COMPARISON OF THE EFFECT OF SILVER SULFADIAZINE AND A NEW HERBAL PREPARATION ON THERMAL INDUCED PARTIAL THICKNESS BURNS IN RABBITS WITH AND WITHOUT SUPPORTIVE THERAPY WITH CISSUS QUADRANGULARIS AND HEMIDESMUS INDICUS

THESIS
SUBMITTED FOR THE DEGREE OF

DOCTOR OF PHYLOSOPHY

(MEDICAL PHARMACOLOGY)

FACULTY OF MEDICINE

DATTA MEGHE INSTITUTE OF MEDICAL SCIENCES

DEEMED UNIVERSITY, NAGPUR

BY

Dr. SWANAND S. PATHAK
DEPARTMENT OF PHARMACOLOGY
JAWAHARLAL NEHRU MEDICAL COLLEGE,
SAWANGI (M), WARDHA

2014

DEPARTMENT OF PHARMACOLOGY
JAWAHARLAL NEHRU MEDICAL COLLEGE,
SAWANGI (M), WARDHA

Certificate

Certified that the work embodied in this thesis for the degree of Ph.D. in Medical Pharmacology of Datta Meghe Institute Of Medical Sciences, Nagpur, entitled ***Comparison of the effect of Silver Sulfadiazine and A New Herbal Preparation on thermal induced partial thickness burns in rabbits with and without supportive therapy with Cissus Quadrangularis and Hemidesmus Indicus*** was undertaken by ***Dr. Swanand S. Pathak*** and carried out in department of Pharmacology,J.N.M.C. Sawangi (M), Wardha, under my direct supervision and guidance.

Dr. S. S. Patel
M.D. Pharmacology,
Supervisor and Guide,
Professor,
Department of Pharmacology,
and Chief Co-ordinator,
DMIMSU, Sawangi (M),
Wardha - 442004.
MAHARASHTRA

Wardha

Date:

DEPARTMENT OF PHARMACOLOGY
JAWAHARLAL NEHRU MEDICAL COLLEGE,
SAWANGI (M),
WARDHA

Certificate

Certified that the work embodied in this thesis for the degree of Ph.D. in Medical Pharmacology of Datta Meghe Institute of Medical Sciences (Deemed University) Nagpur, entitled ***"Comparison of the effect of Silver Sulfadiazine and Herbal Preparation on thermal induced partial thickness burns in rabbits with and without supportive therapy with Cissus Quadrangularis and Hemidesmus Indicus"*** was undertaken by ***Dr. Swanand S. Pathak***, Professor & Head, Department of Pharmacology, Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha. We have great pleasure in forwarding this thesis to Datta Meghe Institute of Medical Sciences (Deemed University) Nagpur

(Dr. Sandeep Shrivastava)
Dean
Jawaharlal Nehru Medical College
Sawangi (Meghe), Wardha

COMPARISON OF THE EFFECT OF SILVER SULFADIAZINE AND A NEW HERBAL PREPARATION ON THERMAL INDUCED PARTIAL THICKNESS BURNS IN RABBITS WITH AND WITHOUT SUPPORTIVE THERAPY WITH CISSUS QUADRANGULARIS AND HEMIDESMUS INDICUS

THESIS
SUBMITTED FOR THE DEGREE OF

DOCTOR OF PHYLOSOPHY

(MEDICAL PHARMACOLOGY)

FACULTY OF MEDICINE

DATTA MEGHE INSTITUTE OF MEDICAL SCIENCES

DEEMED UNIVERSITY, NAGPUR

BY

Dr. SWANAND S. PATHAK

DEPARTMENT OF PHARMACOLOGY
JAWAHARLAL NEHRU MEDICAL COLLEGE,
SAWANGI (M), WARDHA

2014

DEPARTMENT OF PHARMACOLOGY

JAWAHARLAL NEHRU MEDICAL COLLEGE,

SAWANGI (M), WARDHA

Certificate

Certified that the work embodied in this thesis for the degree of Ph.D. in Medical Pharmacology of Datta Meghe Institute Of Medical Sciences, Nagpur, entitled ***Comparison of the effect of Silver Sulfadiazine and A New Herbal Preparation on thermal induced partial thickness burns in rabbits with and without supportive therapy with Cissus Quadrangularis and Hemidesmus Indicus*** was undertaken by ***Dr. Swanand S. Pathak*** and carried out in department of Pharmacology, J.N.M.C. Sawangi (M), Wardha, under my direct supervision and guidance.

Dr. S. S. Patel

M.D. Pharmacology,
Supervisor and Guide,
Emeritus Professor,
Department of Pharmacology,
and Chief Co-ordinator, DMIMSU,
Sawangi (M), Wardha - 442004.
MAHARASHTRA

Date:

Place

DEPARTMENT OF PHARMACOLOGY

JAWAHARLAL NEHRU MEDICAL COLLEGE,

SAWANGI (M), WARDHA

Certificate

Certified that the work embodied in this thesis for the degree of Ph.D. in Medical Pharmacology of Datta Meghe Institute of Medical Sciences (Deemed University) Nagpur, entitled **"*Comparison of the effect of Silver Sulfadiazine and A New Herbal Preparation on thermal induced partial thickness burns in rabbits with and without supportive therapy with Cissus Quadrangularis and Hemidesmus Indicus*"** was undertaken by ***Dr. Swanand S. Pathak***, Professor & Head, Department of Pharmacology, Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha. I have great pleasure in forwarding this thesis to Datta Meghe Institute of Medical Sciences (Deemed University) Nagpur

(Dr. Sandeep Shrivastava)

Dean

Jawaharlal Nehru Medical College

Sawangi (Meghe), Wardha

Acknowledgement…

First and foremost I offer my sincerest gratitude to my fatherly supervisor, Dr. S. S. Patel, Professor Emeritus Pharmacology Dept. and Chief Co-ordinator, DMIMS, Sawangi (M), Wardha who has supported me throughout my thesis with his patience and knowledge whilst allowing me the room to work in my own way. I attribute the level of my Doctorate degree to his encouragement and effort and without him this thesis, too, would not have been completed or written. One simply could not wish for a better or friendlier supervisor.

I place my deep sense of gratitude and thanks to Shri. Dattaji Meghe Chancellor DMIMSU and Shri Sagarji Meghe, DMIMSU, Sawangi (M), Wardha for providing all the required facilities in the institution for research work.

I would like to thank Dr. Ved Prakash Mishra, Chief Advisor, DMIMSU for his timely help and encouragement to carry out research. I would like to thank Dr Dilip Gode, Vice Chancellor, DMIMSU for his encouragement to carry out research. I wish to thank Dr. S. Shrivastava, Dean, JNMC, Sawangi (M), Wardha for his kind cooperation.

I wish to express my deep gratitude to Dr. A. J. Anjankar, Vice Dean, JNMC, Sawangi (M) who deserve special thanks as my thesis committee members and advisors for providing his able guidance.

I wish to extend my gratefulness and thanks to Dr. R K Jha, Prof. Pharmacology Dept, JNMC, Sawangi (M),Wardha for his kind cooperation and support at every stage of my research. I would like to show my gratitude to my collogues Dr. Mrs. S. Totade, Dr. Nitin Gaikwad, Dr. Nitin Pise, Dr. Mrs. Asha Jha, Dr. Mrs. Leena Chimurkar, Dr. Sangita Jogdand ,Dr. Shailesh Nagpure for the kind co-operation extended by them.

I must mention two of my very close friends Dr. Mangal Borkar, and Dr. R. R. Kale Assistan Prof., Dept of Pharmacology, JNMC without fail, who provided friendly support not only for this work but also in the ups and downs of my life.

My sincere thanks to Dr. Jitendra Patel , Dr. Chetna Gupta , Dr. Pallavi Nema, Dr. Vaibhav Gupta , Dr. Diptendu Santra , Dr. Sohin Thaim , Dr. Muzammil Khan , Dr. Sachin Khond , Dr. Ankur Bhute , Dr. Arshiya Tabassum, Dr. Mujawar Jahir P.G. Students, Dept. of Pharmacology, for their kind cooperation and valuable help they extended to me during my research work.

I would like to thank Dr. S. Bhutada, Principal, MGACH and RC, Salod (H) and Dr, Anita Wanjari, Assoc. Prof, Rasashastra and Bhaishijya Kalpana Dept, MGACH and RC, Salod (H), for their kind co-operation and provision for uninterrupted supply of freshly prepared Hemidesmus powder throughout my research .

My sincere thanks go to Mr. Bharambe, Librarian, JNMC,Sawangi (M),Wardha for providing all sorts of library facilities of books, periodicals and digital library liberally.

I would like to thank Mr. Brahmankar, A.O. for his support in urgent repairs of equipments and also many other situations.

In my daily work I have been blessed with a friendly and cheerful group of technicians. Deepali Deshpande, Mamta Narkhedkar and attendant Ravi Padole without whom the work would have been a exhausting and disappointing experiment.

I am thankful to Mr. Sunil Rahate, Mrs. Sandhya Kamadi, Late Mr. Ankush Wankhede, Mrs. Sushila Samudre, staff of Pharmacology Department, for their kind cooperation and help.

My deepest gratitude goes to my parents, Mrs. Swati Pathak and Mr. Shrikant Pathak for their unflagging love and support throughout my life, this dissertation is simply impossible without them. I am indebted to my parents for their care, love and support. They are simply perfect.

