bathing.

SARPI [GHEE]:

As mentioned above, ghee has not been considered as promising healing agent by modern science. But taking into consideration the references from *Ayurveda, ghrita* has been tested with honey in this study. The healing enhancing action can be explained on the grounds of the composition of ghee. The different

ingredients present in the ghee have role in the wound healing. For the same purpose ghee was first analyzed chemically. It has been observed that theses ingredients serve the important functional aspects of healing like antimicrobial activity, moist wound environment of healing, medium for carrying the other ingredients to the cells, collagen synthesis and many others.

- **Anti microbial activity of Ghee**

When analyzed in the laboratory Ghee showed zone of inhibition for *Staphylococcus Aureus* in vitro. The test organisms were incubated in Nutrient Agar and sensitivity was tested using Ghee. *S. Aureus* being the most common organism found in infected wound, the activity found in vitro was of prime importance. Considering this finding an effort was done to find out the antimicrobial factor in the ghee.

Oleic acid, linoeic acid, lauric acid and capric acid are the major fatty acids found in the Ghee along with other fatty acids. Oleic acid has been found to have strong inhibitory action on certain bacteria and viruses. It has been observed that linoleic acid is capable of inhibiting the growth of *Staphylococcus aureus* by altering its protein synthesis, cell wall, nucleic acids and cell membrane during division[17]. As in this study no wound was found to be infected, it can be concluded that ghee acts synergistically with Honey[17]

- **Vit. A**

The other constituents of ghee include Vitamins A, D, E and K. Quantitatively Vit. A is more abundant than others. These are related with proper growth and tissue regeneration. Vitamin A interferes with wound healing causing lysis of lysosomal membranes, stimulation of fibroblasts and collagen deposition[17]. The biological activities of vitamin A have not been completely elucidated, but it appears to act as an anti-inflammatory agent. Its antioxidant properties suggest a protective function for growing cells against free oxygen radicals released by leukocytes[17].

- **Maintenance of moist environment**

Ghee being oily in nature retains the moisture of honey and maintains moist environment at wound site. The role of moist environment is discussed in detail further.

- **Triglycerides**

The cell membrane is made up of lipid bi-layer. The lipophpilic substances are better carried to the cells with lipid media. Here ghee acts as a vehicle for honey to reach to intracellular part. Ghee as mentioned in the literature contains *97%* triglycerides i.e. fatty acids. Fatty acids belong to a class of compounds formed by a long hydrocarbon chain and a terminal carboxylic group. They have three main functions: they are structural components of biological membranes,

they act as precursors of intracellular messengers and they are oxidized producing ATP (adenosine triphosphate)[17].

In early *1970s,* there were studies on the effects of fatty acids on the immune response. Such compounds interfere with various events of the inflammatory process, such as vascular contraction, chemotaxis, adhesion, diapedesis, activation and cell death, where the majority of these occur via arachidonic acids such as prostaglandins, leukotrienes, tromboxanes and lipoxins[17].

During the study early inflammatory response was found to last for a very short period in the test group than control. The period was relatively pain free for patients and less exudation was observed in the test group. These actions can be attributed to ghee. Ghee provided medium for honey by its lipohilic nature to act at the cell level.

- **Essential fatty acids -**

Essential fatty acids´ (EFA) effectiveness on problems related to skin lesions has been studied since *1929,* when the first observation of skin lesions provoked by a shortage of EFA levels in foods were made. [17] *Linn and Shepherd*[17] described the cure of those alterations by topical application of EFAs. Essential fatty acids, linolenic acid and arachidonic acid are polyunsaturated vegetable lipids that cannot be synthesized by the animal organisms, necessitating renewal by diet. [17]

Ghee is a good source of above mentioned fatty acids. Deficiency of these fatty acids leads to impaired healing of wounds. It can be fulfilled by oral as well as topical application of the same. Ghee being a good source of these fatty acids might have contributed in the healing process.

- **Linoleic acid as a pro inflammatory agent and growth factor**

Linoleic acid is a powerful pro-inflammatory mediator that causes migration of granulocytes and macrophages as well as important changes in granulation tissue. [17] According to Glasgow and Eling[17] linoleic acid is essential for regulation of the biochemical events that precede the fibroblastic mitogenesis since it stimulates some factors of cellular growth.

Linoleic acid has also been shown to participate in cell proliferation and inflammatory process, where in the latter it plays a role as a mediator of leukocyte function having chemotactic and stimulatory effects on neutrophils [17].

The acceleration of the inflammatory process can be explained by biological and biochemical features of the linoleic acid. This polyunsaturated fatty acid (18:2n-6) is changed by desaturation and elongation of its molecule to arachidonic acid (20:4n-6) which is metabolized via the 5-lipoxygenase and clyclooxygense pathway in leukotrienes (LTB_4, LTC_4 and LTD4), prostaglandins (PGE_2, PGF_2, PGD_2 and PGI_2) and thromboxane A_2 by polymorphonuclear cells. These substances produced by LA have pro-inflammatory properties that can

stimulate new vascularization locally, cell migration, proliferation and fibroblastic differentiation as well as extracellular matrix synthesis.[17]

When the combination of *Honey and Ghee* is used the action can be summarized as:

- **Moist environment**[18]

The most significant advancement in wound care came with Winter's study in *60's*, which showed that occluded wounds healed much faster than dry wounds and moist wound healing environment optimized the healing rates. He demonstrated that when wounds on pigs are kept moist, epithelialisation is twice as rapid as on wounds allowed to dry by exposure to air. Later *Hinman* and *Maibach* confirmed *Winter's* work on human beings in *1963.* An open wound, which is directly exposed to air, will dehydrate and a scab or escar is formed. This forms a mechanical barrier to migrating epidermal cells and is then forced to move in a deeper level of tissue, which prolongs the healing process. Moist healing prevents the formation of scab as the dressing absorbs wound exudate secreted from the ulcer.

The property of maintaining moist environment at wound site is well known for honey. *Ghee* by its lipid nature helps in retaining the moisture. This was observed in all cases of test group in this study. No dry scab formation was observed in any case of test group. This is consistent with the other studies done using honey in human as well as animals.

- **Anti inflammatory action:**

The moist environment right from beginning of the treatment helped to accelerate the inflammatory response. Inflammatory phase was short lasting in test group compared to the control. It subsided rapidly in test group. By seventh day, inflammation subsided in the test group in all *30* cases while mild to moderate inflammation could be seen in *8* cases of control group. The various anti inflammatory factors in both, *Honey and Ghee*, could act to their full strength in the moist environment. This may be the reason that *Madhu-Sarpi* has been advocated in the cases of burst abdomen and penetrating injuries of abdomen in *Ayurveda*[19]. Also this is the standard treatment of Burns according to *Ayurveda*[20].

- **Analgesic action of the combination**

Pain was not a major symptom in both groups before treatment. As the criteria of wound area was below *9 sq c*ms i. e. the wound area was less and the wounds selected were superficial in nature, the pain was less initially. But still few patients showed mild to severe pain in both groups. After commencement of treatment the status was significantly different. Patients treated with *Madhu Sarpi* showed greater reduction in pain than the other group on third day.

On seventh day, no patients showed pain in the group treated with *Madhu Sarpi*. But some patients from control group showed pain. Thus the efficacy of

Madhu Sarpi application on pain is proved. This analgesic action is in accordance with anti inflammatory action of the drug.

- **Wound contraction**

In *25* cases, wound size reduced by *75 %* on seventh day in test group while in control the figure was only *5*. This clearly indicates the existence of wound healing enhancing agents in the combination of Honey and ghee. Povidone iodine being antiseptic agent only did not result in acceleration of healing in control group. No additional action of povidone iodine has been reported apart from antiseptic action in literature. This difference is significant considered to the partial thickness cutaneous wounds. This indicates that rate of wound contraction is significantly increased by *Madhu Sarpi*. The overall rate of wound healing *(Decrease in wound size)* was *0.614 cm^2 /day* in test group which is significantly better than *0.548 cm^2 /day* of control group.