I have no suitable words to describe the everlasting love of my daughter Swarangi. I remember her constant support when I encountered difficulties and how she has brightened my life.

I deeply appreciate my wife, Dr. Bageshri Pathak for the care, cooperation and encouragement rendered by her.

It is not possible for me to mention name of each relative and friend whose direct or indirect help has contributed to the completion of this study.

Joining JNMC, Sawangi (M) was not only a turning point in my life, but also a wonderful experience. I cherished the prayers and support between me and the people at JNMC, Sawangi (M). I treasured all precious moments we shared and would really like to thank them. Furthermore, I am grateful to come across several life-long friends at work.

Last but not least, thanks to God for my successful life through all tests in the past years. You have made my life more bountiful. May your name be exalted, honored, and glorified forever and for all.

I would like to close with an inspiring quote by Swami Vivekananda

"Those alone live who live for others the rest are more dead than alive"

Dr. Swanand S. Pathak

Dedicated to

All those animals without whom this study was utterly impossible ….

Acknowledgement…

First and foremost I offer my sincerest gratitude to my fatherly supervisor, Dr. S. S. Patel, Professor Emeritus Pharmacology Dept. and Chief Co-ordinator, DMIMS, Sawangi (M), Wardha who has supported me throughout my thesis with his patience and knowledge whilst allowing me the room to work in my own way. I attribute the level of my Doctorate degree to his encouragement and effort and without him this thesis, too, would not have been completed or written. One simply could not wish for a better or friendlier supervisor.

I place my deep sense of gratitude and thanks to Shri. Dattaji Meghe Chancellor DMIMSU and Shri Sagarji Meghe, DMIMSU, Sawangi (M), Wardha for providing all the required facilities in the institution for research work.

I would like to thank Dr. Ved Prakash Mishra, Chief Advisor, DMIMSU for his timely help and encouragement to carry out research. I would like to thank Dr Dilip Gode, Vice Chancellor, DMIMSU for his encouragement to carry out research. I wish to thank Dr. S. Shrivastava, Dean, JNMC, Sawangi (M), Wardha for his kind cooperation.

I wish to express my deep gratitude to Dr. A. J. Anjankar, Vice Dean, JNMC, Sawangi (M) who deserve special thanks as my thesis committee members and advisors for providing his able guidance.

I wish to extend my gratefulness and thanks to Dr. R K Jha, Prof. Pharmacology Dept, JNMC, Sawangi (M),Wardha for his kind cooperation and support at every stage of my research. I would like to show my gratitude to my collogues Dr. Mrs. S. Totade, Dr. Nitin Gaikwad, Dr. Nitin Pise, Dr. Mrs. Asha Jha, Dr. Mrs. Leena Chimurkar, Dr. Sangita Jogdand ,Dr. Shailesh Nagpure for the kind co-operation extended by them.

I must mention two of my very close friends Dr. Mangal Borkar, and Dr. B. P. Kale Assistan Prof., Dept of Pharmacology, JNMC without fail, who provided friendly support not only for this work but also in the ups and downs of my life.

My sincere thanks to Dr. Jitendra Patel , Dr. Chetna Gupta , Dr. Pallavi Nema, Dr. Vaibhav Gupta , Dr. Diptendu Santra , Dr. Sohin Thaim , Dr. Muzammil Khan , Dr. Sachin Khond , Dr. Ankur Bhute , Dr. Arshiya Tabassum , Dr. Mujawar Jahir P.G. Students, Dept. of Pharmacology, for their kind cooperation and valuable help they extended to me during my research work.

I would like to thank Dr. S. Bhutada, Principal, MGACH and RC, Salod (H) and Dr, Anita Wanjari, Assoc. Prof, Rasashastra and Bhaishijya Kalpana Dept, MGACH and RC, Salod (H), for their kind co-operation and provision for uninterrupted supply of freshly prepared Hemidesmus powder throughout my research .

My sincere thanks go to Mr. Bharambe, Librarian, JNMC ,Sawangi (M),Wardha for providing all sorts of library facilities of books, periodicals and digital library liberally.

I would like to thank Mr. Brahmankar, A.O. for his support in urgent repairs of equipments and also many other situations.

In my daily work I have been blessed with a friendly and cheerful group of technicians . Deepali Deshpande, Mamta Narkhedkar and attendant Ravi Padole without whom the work would have been a exhausting and disappointing experiment.

I am thankful to Mr. Sunil Rahate, Mrs. Sandhya Kamadi, Late Mr. Ankush Wankhede, Mrs. Sushila Samudre, staff of Pharmacology Department, for their kind cooperation and help.

My deepest gratitude goes to my parents, Mrs. Swati Pathak. and Mr. Shrikant Pathak for their unflagging love and support throughout my life, this dissertation is simply impossible without them. I am indebted to my parents for their care, love and support. They are simply perfect.

I have no suitable words to describe the everlasting love of my daughter Swarangi. I remember her constant support when I encountered difficulties and how she has brightened my life.

I deeply appreciate my wife, Dr. Bageshri Pathak for the care, cooperation and encouragement rendered by her.

It is not possible for me to mention name of each relative and friend whose direct or indirect help has contributed to the completion of this study.

Joining JNMC, Sawangi (M) was not only a turning point in my life, but also a wonderful experience. I cherished the prayers and support between me and the people at JNMC, Sawangi (M). I treasured all precious moments we shared and would really like to thank them. Furthermore, I am grateful to come across several life-long friends at work.

Last but not least, thanks to God for my successful life through all tests in the past years. You have made my life more bountiful. May your name be exalted, honored, and glorified forever and for all.

I would like to close with an inspiring quote by Swami Vivekananda

"Those alone live who live for others the rest are more dead than alive"

Dr. Swanand S. Pathak

Dedicated to

All those animals without whom this study was utterly impossible ….

I N D E X

LIST OF TABLES

LIST OF TABLES

LIST OF TABLES

LIST OF GRAPHS

LIST OF GRAPHS

LIST OF FIGURES

LIST OF FIGURES

1. INTRODUCTION

The successful treatment of burns is a very recent development. As late as 1939 it was thought that someone with burns over one third of their body would probably die, whilst today we expect to successfully treat someone with 65% burns of total body surface area. This is due to expanding knowledge of the pathophysiology of thermal injury and its systemic consequences, medical technology advances and improved surgical techniques.[1]

Until recently the majority of topical treatments were obtained from nature, plant or animal byproducts or mineral/chemical agents. Amongst physicians there were two schools of topical treatment, those who sought to dry the wound and promote the formation of scabs and those who saw greater benefit in keeping the wound moist with ointments and poultices.[2]

Curatives such as lard, honey, milk, butter and eggs have been widely used since ancient times and are still used as home remedies today whilst pure lard was the most commonly used. Following World War II the use of any oil was increasingly discouraged because it is not water soluble and therefore difficult to remove. The use of live animal sources were also used in the 20th Century through the use of maggots to debride burn wounds as they are able to distinguish the dead tissue, which they eat and the live tissue, which they leave.[3]

Agents such as plant oils, potatoes, flour, apples, onions, leaves, vinegar and turpentine have also been recommended as curatives. Carron oil, a mixture of equal parts of linseed or olive oil and 'lime water' (an aqueous suspension of calcium carbonate and calcium oxide or quick lime) was recommended in most burn care studies in the 19th and early 20th Centuries. Alkaline pH of lime water, and its healing and soothing properties made it ubiquitous until the advent of antiseptics following Lister's studies in the 1870's.During the 19th Century emollient poultices made largely from ingredients such as potatoes, apples, onions and leaves were used alongside bread and milk poultices to slough wounds and were the treatment of choice during the American Civil War (1861). Also during this time vinegar or turpentine use was still prevalent having first been used in ancient Greek and Roman times with its proposed beneficial effect due to the cooling process incidental to its evaporation.[4]

Surprisingly, lead treatments were used extensively during the 19th Century as an escarotic agent until toxic effects were noted. Of less toxic effect ice and iced water is mentioned occasionally throughout history for its cooling and anti-swelling properties until it was realized at the end of the 20th Century that it led to burn shock. The middle of the 20th Century saw the development of petroleum based products and especially petrolatum or petroleum jelly, which were used to relieve pain and hyperemia. During World War II the branded Vaseline impregnated gauze was the standard topical treatment for burn injuries. The

introduction of carbolic acid or phenol marked an important advance in burns treatment towards bacterial control. It had many beneficial qualities to recommend it to 19th century physicians experimenting in this field, unfortunately there were many toxic effects including burning tissue. It was not until 1965 that the first effective bactericide was produced, 0.5% silver nitrate, followed by mafenide and then silver sulphadiazine (1968). The latter proving less toxic and less painful to apply whilst reducing burn wound contamination and blocking sepsis. (5)

Wound is defined as disruption of cellular, anatomical, and functional continuity of a living tissue. It may be produced by physical, chemical, thermal, microbial, or immunological insult to the tissue. When skin is torn, cut, or punctured it is termed as an open wound and when blunt force trauma causes a contusion, it is called closed wound, whereas the burn wounds are caused by fire, heat, radiation, chemicals, electricity, or sunlight (6)

Burn is defined as tissue damage caused by a variety of agents such as heat, chemicals, electricity, sunlight or nuclear radiation .The most common are burns caused by scalds, building fires and flammable liquids and gases. Thermal burn and related injuries have remained a major cause of death and disability. Although small burns are not usually life threatening, they need the same attention as large burns, in order to achieve functional and cosmetic outcome. (7)

Thermal burns are classified into three types namely first degree, second degree and third degree burns based on various characteristics

1st degree is the superficial burns caused by Sun or minor flash leading to the formation of dry blister and erythmatus skin and it is painful

2nd degree burns are caused due to the flash, hot metal or liquids leading to the formation of blisters which are moist. The wound developed is motted red in colour and painful.

3rd degree burns are caused due to the high temperature flame. The wound developed is dry, partly white and charred, there is little pain. (8)

Wound healing is the interaction of a complex cascade of cellular and biochemical actions leading to the restoration of structural and functional integrity with regaining of strength of injured tissues. It involves continuous cell-cell interaction and cell-matrix interactions that allow the process to proceed in different overlapping phases and processes including inflammation, wound contraction, reepithelialization, tissue remodelling, and formation of granulation tissue with angiogenesis. The phases of wound healing normally progress in a predictable, timely manner, and if they do not, healing may progress inappropriately to either a chronic wound such as a venous ulcer or pathological scarring such as a keloid scar (9)

Medicinal Plants have the immense potential for the management and treatment of wounds. A large number of plants are used by tribal and folklore in many countries for the treatment of wounds and burns. These natural agents induce healing and regeneration of the lost tissue by multiple mechanisms. These phytomedicine are not only cheap and affordable but are also safe. The presence of various life-sustaining constituents in plants has urged scientist to examine these plants with a view to determine potential wound healing properties .The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body . The screening of herbal extracts has been of great interest to the scientists for the discovery of new effective drugs [(10)]

The gold standard in topical burn treatment is Silver sulfadiazine a useful antibacterial agent for burn wound treatment. It is 1% cream which is soft, white, water-miscible containing the antimicrobial agent silver sulfadiazine in micronized form. Each gram of silver sulfadiazine cream 1% contains 10 mg of micronized silver sulfadiazine. The cream vehicle consists of white petrolatum, stearyl alcohol, isopropyl myristate, sorbitan monooleate, polyoxyl 40 stearate, propylene glycol, and water with methylparaben 0.3% as a preservative. Silver sulfadiazine cream 1% spreads easily and can be washed off readily with water. Gives broad spectrum prophylaxis against bacterial colonization and is particularly effective against Pseudomonas aeruginosa and also methicillin

resistant Staphylococcus aureus. Silver sulfadiazine cream is the best present treatment of choice for the burn wounds. [11]

Recent findings, however, indicate that the compound silver sulfadiazine delays separation of scar, delays reepithelization, responsible for developing the post burn contractures, stains the skin to black, further the cream is contraindicated in the patients who are hypersensitive to sulfa drugs, it tends to increase the possibility of kernicterus if used in the pregnant women approaching term or in premature infants. The use of silver sulfadiazine cream 1% in some cases of glucose-6-phosphate dehydrogenase-deficient individuals may be hazardous, as hemolysis may occur. Other infrequently occurring events include skin necrosis, erythema multiforme, burning sensation, rashes, and interstitial nephritis. Reduction in bacterial colonization has caused delayed separation, in some cases necessitating escharotomy in order to prevent contractures. [12]

Further Pseudomonas aeruginosa is a major cause of infection and a contributing factor in the death of patients with severe burns. The impression that these infections are the result of contamination by the patient's own intestinal flora is common even though the incidence of intestinal carriage among normal subjects and patients not associated with pseudomonas infections has been reported to be only 3 percent to 11 percent. The high infection rate in burns points to hospital contamination as a more probable source of the bacteria **[13].**

A burn patient who receives the best of treatment is expected to heal without any

contractures. The incidence of post-burn contractures is extremely high in our country. An understanding of the burn wound healing is fundamental not only to the management of the acute burn wound, but also for the prevention, minimization and treatment of post-burn scars and scar contractures. So, there is a growing need to develop drugs which will decrease the complications and prevent infections more effectively than the presently used drugs. [14]

Azadirachta Indica Locally called as 'Neem' The plant is widely found in India. Almost all the parts of this plant have the medicinal properties but leaves are most commonly used .Leaves are antiseptic, astringent, insecticidal. They are important in the vital conditions of pitta, burning sensation, leprosy, skin diseases and ulcers. [15]

Shorea robusta Gaerthn.F. bark is astringent, acrid, cooling. Useful in vitiated conditions of kapha and pitta, ulcers, wounds, bacterial infections, leprocsy. Resin is antibacterial, astringent, cooling. It is useful in wound ulcers, burns, neuralgia. [16]

Linum usitatissmum L. oil is used for external application. It is a common base for liniments. Crushed linseed is applied in the form of poultice for the relief of local inflammations, ulcers, boils and carbuncles. [17]

Reviewing the medicinal properties to these plants we developed an ointment indigenously using the plants *Azadirachta Indica A.Juss, Shorea robusta Gaerthn.f* and *Linum usitatissmum L.* which can be used for the treatment of the

burn wounds of second degree character.

Herbal Antioxidants are another group of promising drugs since ages for antagonizing the tissue injuries as evident from the ancient story of Maharshi Chawan who suffered the premature aging and related health problem. He was benefited from use of the formulation provided to him by Ashwini twins and his formulation was eventually known as Chyawanaprash. It can also be inferred that, Chyawanaprash is a formulation which is capable of arresting the degenerative process. The mythological linkage of Chyawanaprash thus brings forth, its specific use attributed and its effectiveness in arresting the degenerative process. Charaka samhita reiterates its role as a rasayana citing the instance of maharshi Chyawana. The regular use of Chyawanaprash promotes intellectual memory complexion and overall health. It is also said to contribute to longevity to strengthen sensory organs, to promote sexual vigor, digestive and metabolic fire. Chyawanaprash exhibits antioxidant properties. Due to its free radical scavenger activity, Chyawanprash might help in modification of risk factors of myocardial iscimia and may also be responsible for its anti aging effect. This clearly indicates the role of antioxidants in arrest of the degenerative process by scavenging the free radicals produced by oxidative stress. [18]

Burns is definitely one of the conditions where there is increased oxidative stress in the body hence we decided to use two proven antioxidant plant formulations *Hemidesmus indicus* and *Cissus quadrangularis* along with the test ointment to

observe the effect on the healing of the second degree burn wounds

Hemidesmus indicus, locally called as Anantamul (Indian Sarsaparilla) is widely distributed throughout India. Hemidesmus indicus root extract is traditionally used as antioxidant, anti-inflammatory, immunomodulatory and antidote in the Indian System of Medicine. Borkar and Patel showed the beneficial effect of Hemidesmus indicus in decreasing oxidative stress of TB and hypertension. [19]

Cissus quadrangularis is a medicinal herb used in Siddha and Ayurvedic medicine since ancient times in Asia, as a general tonic and analgesic, especially for bone fracture healing. Recently, *Cissus quadrangularis* has been linked to several health benefits such as antiobesity, reduction of pro inflammatory cytokines, anti-inflammatory, antioxidant, and antidiabetic properties. The antioxidant properties of the plants *Hemidesmus indicus, Cissus quadrangularis* could be explored to enhance the healing of the burn wounds. [20]

As such effective and affordable (cheap) ointment is needed for the management of burn injuries.

The present study was carried out to evaluate the effect of the test ointment with and without the treatment with *Hemidesmus indicus* and *Cissus quadrangularis* in second degree thermal burns and compare it with ointment Silver Sulfadiazine, the present standard ointment used in treatment of burns.

Since the ointment will be used first time for the treatment of the burn wounds it was decided to perform the study on animals. New Zealand white Rabbits are the animals chosen for the study

3. AIM AND OBJECTIVES

3.1 Aim:

To compare the second degree burn wound healing property of a New Herbal Ointment with Silver sulfadiazine with and without the supportive therapy of Cissus quadrangularis and Hemidesmus indicus.

3.2 Objectives:

1. To prepare a Herbal Ointment with new formulation for the treatment of burns using Azadirachta indica, Shorea robusta and Linum usitatissumum oil.

2. To compare the efficacy of healing the second degree burn wounds using the New Herbal Ointment and silver sulfadiazine with and without the supportive therapy with Cissus quadrangularis and Hemidesmus indicus with respect to following parameters

    2.1 Percentage of wound contraction

    2.2 Period of repithelization

    2.3 Wound healing scores based on histopathology

2.4 Epithelial regeneration grading based on histopathology

2.5 Wound swab culture for presence of infection

3. To compare the oxidative stress when second degree burn wounds were treated using the New Herbal Ointment and silver sulfadiazine with and without the supportive therapy with Cissus quadrangularis and Hemidesmus indicus with respect to following parameters

3.1 MDA

3.2 SOD

3.3 Stress index

3.4 Protective index

4. To analyze the Pharmacoeconomics of therapies when second degree burn wounds were treated using the New Herbal Ointment and silver sulfadiazine with and without the supportive therapy with Cissus quadrangularis and Hemidesmus indicus with respect to the following parameters

4.1 Cost per dose

4.2 Total cost of the therapy

4.3 Cost effectiveness

5. To find out correlation between protective index and percentage of burn wound healing.

2. REVIEW OF LITERATURE

2.1. Extent of the Problem:

Burns remain a major public health issue all over the world, especially in developing countries. Superficial burns comprise a spectrum of injury severity depending on the depth of the wound and proportion of the body affected**.** While current approach to burn injury management has improved patient prognosis, however increased morbidity and mortality remain a major challenge for clinicians [(21)].