- **Discharge:**

The anti-inflammatory activity of honey gives another advantage that is of practical importance in managing wounds that have a high degree of inflammation. Factors produced in the inflammatory process open up blood vessels so serum flows out into the surrounding tissues. Where the skin is broken the serum exudes from the wound and the quantity of exudate can be large enough to create practical difficulties in absorbing it. If honey is kept on the wound (i.e.

not allowed to be washed away by the exudate) its anti-inflammatory activity soon suppresses the inflammation so that the quantity of exudate decreases[21].

In this study, mild to moderate discharge was observed in only *4* cases in test group while it was in *12* cases in control group on *3rd* day. On seventh day no discharge was observed in test group but it was observed in *10* cases of control. The discharge decreased in all cases of test group but there was increase in discharge in *4* cases from *1st to 3rd* day. Qualitatively it was of serous in nature, in both groups. Early cessation of discharge is in accordance with rapid subsidence of inflammation in test group.

- **Granulation tissue formation**

Early granulation tissue formation was observed in test group. Complete healing was observed in *25* patients of test group and *15* patients of control within *10* days. Almost *15* out of *30* cases took more than *10* days to heal in control which indicates relatively delayed healing. As no wound was infected in the study in both groups the factor responsible for delay was not infection. The wounds which showed delayed healing actually showed deficient granulation tissue which could be observed clinically. The rapid formation of granulation tissue and rapid collagen deposition due to Honey and Ghee was well observed in the study.

Wounds completely healed in all patients in both groups in *15* days. But remarkable difference was observed in the quality and dimensions of scar. There was more discoloration, more thickness of scar in the control. Relatively small scars and less discoloration were observed in test group. Honey is a known agent

which prevents hypertrophication. The action of maintaining normal pigmentation and hence the cosmetic effect is in accordance with the actions of *Ghee* described in *Ayurveda i.e. Varnya (Savarnikara).*

No wound was disrupted as dressing did not adhere to the wound in any case in test group. Adhesion of dressing and disruption of wound was observed in the control group which is a routine observation in day to day practice also.

The drug used for the control group was an antiseptic, a solution of polyvinyl pyrrolidone iodine (PVP-1). Antiseptics have long and commonly been used on wounds to prevent or treat infection. Antiseptics are agents that destroy or inhibit the growth and development of microorganisms in or on living tissue. Unlike antibiotics, which act selectively on a specific target, antiseptics have multiple targets and a broader spectrum of activity, which include bacteria, fungi, viruses, protozoa, and even prions. Several antiseptic categories exist, including alcohols (ethanol), anilides (triclocarban), biguanides (chlorhexidine), bisphenols (triclosan), chlorine compounds, iodine compounds, silver compounds, peroxygens, and quaternary ammonium compounds. The most commonly used products in clinical practice today include povidone iodine, chlorhexidine, alcohol, acetate, hydrogen peroxide, boric acid, silver nitrate, silver sulfadiazine, and sodium hypochlorite[22].

[22]The main rationale for using antiseptics on open wounds is prevention and treatment of infection and, therefore, increased rate of the healing process. It is established that infections may delay healing, cause failure of healing, and even

cause wound deterioration. Microbial pathogens delay wound healing through several different mechanisms, such as persistent production of inflammatory mediators, metabolic wastes, and toxins, and maintenance of the activated state of neutrophils, which produce cytolytic enzymes and free oxygen radicals. This prolonged inflammatory response contributes to host injury and delays healing. Moreover, bacteria compete with host cells for nutrients and oxygen necessary for wound healing.javascript:newshowcontent('active','references'); Wound infection can also lead to tissue hypoxia, render the granulation tissue hemorrhagic and fragile, reduce fibroblast number and collagen production, and damage re-epithelization. Consequently, although creation of an optimal environment for the wound healing process is currently the primary objective of wound care, addressing infection still plays a critical role in wound management.

[22]Since the first discovery of the natural element iodine in *1811* by the chemist *Bernard Courtois*, iodine and its compounds have been broadly used for prevention of infection and treatment of wounds.[22] However, molecular iodine can be very toxic for tissues, so formulations composed by combination of iodine with a carrier that decreases iodine availability were developed. Povidone iodine (PVP-I) results from the combination of molecular iodine and polyvinylpyrrolidone. Povidone iodine is available in several forms (solution, cream, ointment, scrub). The scrub form contains detergent and should be used only on intact skin.

[22]Two official guidelines have been released recently concerning antiseptic use on wounds. Povidone iodine has been approved by Food and Drug Administration (FDA) - for short-term treatment of superficial and acute wounds. The statement includes that povidone iodine has not been found to either promote or inhibit wound healing. On the other hand, guidelines for the treatment of pressure ulcers by the US Department of Health and Human Services, strongly discourage the use of antiseptics and promote the use of normal saline for cleansing pressure ulcers.

This study also revealed the inability of the antiseptic to promote healing. The antimicrobial activity was well observed but delay in healing of wounds in control group clearly demonstrated the limitations of the drug as a *'wound healing promoting agent'*.

All the other antiseptics are having more or less same mechanism as that of povidone iodine. Cytotoxity is the major issue of concern related to these antiseptics. The strongest argument against the use of antiseptics on wounds is that antiseptics have been found, primarily using *in-vitro* models, to be cytotoxic to cells essential to the wound healing process, such as fibroblasts, keratinocytes, and leukocytes.[22] However, this cytotoxicity appears to be concentration dependent, as several antiseptics in low concentrations are not cytotoxic, although they retain their antibacterial activity *in vitro.*[22]

A second reason against the use of antiseptics on open wounds, as first stated by *Fleming in 1919*, is that antiseptics are not as effective against bacteria

invading wounds as they are against bacteria *in vitro.* The presence of exudate, serum, or blood seems to decrease their activity. It was observed in the control group that the wounds which were having serous discharge showed less granulation tissue formation and delayed healing. In this particular study, it was observed that *Madhu Sarpi* acted even in the presence of exudates. In fact, the action of honey is increased in presence of exudates and it has been proved in previous trials.

In the present era where the use of not only polyvinyl pyrrolidone Iodine (PVP-1) but also the other antiseptics on wounds is being viewed with skepticism, the combination of *Madhu Sarpi* can answer all the controversies related with topical agents for wound healing. It alone can be used as an antimicrobial agent as well as healing promoting agent in cutaneous wounds

- **References**

1. *Sushruta samhita Chi.1/8*
2. *Sushruta samhita Chi. 1/3*
3. *Sushruta samhita Chi. 1/130*
4. *Sushruta sutrasthana 45/96*
5. *Sushruta sutrasthana 45/132*
6. *Cha. Su. 27/244, 5*
7. *Ashtang Sangrha sutrasthana 25*

8. *Sushruta samhita Chi. 3/14*

9. *Cha. Chi. 25/65*

10. *As. Hr. 26/29*

11. Waikato Honey Research Unit, published on official website of The University of Waikato, Department of Biological Science.