2.2 Epidemiology:

India, the second most populous country in the world with over a billion people has an estimated annual burn incidence of 6-7 million, based on data from major hospitals when extrapolated to whole of the country, which is the second largest group of injuries after road accidents. Nearly 10% of these are life threatening and require hospitalization. Approximately 50% of those hospitalized succumb to their injuries. Nearly 1 to 1.5 lac people get crippled and require multiple surgeries and prolonged rehabilitation. Seventy percent of the burn victims are in most productive age group of 15 to 40 years and most of the patients belong to poor socioeconomic strata [(22)].

Burns happen everywhere, but are somewhat more common in the rural areas. The main reason is the insufficient electric power supply leading to popular use of kerosene lamp for lighting which falls down frequently spilling kerosene and causing burns [23].

Kerosene stove continues to be another common cause of flame burns as liquid petroleum gas supply to rural area is insufficient and biogas or wood as fuel has to be supplemented with quick lighting kerosene a highly flammable liquid, is available in every household and unfortunately is used in most intentional burn injuries .Children do get scalded due to floor level storing of hot liquids like water, milk and food [24].

2.3 Definition:

Burn is defined as tissue damage caused by a variety of agents such as heat, chemicals, electricity, Sunlight, or nuclear radiation. Burn injuries can be accidental, suicidal and homicidal [25] .

2.4 Classification:

2.4.1. Depending on the causative agent burns are classified as follows [26]:

(a) Physical

(b) Thermal burns

i. By dry heat - Flame burns

ii. By wet heat – Scalds

(c) Electrical burns

i. Contact burns

ii. High voltage

iii. Low voltage

iv. Flash burns

(d) Radiation burns

(e) LASER burns

(f) Chemical burns

i. Acid burns

ii. Alkali burns

2.4.2. Burn wounds can be classified according to involvement of skin and deeper tissues as follows [27]

(a) First-degree burn or epithelial burns - Skin is erythematic without vesication.

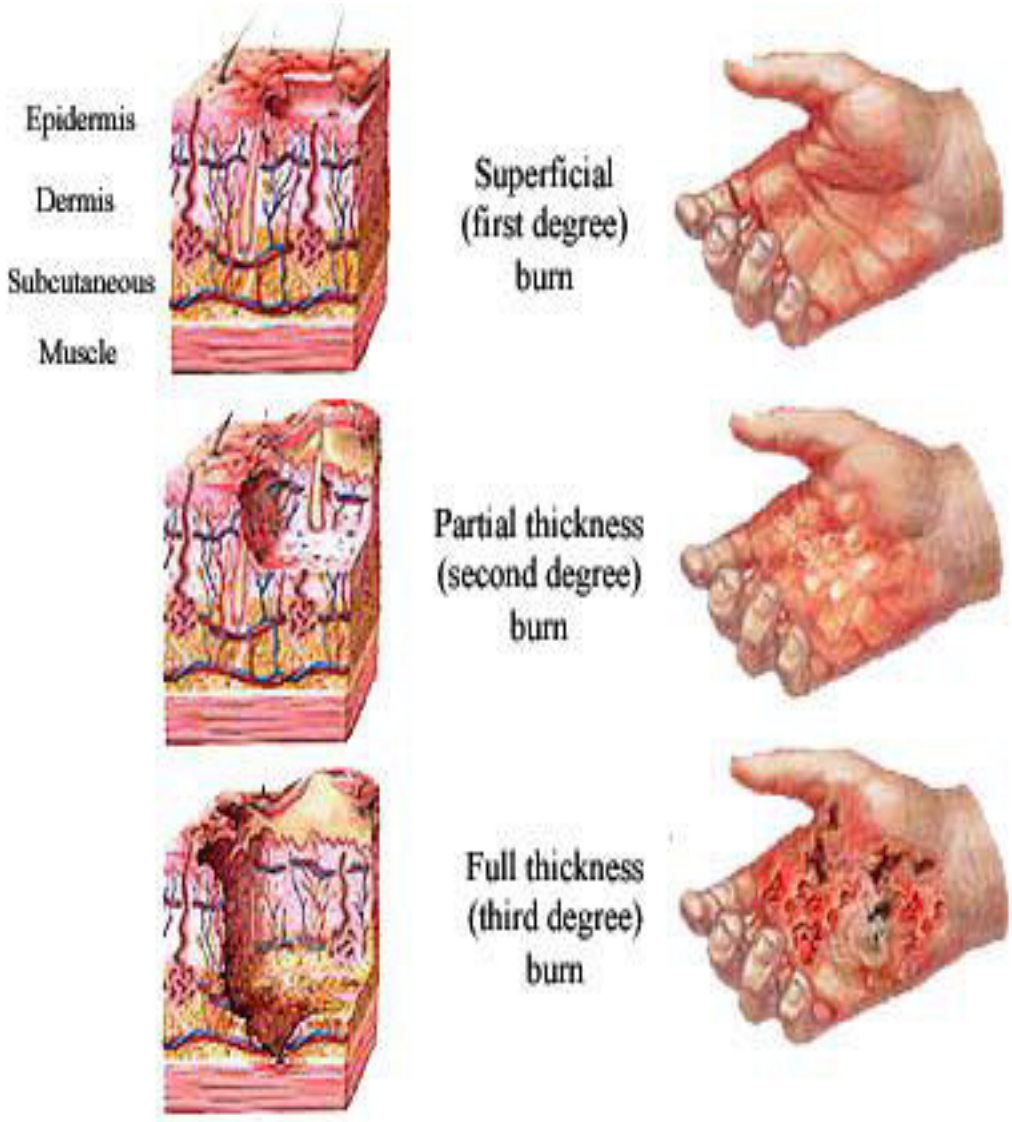
(b) Second-degree burns - Involving epidermis and variable thickness of dermis.

This is further divided into

1. Second-degree superficial -where vesication and inflammation is seen in skin as only papillary dermis is involved.
2. Second-degree deep -eschar formation is seen as it involves deep reticular dermis.

(c) Third-degree burn - Also known as full thickness burns invoving the layers of skin and deeper tissue - echar formation is present in these burns.

Figure: 1 – Classification of burns according to involvement of skin and deeper tissues



2.4.3. Classification depending on the severity of burns is as follows [28]

(a) Minor burns are classified as less than 15% Total Body Surface Area in adults and less than 10% in children.

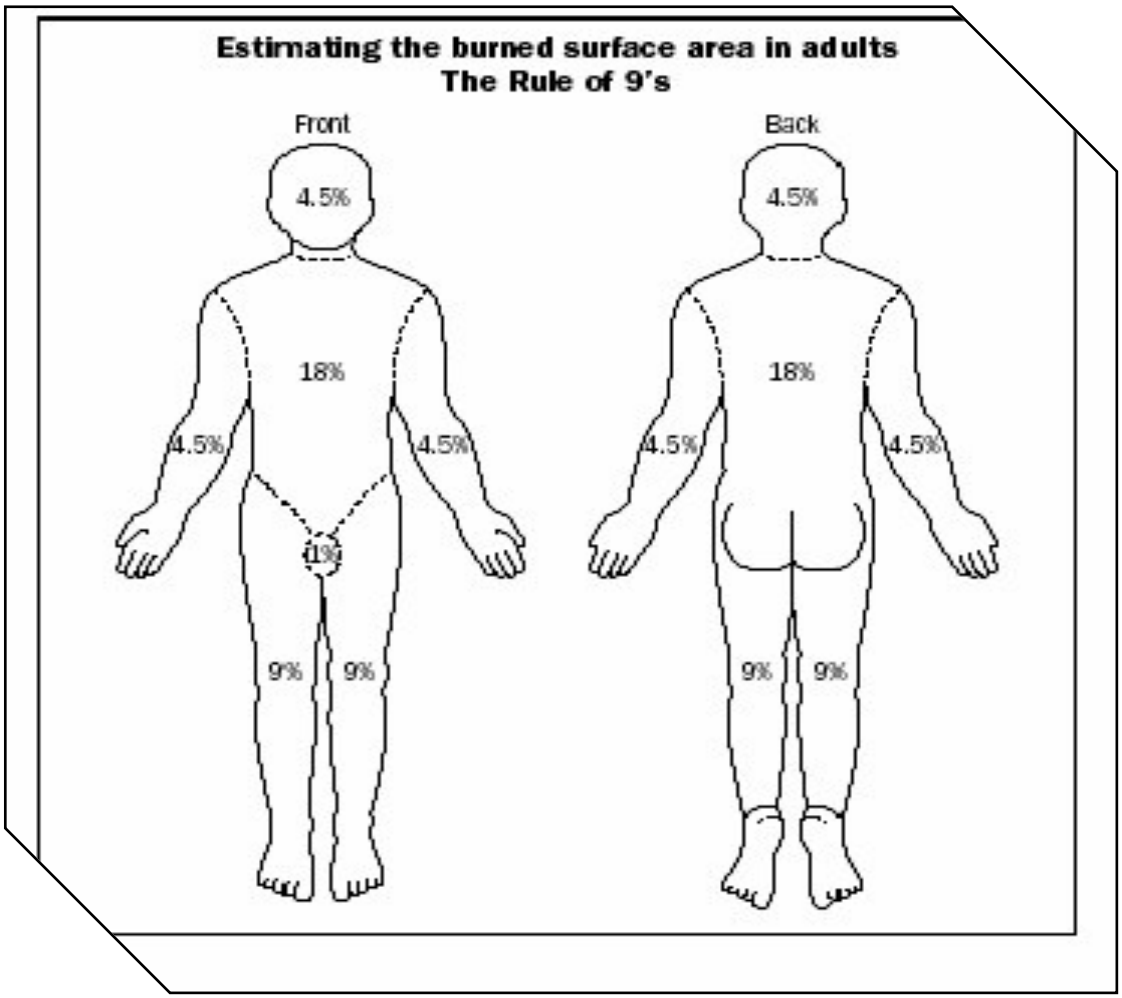
(b) Major burns are more than 15% burns up to 35% in adults and more than 10% and less than 30% in children.

(c) Critical burns or life-threatening burns are classified as more than 35% burns in adults and more than 30% in children.

2.5 Estimation of Burned Surface Area (Wallace's Rule of 9's)

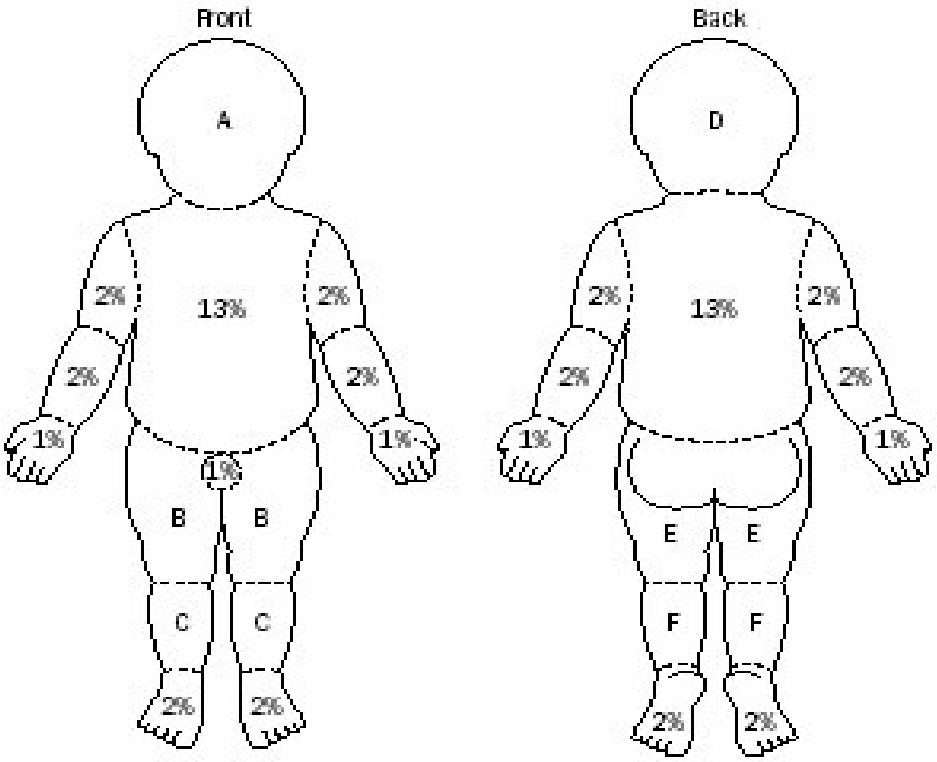
The "Rule of 9's" is commonly used to estimate the burned surface area in adults. The body is divided into anatomical regions that represent 9% (or multiples of 9%) of the total body surface. The outstretched palm and fingers approximates to 1% of the body surface area [27].

Figure : 2 - Estimation of Burn Surface Area in Adults



The 'Rule of 9's' method is too imprecise for estimating the burned surface area in children because the infant or young child's head and lower extremities represent different proportions of surface area than in an adult

Figure : 3 - Estimation of Burn Surface Area in Children



Area	By age in years			
	0	1	5	10
Head (A/D)	10%	9%	7%	6%
Thigh (B/E)	3%	3%	4%	5%
Leg (C/F)	2%	3%	3%	3%

Figure 8

2.6. Body's Response to Burn:

Burn injuries result in both local and systemic response.

2.6.1. Local Response [29]

The three zones of a burn were described by Jackson in 1947.

Zone of Coagulation*:*

This occurs at the point of maximum damage. In this zone there is irreversible tissue loss due to coagulation of the constituent proteins.

Zone of Stasis*:*

The surrounding zone of stasis is characterized by decreased tissue perfusion. The tissue in this zone is potentially salvageable. The main aim of burns resuscitation is to increase tissue perfusion and prevent any damage becoming irreversible. Additional insults—such as prolonged hypotension, infection, or oedema—can convert this zone into an area of complete tissue loss.

Zone of Hyperemia:

In this outermost zone tissue perfusion is increased. The tissue here will invariably recover unless there is severe sepsis or prolonged hypoperfusion.

These three zones of a burn are three dimensional, and loss of tissue in the zone of stasis will lead to the wound deepening as well as widening.

Figure: 4 - Clinical image of burn zones.

There is central necrosis surrounded by the zones of stasis and of hyperemia

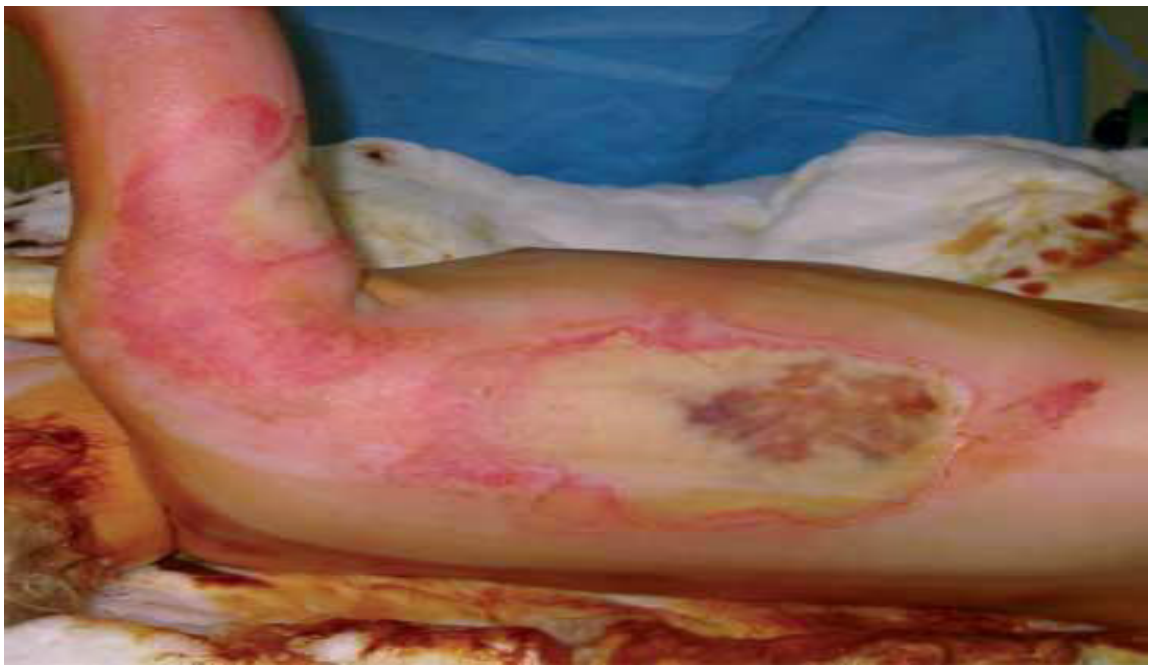
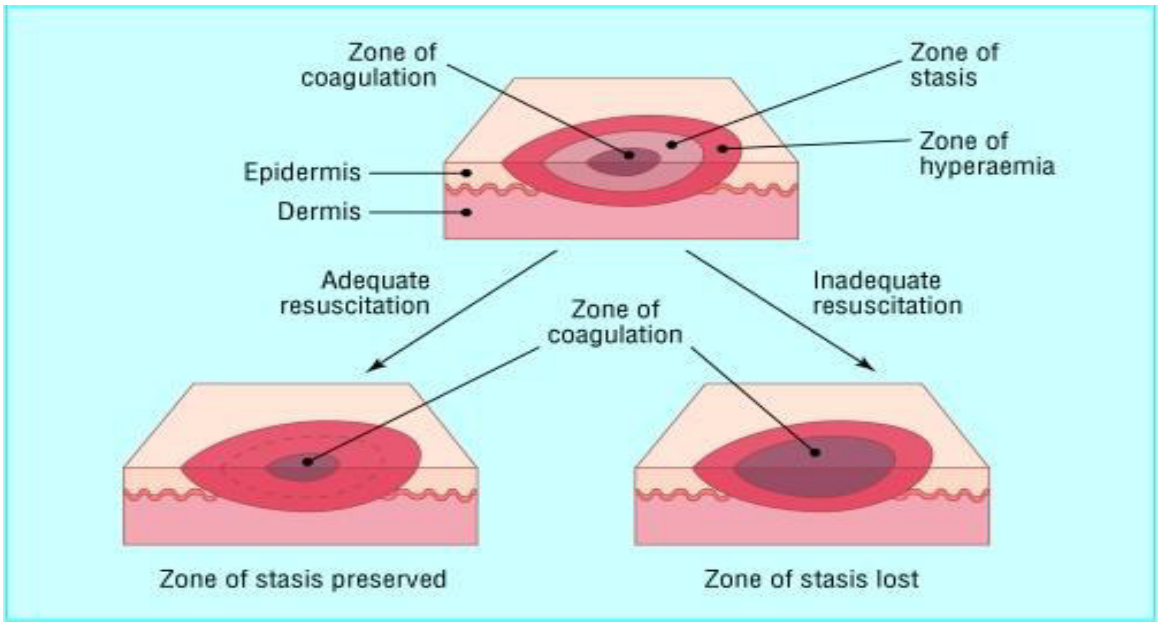


Figure: 5 - Jackson's Burn Zones



2.6.3. Systemic Response [30]

The release of cytokines and other inflammatory mediators at the site of injury has a systemic effect once the burn reaches 30% of total body surface area.