12. Honey based wound ointment for wound healing and skin disorders with animals, *Dr. Tineke Creemers and Dr. Willem Jan Bosma* , Published in *Dier en Arts Animal and Veterinary, April 2006.*

13. Comparative Study of Therapeutic Effects of Honey and Povidone Iodine in Surgical Wound Healing in Rabbit. Sakhavar N, Khadem N, Published in Shiraz *E-Medical Journal Vol. 9, No. 4, October 2008*

14. Effects of aqueous plant extract mixtures in combination with honey on wound healing in rats: an animal model, International journal of molecular medicine and *advance sciences 2 (1) 61-66, 2006 Med well online 2006*

15. 34th annual European society for dermatological research-ESDR Meeting The Effects of Topical Application of Honey on Wound Healing in Mice: An Experimental Study, Abstract number: 42 Ghaderi R, Afshar M)

16. Acceleration of wound healing by aqueous extract of allium sativum in combination with honey on cutaneous wound healing in rats. International journal of molecular medicine and advance sciences 2 (2) 231-35, 2006 Med well online 2006

17. Effect of a combination of medium chain triglycerides, linoleic acid, soy lecithin and vitamins A and E on wound healing in rats

    Maria Sonia Felício Magalhães; Francisco Vagnaldo Fechine; Rafael Nogueira de Macedo; Diego Levi Silveira Monteiro; Cecília Carvalho Oliveira; Gerly Anne de Castro Brito; Maria Elisabate Amaral de Moraes; Manoel Odorico de Moraes ,Federal University of Ceará (UFC), Brazil published on world wide web.

18. Chitosan and Alginate Wound Dressings: A Short Review
Willi Paul and Chandra P. Sharma, Trends Biomater. Artif. Organs, Vol 18 (1), pp 18-23 (2004) http://www.sbaoi.org

19. Ashtang hridaya chi. 27/48

20. Sushruta sutrasthana 12/13

21. Using honey in wound care, P. C. Molan, Professor of Biological Sciences, and Director of the Honey Research Unit, Department of Biological Sciences, University of Waikato, Hamilton, New Zealand.

22. Antiseptics on Wounds: An Area of Controversy Anna Drosou, MD, Anna Falabella, MD, Robert S. Kirsner, MD;Published: 06/11/2003 in wounds.

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SUMMARY

The study was initiated to evaluate the efficacy of *Madhu Sarpi* which is most appreciated drug in *Ayurveda.* The drugs are in use from centuries in isolated forms. As they are effective individually, the combined use of both was not paid much attention. In our knowledge, this combination has not been tested on humans in a scientific way. To establish it as a local applicant for fresh wounds this clinical trial was undertaken.

In *Ayurveda*, drug has been advocated in almost all types of fresh wounds and burns. Taking these references, the work was actually started with chemical analysis of both the ingredients, to use their standardized forms. *Madhu (Floral honey)* was used, available in the local market. It was tested for any adulteration. Second ingredient of the combination, *Sarpi (Ghee)*, was prepared by the Author himself according to the method described in the classical *Ayurvedic* texts. The composition of both ingredients was according to national and international standards.

The standards were maintained on modern as well as *Ayurvedic* grounds. The author has himself conducted the animal trial in National Toxicology Centre, Pune, on rats and mice. The results were encouraging. Histo-pathological study of the wounds clearly revealed the effectiveness of *Madhu Sarpi* over other topical applicants including local antiseptics. No systemic toxicity was found in the histo-pathological study of animals after they were sacrificed. Considering the non toxicity and effectiveness of *Madhu Sarpi*, it was decided to conduct a clinical trial. Hence the proposal for human trial was forwarded to the Institutional Ethical Committee. The trial was approved by Institutional Ethical Committee (IEC).

In all, the study was conducted on sixty patients. The patients were divided in two groups i.e. Experimental and Control. Experimental group was treated using the drug *Madhu Sarpi* as local application without any prior processing of the drug. *Madhu Sarpi* is a formulation containing Honey *(50%)* and clarified butterfat i.e. *Ghee (50%)* as its constituents. After studying the literature, both Modern and *Ayurvedic,* of *Madhu (Honey) & Sarpi (Ghee)* and their probable mechanism of action, it was decided to use cotton gauze soaked in *Madhu Sarpi* for local application. The drug was not used directly in the liquid form or any other forms like pouring on the wounds. Polyvinyl pyrrolidone iodine (PVP1) was used in control group. For better comparison, the PVP1 was also used in the form of cotton gauze soaked in the drug. The patients were selected strictly according to the selection criteria. The assessment criteria were finalized using standard and universal methods. Follow up was taken up to the healing of the wound or maximum fifteen days, whichever is earlier. Observations were noted in the CRF of the patient at each follow up.

The data collected after completion of the study and were analyzed using appropriate statistical methods. Some patients who were not regular with their follow up or those who left the trial during the study were excluded from the study in both groups. As the process of healing varies with the wounds on face, head (scalp) and neck, they were excluded from the study. No antibiotic - oral, par-enteral or local – or any other type of supplementary treatment was given to any patient during the study. No surgical debridement was done in any patient. No adverse effects were observed in any patient in both groups.

In both groups the maximum number of patients was in the age group of *13 to 50 yrs.* Males were dominant in both groups. Almost all the wounds were found on upper and lower extremities.

Relatively prolonged inflammatory phase was observed in the control group compared to the test group. The anti inflammatory effect of *Madhu Sarpi* was remarkable. Anti microbial activity of *Madhu Sarpi* is comparable to Povidone iodine as no wound was infected in the test group.

Analgesic effect in the test group was significant compared to control group. Though tolerable, the incidence of pain was seen more in the control group. Pain was seen in the cases in which inflammation was present even after local treatment. It can be said that the pain was due to inflammation. The analgesic effect of the drug is due to its anti inflammatory action. The soothing effect of the drug was observed in this trial.

Discharge was reduced significantly in the test group. The wounds dried early and thick bed of granulation tissue was observed. Whereas, discharge of serous type, increased in a few cases of control. The cleansing action of *Madhu Sarpi* was comparatively better than PVP1.

Maximum wounds were of size *4-6 sq. cms* in both groups. Decrease in the wound size as observed on the 7^{th} *day*, was significant in the test group. Rapid wound contraction, epithelialization and granulation tissue formation were observed in the test group. Early resolution of inflammation and discharge contributed in rapid healing. Relatively poor healing dimensionally as well as qualitatively was observed in the control group.

The overall healing rate in test group was much higher than that of control group. *80 %* wounds healed within a period of *6-10* days in test group whereas only *50 %* wounds healed in the same period in control group. This action of rapid healing is a result of multidimensional properties of *Madhu Sarpi*.

Scar quality in the test group was better than control group. The discoloration was minimal and good cosmetic effect was observed.

The main action of *Madhu Sarpi*, *Kshato Ushmano Nigraha*, is comparable to the resolution of acute inflammation which occurs immediately after injury. *Rakshoghna* property of *Madhu Sarpi* is comparable to Anti microbial effect which has been proved. The actions described in *Ayurveda* like *Shodhana, Ropanaa and Sandhana* were proved on the modern parameters also. These actions are comparable to cleansing, healing and approximating the cut edges respectively. *Savarnikara* effect of *Madhu Sarpi* comparable to good cosmetic effect was observed after treatment.

The remedies described in *Ayurveda*, the ancient Indian Medical Science, are having miraculous results. This study on *Madhu Sarpi* is a very small effort to prove the greatness of this science. But practically this remedy is not used by most of the practitioners. It is expected after this study that it will attract the attention of practitioners of *Ayurvedic* science as well as Modern science. As no drug exists today in such a versatile form, it will emerge as a useful *'wound healing promoting agent'* in the management of fresh wounds.

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Comparison of Madhu Sarpi with the topical agents in common use for wound healing

Action	Madhu sarpi	Povidone iodine	Chlor hexidine	Hydrogen peroxide	Silver compounds
Anti microbial action	Present	Present	Present	Absent	Present
Anti inflammatory action	Present (Antimicrobial & anti-inflammatory factors)	Due to antibacterial action only	Due to antibacterial action	Absent	Due to antibacterial action
Cleansing	Present	Absent	Absent	Present	Absent
Wound contraction	Present	Absent	Absent	Absent	Absent
Granulation tissue formation	Present	Absent	Absent	Absent	Absent
Epithelialisation	Present	Absent	Absent	Absent	Absent
Analgesic action	Present	Absent	Absent	Absent	Absent
De-odorizing	Present	Absent	Absent	Absent	Absent
Burning sensation on applicaton	Absent	Absent	Absent	Present	Absent
Anti oxidant	Present	Absent	Absent	Absent	Absent
Moist environment	Present	Absent	Absent	Absent	Absent
Cost	Low	High	High	Low	High

CONCLUSION

The topical application of honey and ghee to wounds originated with ancient civilizations. The effect of *Madhu Sarpi* appeared to have several important properties that make it ideal as a dressing agent for wounds. It can be suggested that it may be possible to use *Madhu Sarpi* as topical application for the treatment of wounds. It accelerates healing significantly. This drug possesses certain anti microbial, anti inflammatory, analgesic and anti oxidant effects. The quality of healing is better with minimum scar formation and minimum discoloration giving better cosmetic results.