Cardiovascular changes

Capillary permeability is increased, leading to loss of intravascular proteins and fluids into the interstitial compartment. Peripheral and splanchnic vasoconstriction occurs. Myocardial contractility is decreased, possibly due to release of tumour necrosis factor

Respiratory changes

Inflammatory mediators cause bronchoconstriction, and in severe burns adult respiratory distress syndrome can occur.

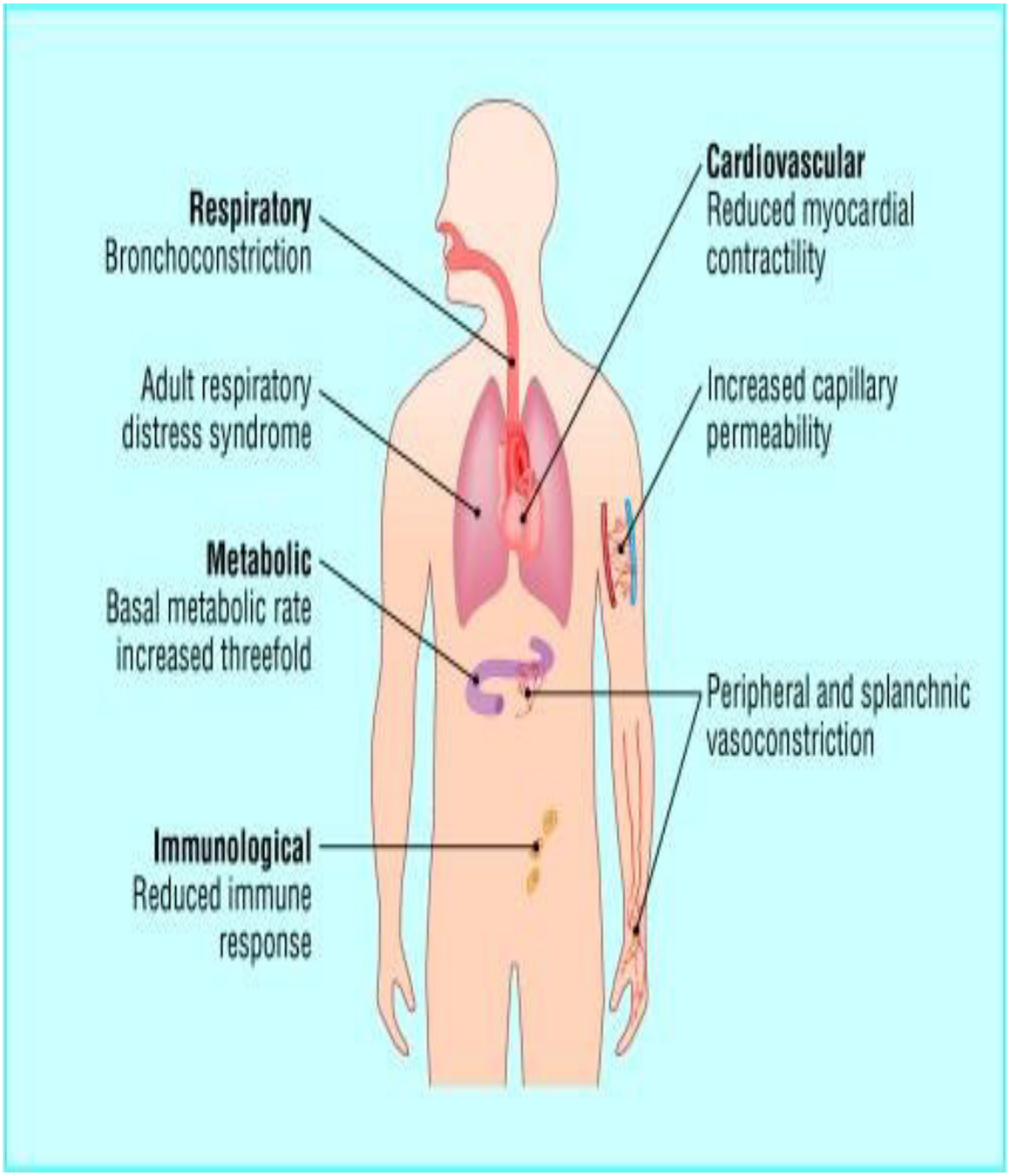
Metabolic changes

The basal metabolic rate increases up to three times its original rate. This, coupled with splanchnic hypoperfusion.

Immunological changes

Non-specific down regulation of the immune response occurs, affecting both cell mediated and humoral pathways.

Figure: 6 -Systemic changes that occur after a Burn Injury



2.11. Oxidative Stress in Burns

Oxidative stress is defined as a state in which oxidation exceeds the antioxidant systems in the body secondary to a loss of the balance between them [31].

Free radicals, reactive oxygen species (ROS) are formed during a variety of biochemical reactions and cellular functions, and act as pro-oxidants. The formation of free radicals is normally balanced by antioxidants. Oxidative stress (OS) results from an imbalance between formation and neutralization of free radicals. Oxidative stress plays an important role in oedema formation after burn injury [32].The pathophysiological mechanisms underlying the development of oedema in burn patients are mainly due to the inflammatory response which activates cytokines, with subsequent stimulation of phagocytic cells. This results in the formation of reactive oxygen species (ROS) leading to lipid peroxidation [33].

Another source of ROS in burns is the enzyme Xanthine Oxidase, produced from Xanthine Dehydrogenase under ischaemic conditions producing ROS, which causes lipid peroxidation [34].

A close relationship has been demonstrated between the intensity of lipid peroxidation and postburn complications, and it has been possible to document the role of ROS leading to lipid peroxidation as a causative agent in the mechanism of

local and systemic complications in burns, including increased vascular permeability. The increase in vascular permeability leads to immediate and continuous loss of substances ranging from water to macromolecules [35].

Various antioxidants are used to antagonize ROS: allopurinol, a well-known XO inhibitor, Melatonin, a product of the pineal gland that has powerful antioxidant action through its direct scavenging effect and its stimulation of antioxidant enzymes and N-acetylcysteine, used in the past as a mucolytic agent that rapidly metabolized to cysteine, a direct precursor in the synthesis of intracellular glutathione (GSH), the natural antioxidant [36].

The involvement of these antioxidants in burns treatment may have improving effects on endothelial dysfunction and burn outcome in general [37].

2.12.Antioxidant:

Antioxidant means "against oxidation." An antioxidant is any substance that retards or prevents deterioration, damage or destruction by oxidation. [38]

2.12.1. Types of Antioxidants:

1. Primary antioxidants: They prevent formation of new free radical species by preventing their formation from other molecules or by converting existing radicals

to harmless substances. (a) Superoxide dismutase: b) Glutathione peroxidase (GPx) (c) Catalese.

2. Secondary antioxidants: They trap free radicals and prevent chain reactions. These include the action of Vitamin E (α-tocopherol), Vitamin C (ascorbate), β-carotene, uric acid , albumin and bilirubin.

3. Tertiary antioxidants: They repair biomolecules damaged by free radicals. These include DNA repair enzymes and Methionine sulphoxide reductase. [39]

Antioxidant vitamins such as vitamin A, E and C also have a number of biological activities such as immune, stimulation inhibition or nitrosamine formation. They prevent genetic changes by inhibiting DNA damage induced by the ROMs. The vitamins C prevent the formation ONOO- by reaction with O2 and may help to release NO from endothelial cells. [40]

Enzymatic antioxidant:

The first lines of defense against O_2·- and H_2O_2 mediated injury are antioxidant enzymes: SOD, GPx, and CAT . Antioxidant enzymes, together with the substances that are capable of either reducing ROMs or preventing their formation, form a powerful reducing buffer which affects the ability of the cell to counteract the action of oxygen metabolites. All reducing agents thereby form the protective mechanisms, which maintain the lowest possible levels of ROMs inside the cell. [41]

2.13. Malondialdehyde (MDA):

Malondialdehyde is a terminal product of lipid peroxidation, which can be measured in plasma and serves as an effective lipid peroxidation marker and an indirect index of reactive oxygen species (ROS) activity. Malondialdehyde (MDA) is the organic compound with the formula $CH_2(CHO)_2$. It is the major reactive Aldehyde resulting from the peroxidation of biological membrane polyunsaturated fatty acid (PUFA). It is also a by-product of prostaglandin biosynthesis which is used as an indicator of tissue damage. It reacts with Thiobarbituric acid and produces red colored product. Some of the workers considered the ratio of MDA and SOD as a determinant of OS. [42,43]