Both the ingredients are easily available in India, being the part of every Indian kitchen. They are cost effective compared to the various products used today for wound management.

Therefore the drug is beneficial for rapid and quality healing of the wound. However further study with newer investigative aids and with large samples are required to elucidate their exact mechanism (s) of the wound healing activity.

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BIBLIOGRAPHY

CLASSICAL TEXTS:

1. *Amarkosha : By Amara Simha 1st Edi., Pub. by Chaukhambha Sanskrit Series Varanasi (1970).*
2. *Astanga Hridaya with Sarvangasundara by Arunadatta and Ayurveda Rasayana by Hemadri (H. S. Paradkar) VI Edi., Chaukhambha.*
3. *Astanga Samgraha (AD Athavale), Atreya Prakasana Pune, 1980 with commentary by Indu.*
4. *Astanga Samgraha (Ram Sastri Kinjavadekar) Citrasala Press, Pune, 1940 with commentary by Indu, Part-III.*
5. *Astanga Samgraha, with Tika by Lalachandra Sastri, I-II Volume, Vaidyanatha Prakasana.*
6. *Atharvaveda Samhita : Translated by W. D. Whitney Harward University, Oriental Series, Cambridge (1905).*
7. *Bhagavad Gita As it is by Narmada Goswami, Bhakti Vedanta book Trust, Mumbai.*
8. *Bhavaprakash Nighantu - By Acharya Bhava Mishra with Hindi commentary by K. Chunekar 7th Edi., Chaukhambha, Amara Bharati Academy, Varanasi, 1986.*
9. *Carak Samhita - with Ayurveda Dipika commentary by Cakrapani Datta Chaukhambha Orientalia, Varanasi (1989).*
10. *Caraka Samhita : Vidyotini Hindi Commentary by Kashinath Shastri and Gorakhnath Chaturvedi, 16th Edi., Chaukhambha Bharati Academy (1989).*
11. *Caraka Samhita : with Ayurveda Dipika commentary by Cakrapanidatta and Jalpa Kalpa Taru commentary by Gangadhara Chaukhambha Orientalia Varanasi. 1991.*
12. *Caraka Samhita : with Carakopascara by Yogindra Nath Sen Vol. IIV, J. N. Sen, Calcutta.*

13. *Madhava Nidana : With Madhukosha and Madhusrava by Narendra Sastri, Motilal Banarasidasa (1994).*
14. *Madhava Nidanam with Madhukosa by Vijayraksita and Srikanthadatta (Acharya Y. T.) Vth Edi., Nirnaya Sagar Press, Mumbai, 1955.*
15. *Rigveda Samhita : Translated by A. B. Keith, Harward University Oriental Series, Cambridge (1920).*
16. *Sarangadhara Samhita : By Acharya Sharangadhara Bhisagvara, Adhamalla*

    Virachita Deepika ,Commentary and Pandita Kashirama Vaidya, Pranita Gudhartha, Deepika Commentary (Sanskrit) by Krishnadas Academy, Varanasi.

17. *Sushruta Samhita : Ayurveda Tatwa Sandipika Hindi Commentary by\ Ambikadatta Sastri, 6th Edi., Chaukhambha Sanskrit Samsthana (1986).*

18. *Sushruta Samhita : with Nibandha Samgraha Commentary by Dalhana and the Nyaya Candraka Panjika of Sri Gaya Dasacarya on Nidana Sthana, Chaukhambha Orientalia, Varanasi. (1986).*

19. *Susruta Samhita : Ayurveda Rasayana Dipika, Hindi Commentary by B.G.Ghanekar. Sutra, Nidana, Sarira Meharchand Lachamandas Pub. New Delhi. (1986).*

20. *Yajurveda Samhita : Translated by A. B. Keith, Harward University Oriental Series, Cambridge (1914).*

REFERENCE BOOKS

1. Baly and Love's Short Practice of surgery, 25th Edition Textbook of Surgery, S. Das,
2. API Medicine editor in chief G. S. Sainani, 4th Eti., 1997.
3. Bhagawan Dash: Concept of Agni in Ayurveda Chaukhambha Amarbharati.

4. Text book of Pathology: 2nd Edi. Harsh Mohan, Jaypee.
5. Textbook of surgery, S. Das, 5th edition.

SABDA KOSHAS

1) *Ayurvediya Sabdakosa (Sanskrit - Sanskrit), Vol. I to II, V. M. Joshi and N. H. Joshi, Mumbai. 1968.*
2) *Amarakosa, by Amarsimha, IInd Edition, 1976.*
3) *Sanskrit English Dictionary, V. S. Apte, Motilal Banarasidas, IIIrd Edition, 1965.*
4) *Sanskrit - English Dictionary, Monier Williams, The Clarendon Press, Oxrord, 1951.*
5) *Sabdakalpadrum, Rajaradhakanta Deva (Vasu H. C.), IIIrd Edition, Chaukhambha Sanskrit Office, Varansi, 1967.*
6) Tabers Cyclopedic Medical Dictionary, Jaypee Brothers, New Delhi.

7) Blakiston's New Gold Medical Dictionary

THESIS REFERRED:

A clinical and experimental study on Karpoor Ghrita in the management of Vrana, by Dr. H.S. Shah, submitted to Gujrat Ayurved University, Jamanagar,2002.

Other books

1. Ayurveda Ka Brihat Itihasa, Atrideva Vidyalankara, Hindi Samitj, Granthamala, 1976.
2. Ayurveda Ka Itihas, Acarya Priyavat Sharma, 4th Edition, Chaukhambha Oriyantaliya, Varanasi.
3. Methods in Biostatistics - B. K. Mahajan, Jaypee Brother, New Delhi, 5th Edition.
4. Davidson's Principles and Practice of Medicine, Edited by Edwards, Boucher, 16th Edition.

5. Essentials of Medical Pharmacology, K. d. Tripathi. Fourth edition.
6. Principles of Anatomy and Physiology, Tortora, Grabowski, 7th Edition.
7. Researches in Ayurveda, Dr. M. s. Baghel, Mridu Publication, Jamnagar, 1997.

Articles referred

2. Dr.H.M.sharma, Indian Journal of Clinical Practice, Vol.1No.2 July 1990.

3. Zumla A, Lulat A. Honey--a remedy rediscovered. *J R Soc Med* 1989; **82**(7): 384-5.

4. Molan PC. The role of honey in the management of wounds. *J Wound Care* 1999; **8**(8): 415-8.

5. Molan PC. The antibacterial activity of honey. Part 1. Its use in modern medicine. *Bee World* 1992; **80**(2): 5-28.

6. Cooper RA, Molan PC, Harding KG. The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds. *J Appl Microbiol* 2002; **93**(5): 857-63.

7. Cooper RA, Halas E, Molan PC. The efficacy of honey in inhibiting strains of Pseudomonas aeruginosa from infected burns. *J Burn Care Rehabil* 2002; **23**(6): 366-70.

8. Cooper RA, Wigley P, Burton NF. Susceptibility of multiresistant strains of Burkholderia cepacia to honey. *Lett Appl Microbiol* 2000; **31**(1): 20-4.

9. Descottes B. De la ruche a l'hospital ou l'utilisation du miel dans l'unité de soins. L'abeille de France et l'apiculture 1990(754):459-460.

10. Burlando F. Sull'azione terapeutica del miele nelle ustioni. *Minerva Dermatol* 1978; 113:699-706.

11. Blomfield R. Honey for decubitus ulcers. *J Am Med Assoc* 1973; 224(6):905.

12. Bergman A, Yanai J, Weiss J, Bell D, David MP. Acceleration of wound healing by topical application of honey. An animal model. *Am J Surg* 1983; 145:374-376.

13. Hamdy MH, El-Banby MA, Khakifa KI, Gad EM, Hassanein EM. The antimicrobial effect of honey in the management of septic wounds, Fourth International Conference on Apiculture in Tropical Climates, Cairo, 1989. International Bee Research Association, London.

14. MS Charde, SV Fulzele, PM Satturwar, SB Joshi, AV Kasture, Indian journal of pharmaceutical science, 2006 Vol: 68, Issue 1,Page 26-31.

15. Market Analysis, in Advanced Wound Care Biologics: World Market Analysis, Chapter 6, PJB Medical Publications Inc. 2002

16. *Honey as an Antimicrobial Agent*, *Waikato Honey Research Unit*, November 16, 2006. *Retrieved on* June 2, 2007

17. Typical honey analysis (Ref: *Questions Most Frequently Asked About Sugar*, *American Sugar Alliance*)

18. National Honey Board. "Carbohydrates and the Sweetness of Honey". http://www.honey.com/downloads/carb.pdf.

19. Nutrition Data. "Nutritional Summary for Honey." Last accessed April 14, 2007. http://www.nutritiondata.com/facts-C00001-01c21Ro.html

20. *Martos I, Ferreres F, Tomás-Barberán F (2000). "Identification of flavonoid markers for the botanical origin of Eucalyptus honey". J Agric Food Chem* ***48*** *(5): 1498-502.* PMID 10820049.

21. *Gheldof N, Wang X, Engeseth N (2002). "Identification and quantification of antioxidant components of honeys from various floral sources". J Agric Food Chem* ***50*** *(21): 5870-7.* PMID 12358452.)

22. *Rainer Krell,. Value-Added Products from Beekeeping (Fao Agricultural Services Bulletin). Food & Agriculture Organization of the UN (FA.* ISBN 92-5-103819-8.)

23. Honey based wound ointment for wound healing and skin disorders with animals, *Dr. Tineke Creemers and Dr. Willem Jan Bosma* , Published in *Dier en Arts Animal and Veterinary, April 2006.*

24. Comparative Study of Therapeutic Effects of Honey and Povidone Iodine in Surgical Wound Healing in Rabbit. Sakhavar N, Khadem N, Published in Shiraz *E-Medical Journal Vol. 9, No. 4, October 2008*

25. Effects of aqueous plant extract mixtures in combination with honey on wound healing in rats: an animal model, International journal of molecular medicine and *advance sciences 2 (1) 61-66, 2006 Med well online 2006*

26. 34th annual European society for dermatological research-ESDR Meeting The Effects of Topical Application of Honey on Wound Healing in Mice: An Experimental Study, Abstract number: 42 Ghaderi R, Afshar M)

27. Acceleration of wound healing by aqueous extract of allium sativum in combination with honey on cutaneous wound healing in rats. International

journal of molecular medicine and advance sciences 2 (2) 231-35, 2006 Med well online 2006

28. Effect of a combination of medium chain triglycerides, linoleic acid, soy lecithin and vitamins A and E on wound healing in rats

    Maria Sonia Felício Magalhães; Francisco Vagnaldo Fechine; Rafael Nogueira de Macedo; Diego Levi Silveira Monteiro; Cecília Carvalho Oliveira; Gerly Anne de Castro Brito; Maria Elisabate Amaral de Moraes; Manoel Odorico de Moraes ,Federal University of Ceará (UFC), Brazil published on world wide web.

29. Chitosan and Alginate Wound Dressings: A Short Review
    Willi Paul and Chandra P. Sharma, Trends Biomater. Artif. Organs, Vol 18 (1), pp 18-23 (2004) http://www.sbaoi.org
30. Using honey in wound care, P. C. Molan, Professor of Biological Sciences, and Director of the Honey Research Unit, Department of Biological Sciences, University of Waikato, Hamilton, New Zealand.

Antiseptics on Wounds: An Area of Controversy Anna Drosou, MD, Anna Falabella, MD, Robert S. Kirsner, MD;Published: 06/11/2003 in wounds

■■■■■■

Abbreviations

1) Cha. Su. : Charak Sutrasthana

2) Cha. Chi. : Charak Chikitsasthana

3) Su.Su. : Sushrut Sutrasthana

4) Su.Chi. : Sushrut Chikitsasthana

5) As.San. : Ashtanga Sangraha

6) As.Hr. : Ashtanga Hridaya

7) Sha.Pu.Kh. : Sharangdhar Samhita Purva Khanda

8) Ma.Ni. : Madhava Nidana

9) Va.Su. : Vagbhata Sutrasthana

10) Ut. : Uttarasthana.

■■■■■■

SWAMI RAMANAND TIRTH MARATHAWADA UNIVERSITY,

VISHNUPURI NANDED-431606

Case record form for the study on Management of Saydovrana by Madhusarpi

SCHOLAR - Vd. R. H. Amilkanthwar **GUIDE** - Dr. Lavekar G.S.

Name: Date:

Age: yrs Sex:

Religion: OPD No.:

Occupation: IPD No.:

Address: Contact No.:

Case No.

Clinical features:

- Wound at :

- Wound since Days ☐

Months ☐

☐

- Pain
- Discharge ☐
- Bleeding ☐
- Swelling ☐
- Discoloration ☐
- Itching ☐

H/O Present illness:

H/O past illness:

Family H/O:

Personal H/O:

Bowel- Diet-

Sleep- Addiction-

Habit-

H/o Immunization:

❖ GENERAL EXAMINATION OF PATIENT:

Pulse: / min. Weight:

B.P.: / mm Hg Eyes:

Nails: Skin:

Nourishment:

Weight: Height:

❖ SYSTEMIC EXAMINATION

R.S.

C.V.S.

C.N.S.

❖ LOCAL EXAMINATION

INSPECTION

- Site of wound :
- Size of wound

Length: cms

Width: cms

Depth: cms

Area: sq. cms

- Discharge: Purulent / Sero-purulent / Serous.
- Floor :
- Edge :
- Margin: well defined / ill defined.
- Shape :
- Surrounding skin:
- Regional lymph nodes:

PALPATION

Edge:

Surrounding skin:

Regional lymph nodes:

Type of Vrana:

China ☐ Bhinna ☐ Viddha ☐

Kshata ☐ Picchita ☐ Ghrishta ☐

Adhishtana of Vrana

Twaka ☐ **Mamsa** ☐

Sira ☐ **Snayu** ☐

Asthi ☐ **Sandhi** ☐

☐

Koshtha ☐ **Marma**

Cause of injury:

❖ **INVESTIGATIONS**

Parameters	**Value**	**Unit**	**Comment**
1.Haemoglobin		Gm/dl	
2.Total leukocyte count		/ cubic mm	
3.Differential count Neutrphils		%	
Lymphocytes		%	
Eosinophils		%	
Basophils		%	
Monocytes		%	
4.ESR		Mm in 1st hour	
5.BSL Fasting		Mg/dl	
Post prandial		Mg/dl	
Random		Mg/dl	
6. Urine routine			
7. B.T.			
8. C.T.			
9. Others			

- **WOUND CULTURE**
- **Intervention:** topical application of madhusarpi.
- **OBSERVATIONS**

Day	Pain	Discharge	Inflammation	Size
1st				
2nd				
3rd				
4th				
5th				
6th				
7th				

Complication:

Result:

Research scholar

Vd. R. H.Amilkanthwar

Lecturer, Dept. of Shalya tantra,Dean,

Govt. Ayurved College.