2.14. Superoxide dismutase (SOD):

SODs are a family of Metalloenzymes that convert $O_2\cdot-$ to H_2O_2 according to the following reaction:

$$O_2\cdot- + O_2\cdot- \xrightarrow[\text{SOD}]{2H} H_2O_2 + O_2$$

SOD is the most important enzyme because it is found virtually in all aerobic organisms. There are four families of SOD: Cu-SOD, Cu-Zn-SOD; Mn-SOD, and Fe-SOD. Human SOD is the Cu-Zn-SOD enzyme. The transition metal of the

enzyme reacts with O_2·- taking its electron. O_2·- is the only known substrate for SOD. [44]

Cu-Zn-SOD is found in the cytosol of most eukaryotic cell. A different form of Cu-Zn-SOD is found in extra cellular fluids, where it is called ECSOD. Mn-SOD is located in the matrix and bacteria, while mitochondrial Fe-SOD is present in many aerobic bacteria. Cu-Zn-SOD is sensitive to cyanide but resistant to chloroform-ethanol treatment. In contrast Mn-SOD is resistant to cyanide, but is destroyed by the treatment with chloroform plus ethanol. Human encoding CU, Zn, SOD and Mn, SOD are found on chromosome 21q 22.1 and 6q21, respectively. [45]

Moreover, SOD is considered to be a stress protein which is synthesized in response to oxidative stress. SOD has been detected in a large number of tissues and organisms, and is thought that it is present to protect the cell from damage caused by O_2·- OH or Fe (II)O generated from the metal-catalysed interaction of O_2·- with H_2O_2, the in vitro process is inhibited by SOD, or catalyses, or by chelating agents. The Cu-Zn-SOD is reported to inhibit ·OH production. [46]

2.15. Glutathione peroxidase (GPx):

Glutathione peroxidase enzyme is a well-known first line of defence against oxidative stress, which in turn requires glutathione as a cofactor. Among the many functions of glutathione, it is involved in the generation of the nucleotide

precursors of DNA via the reduction of ribonucleotides to deoxyribonucleotides. GPx catalyses the oxidation of the expense of H_2O_2. By its selenium (Se) - dependency, GPx divided in to two forms; Sedependent GPx and Se-independent GPx. The former is a tetramer of MW 84000 with very high activity toward both H_2O_2 and organic hydroperoxides. It is found in both cytosol (70%) and mitochondria (30%) of various tissues. Iodoacetate, cyanide and O_2·- are considered as inhibitors of this enzyme. [47] The gene encoding Se-dependent GPx is located at chromosome 3p13-q12, while the genes encoding GPx are found to be on 6p12.2 and 11q13qter Since selenium is an integral component of GPx, the measurement of this enzyme has been used as a functional index of selenium level. GPx activity being reduced in Selenium deficiency. [48]

2.16. Catalase :

Catalase is an enzyme, which is present in most cells, and decomposition at hydrogen peroxide to water and oxygen. CAT is a heme-containing protein. The mechanism of the action is:

$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O2$$

CAT is found to act 10^4 times faster than peroxidase. It is localized mainly in mitochondria and in subcellular respiratory organelles CAT is present in

peroxisome (80%) and cytosol (20%). It has 240,000 molecular weight and consists of four protein subunits, each containing a heme Fe (III)- protoporphyrin group bound to its active site**.** The gene encodes human CAT found on chromosome. [49]

2.17. Wound Healing

2.17.1. Definition:

Wound healing is the interaction of a complex cascade of cellular and biochemical actions leading to the restoration of structural and functional integrity with regain of strength of injured tissues. It involves continuous cell-cell interaction and cell-matrix interactions that allow the process to proceed in different overlapping phases and processes including inflammation, wound contraction, reepithelialization, tissue remodelling, and formation of granulation tissue with angiogenesis [50].

2.17.2. Healing Process of Burn Wound:

Healing of burn wound depends on the depth of burns. In first degree and second-degree superficial burns, healing is by primary intention. Second-degree superficial burns heal from epithelium of hair follicle remnants, which are in plenty in the superficial dermis. Healing is complete within 5-7 days and is almost

scar less. In second-degree deep and third-degree burns, healing is by secondary intention, which involves the process of epithelization and contraction [51].

Inflammatory (reactive), proliferative (reparative) and maturation (remodelling) constitute the three phases in wound healing. This is same for all types of wounds, the only difference being in duration of each stage [52].

a) Inflammatory Phase [53]

This is same in all traumatic wounds. Immediately after the injury, inflammatory response of body begins which has vascular and cellular components.

- Vascular response: Immediately after burns there is a local vasodilatation with extravasation of fluid in the third space. In extensive burn injury increased capillary permeability may be generalized leading to massive extravasation of plasma requiring fluid replacement.
- Cellular response: Neutrophils and monocytes are the first cells to migrate at the site of inflammation. Later on neutrophils start declining and are replaced by macrophages. The migration of these cells is initiated by chemotactic factors like kallkireins and fibrin peptides released from coagulation process and substances released from the mast cells like tumour necrosis factor, histamine, proteases, leukotreins and cytokines. Cellular response helps in phagocytosis and cleaning of dead tissue and toxins released by the burned tissue.

b) Proliferative Phase [54]

In partial thickness burns re-epithelialization starts in the form of keratinocyte migration from viable skin appendages in dermis few hours after injury, this usually covers the wound within 5-7 days. After re-epithelialization the basement membrane zone forms between dermis and epidermis. Angiogenesis and fibrogenesis help in dermal reconstitution.

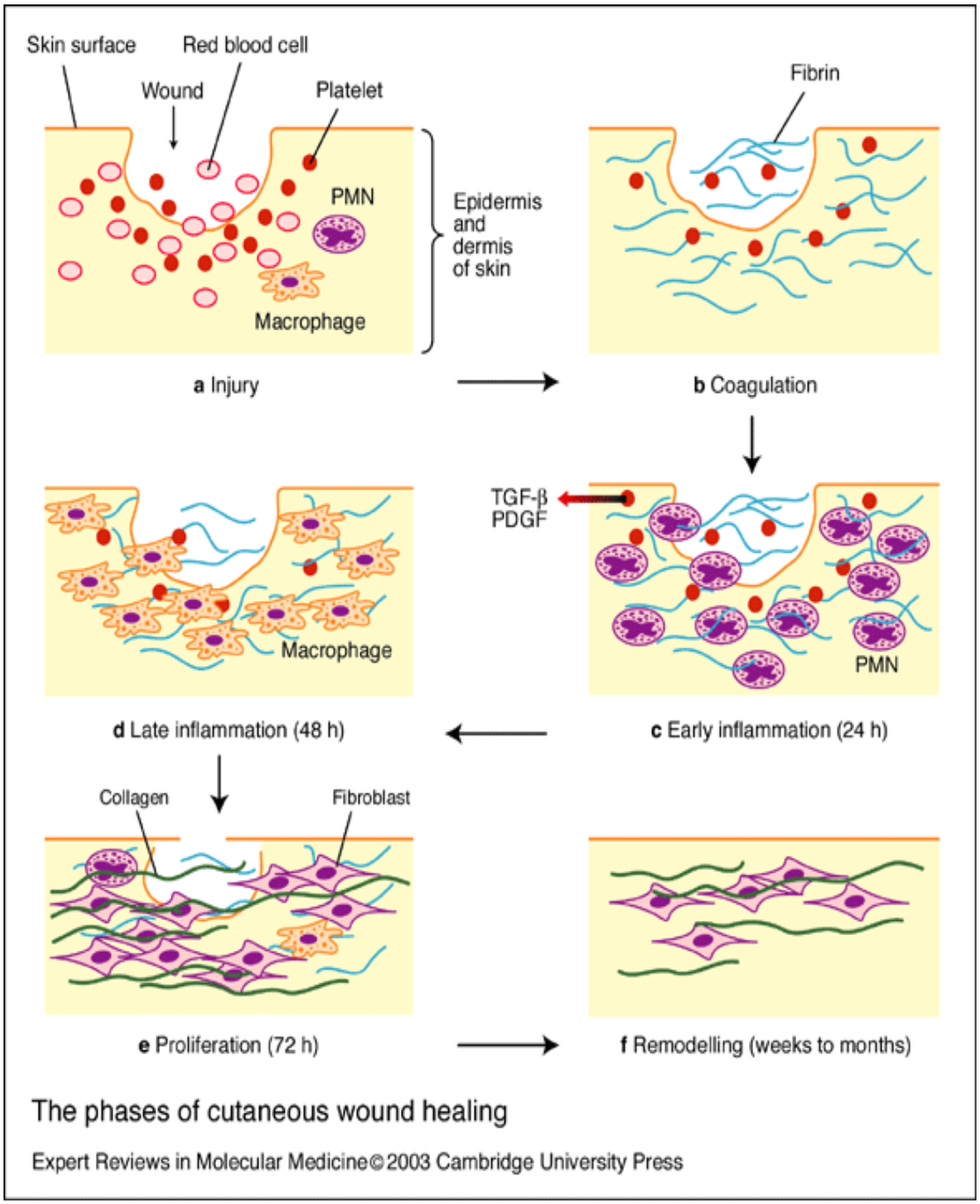
Healing after burn excision and grafting: In deep burns after primary excision and grafting healing is by delayed primary intention. Take of skin graft after primary excision is the part of proliferative phase of wound healing.

c) Remodelling Phase [55]

Remodelling phase is the third phase of healing wherein the maturation of graft or scar takes place. In this final phase of wound healing initially there is laying down of fibrous structural proteins i.e., collagen and elastin around epithelial, endothelial and smooth muscle as extracellular matrix. Later on in the resolution phase this extracellular matrix remodels into scar tissue and fibroblast become myofibroblast phenotype which is responsible for scar contraction.