Nanded.

Guide

Dr. Lavekar G.S.

Govt. Ayurved College,

Nanded.

Master chart (Experimental group)

Sr. No.	Name	Age/sex	**Date**	IPD/OPD	Wound at	Pain			
						1st	3rd	7th	15th
1	***Ravindra Ingale***	***27/M***	***27/02/08***	***3228***	***Rt. Fore -arm***	---	---	---	---
2	***Pradip Lokhnde***	***32/M***	***27/02/08***	***3288***	***Lt. fore -arm***	+	---	---	---
3	***Deepa Parmar***	***20/F***	***10/03/08***	***3875***	***Lt. leg***	+++	+	---	---
4	***Sonali Vidhate***	***15/F***	***03/04/08***	***5302***	***Rt. For earm***	+++	++	---	---
5	***Rahul Adsul***	***19/M***	***05/04/08***	***5438***	***Rt. leg***	---	---	---	---
6	***Sudarshan Chavan***	***23/M***	***11/04/08***	***5832***	***Rt. leg***	---	---	---	---
7	***Mahadev Valake***	***27/M***	***23/04/08***	***6413***	***Rt. Elbow***	---	---	---	---
8	***Sandip Pujari***	***19/M***	***03/05/08***	***7046***	***Lt. leg***	---	---	---	---
9	***Vasant Yadav***	***65//F***	***14/05/08***	***7609***	***Rt. Ankle***	---	---	---	---
10	***Sushila Ingale***	***60/M***	***22/05/08***	***8336***	***Rt. Leg***	---	---	---	---
11	***Amol Vidhate***	***22/M***	***20/07/07***	***14956***	***Rt. Leg***	---	---	---	---
12	***Ganesh Sontakke***	***27/M***	***23/0708***	***15168***	***Rt. Foot***	+	---	---	---
13	***Dhanaraj Patil***	***33/M***	***28/07/07***	***15554***	***Lt. forearm***	---	---	---	---
14	***V.V. Gholap***	***48/M***	***10/08/07***	***16495***	***Lt. Toe***	+	---	---	---

15	***Vitthal Karhale***	***20/M***	***28/08/07***	***17723***	***Lt. foot***	---	---	---	---
16	***Shubhangi Salunke***	***14/F***	***12/09/07***	***18787***	***Lt. Palm***	+++	+	---	---
17	***Yashoda Ranadive***	***40/F***	***22/09/07***	***19402***	***Lt. leg***	++	---	---	---
18	***Dada Chandak***	***19/M***	***19/10/07***	***21329***	***Rt. Forearm***	++	---	---	---
19	***Prashant Bondre***	***16/M***	***16/11/07***	***22997***	***Lt. Forearm***	+	---	---	---
20	***Abdul Shaikh***	***15/M***	***15/12/07***	***24591***	***Lt. Forearm***	+++	++	---	---
21	***Rekha Gundagire***	***20/F***	***04/10/08***	***17201***	***Lt. foot***	---	---	---	---
22	***Ashok Gajare***	***50/M***	***14/10/08***	***17670***	***Lt. foot***	---	---	---	---
23	***Ganesh Panchal***	***14/M***	***23/10/08***	***18503***	***Rt. Arm***	+	---	---	---
24	***Bharat Warkad***	***40/M***	***04/11/08***	***19123***	***Rt. Index finger***	---	---	---	---
25	***Shrimant Garad***	***26/M***	***23/11/08***	***20361***	***Lt. little finger***	---	---	---	---
26	***Suhas Shalu***	***32/M***	***07/09/06***	***2209 (IPd)***	***Rt. Upper arm***	+	---	---	---
27	***Asha Tike***	***20F***	***08/09/06***	***24810***	***Lt.leg dorsal***	+	---	---	---
28	***Shripati Tawade***	***44M***	***25/09/06***	***27002***	***Rt. Leg lateral***	+	---	---	---
29	***Tejas Karwal***	***24M***	***26/09/06***	***27270***	***Lt. Forearm***	+	---	---	---
30	***Manmath Pawar***	***21M***	***20/10/06***	***29432***	***Rt. Palm***	+	---	---	---

Sr.	**Discharge**				**Inflammation**				**Rate of healing**		
no.	**1st**	**3rd**	**7th**	**15th**	**1st**	**3rd**	**7th**	**15th**	**Area**	**Days**	**Rate**
1	---	---	---	---	---	---	---	---	3*1.5*.5	6	0.75

2	+	---	---	---	---	---	---	---	2*1.5*.5	7	0.42
3	+++	++	---	---	---	---	---	---	2.5*2*1	12	0.375
4	+++	++	---	---	+	---	---	---	2.5*1.5*1	14	0.267
5	---	---	---	---	---	---	---	---	2*2*.5	4	1.0
6	+	---	---	---	---	---	---	---	3*2*.5	6	1.0
7	---	---	---	---	---	---	---	---	2.5*2.5*.5	6	1.041
8	+	---	---	---	---	---	---	---	3*2*.75	8	0.75
9	+	---	---	---	+	---	---	---	2.5*1*1	7	0.357
10	+	---	---	---	+	+	---	---	2.5*2.5*1	6	1.041
11	+	---	---	---	---	---	---	---	3*2*.5	8	0.75
12	++	---	---	---	---	---	---	---	3*2*.75	7	0.85
13	---	---	---	---	---	---	---	---	3*2*.5	3	2
14	+	---	---	---	+	+	---	---	1*1*.5	8	0.125
15	---	---	---	---	---	---	---	---	2*1*.5	2	1
16	+++	++	---	---	---	---	---	---	2*2*.5	11	0.36
17	++	+	---	---	+	+	---	---	2*2*1	6	0.67
18	++	+	---	---	---	---	---	---	3*1.5*1	13	0.35
19	+	---	---	---	---	---	---	---	2*2*1	7	0.57
20	++	+	---	---	---	---	---	---	2.5*2.5*1	12	0.52
21	+	---	---	---	---	---	---	---	2.5*1*.5	7	0.36
22	+	---	---	---	---	---	---	---	2*1*.75	8	0.25
23	+	---	---	---	---	---	---	---	3*2*.5	8	0.75
24	+	---	---	---	---	---	---	---	2*2*.5	7	0.57
25	+	---	---	---	---	---	---	---	2*1*.5	6	0.33
26	+	---	---	---	---	---	---	---	2.5*1*.75	7	0.36
27	---	---	---	---	---	---	---	---	2*1*.5	7	0.29
28	+	---	---	---	+	+	---	---	3*1*1	9	0.33
29	---	---	---	---	---	---	---	---	2*1.5*.5	6	0.50
30	+	---	---	---	---	---	---	---	2*2*.75	8	0.50

Master chart (Control group)

Sr. No.	Name	Age/sex	**Date**	IPD/OPD	Wound at	Pain			
						1st	3rd	7th	15th
1	***Vijaya Chougale***	***14/M***	***28/02/08***	***3322***	***Left Leg***	***+***	***+***	***---***	***---***
2	***Sachin Sakhare***	***24/M***	***03/03/08***	***3435***	***Rt. Thigh***	***+***	***---***	***---***	***---***
3	***Akanksha Upare***	***20/ F***	***14/03/08***	***4239***	***Lt. foot Dorsal Aspect***	***---***	***---***	***---***	***---***
4	***Mahadev Suryavanshi***	***19/M***	***17/03/08***	***4351***	***Rt. Forehead***	***---***	***---***	***---***	***---***
5	***Anjana Kumbhar***	***47/F***	***01/04/08***	***5196***	***Rt. Great toe***	***---***	***---***	***---***	***---***
6	***Ram Jadhav***	***21/M***	***07/04/08***	***5471***	***Right Leg***	***---***	***---***	***---***	***---***
7	***Vikas Lokhande***	***18/M***	***10/04/08***	***6763***	***Right Leg***	***+***	***---***	***---***	***---***
8	***Ashwin Chavan***	***20/M***	***07/05/08***	***7262***	***Left Leg***	***---***	***---***	***---***	***---***
9	***Viabhav Vidhate***	***19/M***	***12/05/08***	***7619***	***Left for-arm***	***++***	***+***	***++***	***+***
10	***Samadhan Tambe***	***21M***	***28/05/08***	***8684***	***Rt Little finger***	***---***	***---***	***---***	***---***
11	***Deepali Kadam***	***13/F***	***23/07/07***	***15108***	***Lt. knee***	***+++***	***++***	***+***	***---***
12	***Suresh Sarawde***	***35/M***	***20/07/07***	***15299***	***Rt. Foot***	***---***	***---***	***---***	***---***
13	***Vitthal pawar***	***55/M***	***06/08/07***	***16171***	***Lt. elbow***	***---***	***---***	***---***	***---***

14	***Latabai Pawar***	***31/F***	***24/08/07***	***17439***	***Rt. Middle Finger***	***+++***	***++***	***++***	***+***
15	***Wahid shaikh***	***22/M***	***01/09/08***	***18014***	***Lt. foot dorsal***	***+***	***+***	***---***	***---***
16	***Shivadas Waghamare***	***15/M***	***06/09/07***	***18407***	***Lt. index finger***	***+***	***+***	***+***	***---***
17	***Shahid shaikh***	***37/M***	***27/09/07***	***19786***	***Rt. Wrist***	***+++***	***++***	***++***	***+***
18	***Kedar shinde***	***16/M***	***01/10/07***	***20062***	***Rt. Thumb***	***+***	***+***	***+***	***---***
19	***Sarubai Kore***	***40/F***	***01/11/07***	***22239***	***Rt. Thumb***	***+***	***+***	***---***	***---***
20	***Vishwas More***	***19/M***	***05/12/07***	***24105***	***Lt. index finger***	***---***	***---***	***---***	***---***
21	***Sushil Jadhav***	***17/M***	***10/10/08***	***17503***	***Rt. Sole***	***++***	***++***	***++***	***+***
22	***Suraj Kadam***	***20/M***	***18/10/08***	***18138***	***Lt. thumb***	***---***	***---***	***---***	***---***
23	***Balasaheb Lagadive***	***19/M***	***04/11/08***	***19050***	***Rt.arm***	***---***	***---***	***---***	***---***
24	***Vaijnath Bhure***	***16/M***	***07/11/08***	***19608***	***Rt. Ankle***	***+***	***---***	***---***	***---***
25	***Mahesh Gangane***	***25/M***	***21/11/08***	***20056***	***Rt. Ring Finger***	***---***	***---***	***---***	***---***
26	***Sahadev Kamble***	***37M***	***31/10/06***	***30042***	***Rt.wrist Dorsal***	***---***	***---***	***---***	***---***
27	***Bhimarao Lagadive***	***35M***	***07/11/06***	***30251***	***Left upper arm***	***---***	***---***	***---***	***---***
28	***Machhindra Munde***	***65M***	***17/10/06***	***29042***	***Rt.leg lat aspect***	***---***	***---***	***---***	***---***
29	***Rohit Kale***	***17M***	***02/12/06***	***33401***	***Left forearm dorsal***	***++***	***++***	***+***	***---***
30	***Sakharam Korale***	***22M***	***03/12/06***	***33470***	***Rt.forearm medial***	***---***	***---***	***---***	***---***

Sr.	Discharge				Inflammation				Rate of healing		
no.	1st	3rd	7th	15th	1st	3rd	7th	15th	Area	Days	Rate
1	---	---	---	---	---	---	---	---	***2*2*0.5***	***6***	***0.66***
2	---	+	+	---	---	---	---	---	2*2.5*0.5	7	0.71
3	---	---	---	---	+	---	---	---	2*1.5*1	6	0.50
4	---	+	---	---	+++	++	++	+	1.5*1.5*.5	3	0.75
5	+	++	+	---	+++	++	++	+	2*1.5*0.5	6	0.50
6	---	---	---	---	---	---	---	---	3*1*0.5	8	0.37
7	---	+	---	---	+	---	---	---	1.5*1*1	4	0.37
8	---	---	---	---	---	---	---	---	3.5*2.5*1	11	0.79
9	---	---	---	---	+	---	---	---	4*2*0.5	12	0.66
10	+	+	+	---	+	---	---	---	2.5*2*1	8	0.625
11	+	++	+	---	+	---	---	---	2*2*1	8	0.50
12	---	---	---	---	+	---	---	---	3.5*2.5*0.5	13	0.67
13	---	+	+	---	++	+	+	---	1.5*1*0.5	12	0.125
14	---	---	---	---	---	---	---	---	4*1.5*0.5	14	0.428
15	+	+	+	---	+	+	+	---	2.5*2.5*0.5	12	0.520
16	---	---	---	---	---	---	---	---	***2*2*0.5***	***6***	***0.66***
17	---	---	---	---	+++	+++	++	+	***2.5*2*.5***	***7***	***0.71***
18	+	++	+	---	++	++	++	+	***3*2*0.5***	***6***	1.00
19	---	---	---	---	++	+	+	---	***3.5*1.5*0.5***	***6***	***0.875***
20	---	---	---	---	+	---	---	---	***1.5*.5*.5***	***9***	***0.083***
21	---	---	---	---	++	+	---	---	***3.5*2*.5***	***12***	***0.58***
22	---	---	---	---	+	---	---	---	***2..5*2*.5***	***11***	0.46
23	---	---	---	---	+	---	---	---	***2*2.5*0.5***	***13***	***0.38***
24	---	---	---	---	+	---	---	---	***3.5*2.5*1***	***14***	0.625
25	---	---	---	---	+	+	---	---	***3*2*0.5***	***12***	***0.50***
26	---	+	+	---	+	---	---	---	***1*1*0.5***	***11***	***0.090***
27	---	+	+	---	+	+	+	---	***2*2*0.5***	***14***	*0.29*
28	---	---	---	---	---	---	---	---	***3.5*2*1***	***9***	***0.78***
29	+	++	+	---	+	+	---	---	***2.5*2.5*0.5***	***13***	0.48
30	---	---	---	---	---	---	---	---	***4*2.5*1***	***13***	0.769

Annexure

ATREYA PRATISHTHAN

Atreya Sankul Plot No 39, Gut No 72, Deolai Parisar. Aurangabad -5

Ph – 0240 – 2653131, Web – Jeevanjyoti.net,

E-mail – jeevanjyoti5204@rediffmail.com

Antibacterial activity of Honey Swami samrtha

Sample	Anti bacterial activity against	Zone of inhibition
Honey	E.Coli	No ZOI
	Psedomonas	No ZOI
	Salmonella	No MZI ? probiotic
	S.aureus	No ZOI

Dose 1mg/ml

Sample Provided by

Dr.Amilkanthwar

25/09/06

Dr.M.S.Sabnis

M.D.Ras shastra

M.Sc Micro

Mrs.Manjiri Kulkarni

Annexure

Sample	Anti bacterial activity against	Zone of inhibition
Honey	E.Coli	No ZOI
	Psedomonas	No ZOI
	Salmonella	No MZI
	S.aureus	No ZOI

ATREYA PRATISHTHAN

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Ph – 0240 – 2653131, Web – Jeevanjyoti.net,

E-mail – jeevanjyoti5204@rediffmail.com

Antibacterial activity of Honey Gurukul

Dose 1mg/ml

Sample Provided by

Dr.Amilkanthwar

25/09/06

Annexure

Dr.M.S.Sabnis Mrs.Manjiri Kulkarni

M.D.Ras shastra

M.Sc Micro

ATREYA PRATISHTHAN

Atreya Sankul Plot No 39, Gut No 72, Deolai Parisar. Aurangabad -5

Ph – 0240 – 2653131, Web – Jeevanjyoti.net,

E-mail – jeevanjyoti5204@rediffmail.com

Date 24/09/06

Sodium , Potassium and calcium estimation of samples of Honey

S.No	Code of Sample	Na	K	Ca
1	Honey Gurukul	355 Meq/L	965 Meq/L	3.5 Meq/L
2	Honey Swami samartha	430 Meq/L	580 Meq/L	6.5 Meq/L

Sample provided by Dr.Amilkanthwar

Dr.M.S.Sabnis

Mr.Shakil Shaikh

M.D Ras shastra

M.Pharma

ATREYA PRATISHTHAN

Atreya Sankul Plot No 39, Gut No 72, Deolai Parisar. Aurangabad -5

Ph – 0240 – 2653131, Web – Jeevanjyoti.net,

Annexure

E-mail – jeevanjyoti5204@rediffmail.com

Date 24/09/06

Spectral study of Honey

Sample provided by

S.No	**Honey Swamisamartha**	Absorbance
1	Wave lengeth 214nm	0.885
2	Wave lengeth 278 nm	0.405
	Honey Gurukul	
1	Wave length 219	0.839
2	Wave length 281	1.165

Dr.Amilkanthwar

Dr.M.S.Sabnis

Mr.Shakil Shaikh

M.D Ras shastra

M.Phar

ATREYA PRATISHTHAN

Atreya Sankul Plot No 39, Gut No 72, Deolai Parisar. Aurangabad -5

Ph – 0240 – 2653131, Web – Jeevanjyoti.net,

E-mail – jeevanjyoti5204@rediffmail.com

Date 24/09/06

Spectral study of

Ghee

Sample by

S.No	**Ghee**	Absorbance
1	Wave lengeth 253nm	7.0
2	Wave lengeth 266 nm	1.72

provided

Dr.Amilkanthwar

Dr.M.S.Sabnis

Mr.Shakil Shaikh

M.D Ras shastra

M.Pharma

ATREYA PRATISHTHAN

Atreya Sankul Plot No 39, Gut No 72, Deolai Parisar. Aurangabad -5

Ph – 0240 –2653131, Web – Jeevanjyoti.net,

E-mail – jeevanjyoti5204@rediffmail.com

Date - 26/09/06

Sample provided by Dr.Amilkanthwar

Ghee	**SPECIFICATIONS**
PARAMETERS	
Identification	
Sodium	1939 Meq/L
Potassium	1135 Meq/L
Calcium	100 Meq/L

Dr.M.S.Sabnis

Mr.Shakil Shaikh

M.D Ras shastra

M.Pharma

ATREYA PRATISHTHAN

Atreya Sankul Plot No 39, Gut No 72, Deolai Parisar. Aurangabad -5

Ph – 0240 –2653131, Web – Jeevanjyoti.net,

E-mail – jeevanjyoti5204@rediffmail.com

Date - 26/09/06

Sample provided by Dr.Amilkanthwar

Ghee	**SPECIFICATIONS**
PARAMETERS	
Identification	
Sponification value	274
Acid value	7.62

Dr.M.S.Sabnis

Mr.Shakil Shaikh

M.D Ras shastra

M.Pharma

Annexure

ATREYA PRATISHTHAN

Atreya Sankul Plot No 39, Gut No 72, Deolai Parisar. Aurangabad -5

Ph – 0240 – 2653131, Web – Jeevanjyoti.net,

E-mail – jeevanjyoti5204@rediffmail.com

Date - 26/09/06

Honey Gurukul	**SPECIFICATIONS**
PARAMETERS	
Identification	
Free acidity	32.4 meq/kg
pH	4.0
Moisture	16.7%
Reducing sugar	83.8 gm%

Annexure

Dr.M.S.Sabnis

Mr.Shakil Shaikh

M.D Ras shastra

M.Pharma

ATREYA PRATISHTHAN

Atreya
Sankul
Plot No
Gut No
Deolai

39,
72,

Honey Sawmi samartha	**SPECIFICATIONS**
PARAMETERS	
Identification	
Free acidity	33.2 meq/kg
pH	4.12
Moisture	17.1 %
Reducing sugar	79 gm%

Parisar. Aurangabad -5

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Date - 26/09/06

Dr.M.S.Sabnis

Mr.Shakil Shaikh

M.D Ras shastra

M.Pharma

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25/09/06

Annexure

Antibacterial activity of Ghee

Dose 1mg/ml Sample

Provided by Dr.Amilkanthwar

Sample	Anti bacterial activity against	Zone of inhibition
Ghee	E.Coli	2mm ZOI
	Psedomonas	minimal ZOI
	Salmonella	No ZOI
	S.aureus	1mm ZOI

Dr.M.S.Sabnis

M.D.Ras shastra

M.Sc Micro

Mrs.Manjiri Kulkarni

Annexure

Dr. Avinash Pradhan (MD (Path). D.C.P.
Consulting Histopathologist
KEM Hospital, Pune

Pradhan Surgical Pathology Laboratory
314, Narayan Peth, Opp. Daily Prabhat
Pune - 411 030 . Tel. 24457487

Histological Changes in Liver and Kidney		
Animal No.	Liver	Kidney
DJM1	Congestion. Mild focal MNC and few polymorphs	Mild cloudy change
DJM2	Sinusoidal congestion and focal haemorrhages	Mild cloudy change
DJM3	Sinusoidal congestion. Mild focal MNC.	Mild cloudy change. Mild congestion
DJF4	Focal MNC and polymorph infiltrate	Severe congestion. Focal haemorrhages and cloudy change
DJF5	Mild focal MNC	Congestion and cloudy change
DJF6	Severe sinusoidal congestion	Mild cloudy change and congestion
NAD -	No abnormality detected	
MNC -	Mononuclear cells	
Conclusion - The above histological changes in liver needs biochemical corelation specially liver function tests.		

Histological Changes in Skin		
Animal No.	Skin	
DJMS1	NAD	
DJMS2	Dermal fibrosis. Epidermal hyperkeratosis with crust formation	
DJMS3	NAD	
DJFS4	Dermal fibrosis. Focal hyperkeratosis	
DJFS5	Mild focal dermal fibrosis	
DJFS6	NAD	
NAD -	No abnormality detected	
MNC -	Mononuclear cells	
Conclusion - The above histological changes in skin in form of dermal fibrosis and epidermal crust formation		

Annexure

Dr. Avinash Pradhan (MD (Path).D.C.P)
Consulting Histopathologist,
Tel. 24457487

Pradhan Surgical Pathology Laboratory
314, Narayan Peth, Opp. Daily Prabhat
Pune 411030

Histological changes in vital organs

Animal Number	Liver	Kidney	Skin
RP2G1-1	NAD	Mild cloudy change	Dermal fibrosis and mononuclear cell infiltration at the interface of dermis and subcutaneous fat
RP2G1-2 T	NAD	Mild cloudy change	
RP2G2-1	Sinusoidal congestion	Mild cloudy change and congestion	Dermal fibrosis and mononuclear cell infiltration at the interface of dermis and subcutaneous fat with foreign body giant cells with foreign body in it
RP2G2-2 B	Sinusoidal congestion	Mild cloudy change and haemorrhages	
RP2G3-1 C	Sinusoidal congestion. MNC around portal tracts	Mild cloudy change	Dermal fibrosis and mononuclear cell infiltration at the interface of dermis and subcutaneous fat
RP2G3-2	Sinusoidal congestion MNC around portal tracts	Mild cloudy change	Dermal fibrosis and mononuclear cell infiltration at the interface of dermis and subcutaneous fat

NAD- No abnormality detected
MNC – Mononuclear cell infiltration
Comment: Changes in the liver and the skin requires correlation.