In second-degree deep dermal and full thickness burns which are left to heal of their own this resolution phase is prolonged and may take years and is responsible for hypertrophic scarring and contractures. Hyperpigmentation seen in superficial burns is due to overactive response of melanocytes to burn trauma and hypopigmentation seen in deep burns is due to destruction of melanocytes of the skin appendages.

Figure : 7 – Phases of Burn Wound Healing



2.17.3 Management

First Aid [56]

- If the patient arrives at the health facility without first aid having been given, drench the burn thoroughly with cool water to prevent further damage and remove all burned clothing.
- If the burn area is limited, immerse the site in cold water for 30 minutes to reduce pain and oedema and to minimize tissue damage.
- If the area of the burn is large, after it has been doused with cool water, apply clean wraps about the burned area (or the whole patient) to prevent systemic heat loss and hypothermia.
- Hypothermia is a particular risk in young children.
- First 6 hours following injury are critical; transport the patient with severe burns to a hospital as soon as possible.

Initial Treatment [57]

- Initially, burns are sterile. Focus the treatment on speedy healing and prevention of infection.
- In all cases, administer tetanus prophylaxis.

- Except in very small burns, debride all bullae. Excise adherent necrotic (dead) tissue initially and debride all necrotic tissue over the first several days.
- After debridement, gently cleanse the burn with 0.25% (2.5 g/litre) chlorhexidine solution, 0.1% (1 g/litre) cetrimide solution, or another mild water-based antiseptic.
- Do ***not*** use alcohol-based solutions.
- Gentle scrubbing will remove the loose necrotic tissue. Apply a thin layer of antibiotic cream (silver sulfadiazine).
- Dress the burn with petroleum gauze and dry gauze thick enough to prevent seepage to the outer layers.

Daily Treatment [58]

- Change the dressing daily (twice daily if possible) or as often as necessary to prevent seepage through the dressing. On each dressing change, remove any loose tissue.
- Inspect the wounds for discoloration or haemorrhage, which indicate developing infection.
- Fever is not a useful sign as it may persist until the burn wound is closed.
- Cellulitis in the surrounding tissue is a better indicator of infection.
- Give systemic antibiotics in cases of haemolytic streptococcal wound infection or septicaemia.

- *Pseudomonas aeruginosa* infection often results in septicaemia and death. Treat with systemic aminoglycosides.
- Administer topical antibiotic chemotherapy daily. Silver nitrate (0.5% aqueous) is the cheapest, is applied with occlusive dressings but does not penetrate eschar. It depletes electrolytes and stains the local environment.
- Use silver sulfadiazine (1% miscible ointment) with a single layer dressing. It has limited eschar penetration and may cause neutropenia.
- Mafenide acetate (11% in a miscible ointment) is used without dressings. It penetrates eschar but causes acidosis. Alternating these agents is an appropriate strategy.
- Treat burned hands with special care to preserve function.
- Cover the hands with silver sulfadiazine and place them in loose polythene gloves or bags secured at the wrist with a crepe bandage;
- Elevate the hands for the first 48 hours, and then start hand exercises;
- At least once a day, remove the gloves, bathe the hands, inspect the burn and then reapply silver sulfadiazine and the gloves;
- If skin grafting is necessary, consider treatment by a specialist after healthy granulation tissue appears.

Healing Phase [59]

- The depth of the burn and the surface involved influence the duration of the healing phase. Without infection, superficial burns heal rapidly.

- Apply split thickness skin grafts to full-thickness burns after wound excision or the appearance of healthy granulation tissue.
- Plan to provide long term care to the patient.
- Burn scars undergo maturation, at first being red, raised and uncomfortable. They frequently become hypertrophic and form keloids. They flatten, soften and fade with time, but the process is unpredictable and can take up to two years.

In Children [60]

- The scars cannot expand to keep pace with the growth of the child and may lead to contractures.
- Arrange for early surgical release of contractures before they interfere with growth.
- Burn scars on the face lead to cosmetic deformity, ectropion and contractures about the lips. Ectropion can lead to exposure keratitis and blindness and lip deformity restricts eating and mouth care.
- Consider specialized care for these patients as skin grafting is often not sufficient to correct facial deformity.

Nutrition [61]

- Patient's energy and protein requirements will be extremely high due to the catabolism of trauma, heat loss, infection and demands of tissue

regeneration. If necessary, feed the patient through a nasogastric tube to ensure an adequate energy intake (up to 6000 kcal a day).

- Anaemia and malnutrition prevent burn wound healing and result in failure of skin grafts. Eggs and peanut oil and locally available supplements are good.

2.18. Management of Wound Healing with Silver and Herbs

2.18.1. Silver

Historically, Silver metal has been used widely across the civilizations for different purposes. Many societies use silver as jewelry, ornamentation and fine cutlery. Silver, jewelry, wares and cutlery was considered to impart health benefits to the users. In ancient Indian Medical System, Silver has been described as therapeutic agent for many diseases. There is an increasing use of Silver as an efficacious antibacterial and antifungal agent in wound care products. Another application is to synthesize composites for use as water disinfecting filters.

Several products have incorporated Silver for use as a topical antibacterial agent, such as Silver Nitrate, Silver Sulphadiazine (SSD), Silver Sulphadiazine/ Chlorhexidine, SSD with Cerium Nitrate and Silver Sulphadiazine impregnated lipidocolloid wound dressing [(62)].

In contrast to these Silver agents, newly developed products such as ActicoatTM (Westaim Biomedical Inc., Fort Saskatchewan, Alberta, Canada) and Silverlon1 (Argentum Medical, L.L.C., Lakemont, Georgia) have a more controlled and prolonged release of nanocrystalline silver to the wound area [(63)].

In 1968 , Fox, first described the use of Silver Sulfadiazine for burn treatment, which has since become a standard antimicrobial for burn care [(64)].

Silver Sulfadiazine (SSD) cream 1% is the most widely used topical treatment for burn injury. The antimicrobial efficacy of SSD is probably the main reason for the widespread use of this agent [(65)].

Delayed wound healing is the major clinical adverse effect of silver topical agent following treatment. Several adverse reactions and side effects have been reported, such as resistance to SSD, renal toxicity, and leukopenia. Sloughing of dead tissue in partial thickness burns is retarded because SSD delays or prevents colonization by microorganisms. Prolonged application on partial thickness burn wounds results in high patient care cost and complicates wound healing because inpatient follow up is needed [(66)].

2.18.2. Herbs

A number of studies indicate that traditional herbs are potential wound-healing agents and are largely preferred because of their widespread availability, the absence of unwanted side effects, and their effectiveness [(67)].

Plants have the immense potential for the management and treatment of wounds. A large number of plants are used by tribal and folklore in many countries for the treatment of wounds and burns. These natural agents induce healing and regeneration of the lost tissue by multiple mechanisms. These phytomedicine are not only cheap and affordable but are also safe. The presence of various life sustaining constituents in plants has urged scientist to examine these plants with a view to determine potential wound healing properties. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body. These constituents include various chemical families like alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, and phenolic compounds. The screening of herbal extracts has been of great interest to the scientists for the discovery of new effective drugs [(68)].

A number of reports concerning the antibacterial, antiinflammatory, and wound healing activity of various plants have appeared in the literature [(69)].

Various pharmacological reports are available on plants employing different wound healing models and its underlying molecular mechanism for the validation of their traditional claims and development of safe and effective and globally accepted herbal drugs for wounds [(70)].

Figure 8 : Hemidesmus indicus plant

2.18.2.1. Hemidesmus indicus [71]

Commonly known as Indian Sarasparilla, belonging to the family Asclepiadaceae, is a slender laticiferous, twining, sometimes prostrate or semi erect shrub, occurring over the greater part of India. Roots are woody and aromatic, stem numerous, slender, terete, thickened at the nodes, leaves opposite, short-petioled, very variable, elliptic-oblong to linear-lanceolate often variegated with white above, sometimes silvery white and pubescent beneath; flowers are greenish outside, purplish inside, crowded in subsessile axillary cymes; follicles are slender, four inches long, cylindrical, sometimes curved, divaricate; seeds numerous, black, flattened, with a silvery white coma. This is a common medicinal plant widely used in Indian System of Medicine (Anonymous, 1997) and also an official drug in Indian Pharmacopoeia (Anonymous, 1996) and British Pharmacopoeia (Anonymous, 2003).

Scientific Classification [72] :

Kingdom: Plantae

Order: Gentianales

Family: Apocynaceae

Subfamily: Asclepiadaceae

Genus: *Hemidesmus*

Species: *H. indicus*

Vernacular Names [73] :

Table 1: Vernacular Names of Hemidesmus indicus

Sr. No.	**Language**	**Name**
01.	Sanskrit	Sarivaa
02.	Hindi	Saalsaa
03.	English	Indian Sarasparilla
04.	Marathi	Anantmoola
05.	Gujrati	Saarivaa
06.	Bengali	Anantmoola
07.	Malyalam	Nannari
08.	Tamil	Nannari
09.	Telgu	Sugandhipala

Phytochemical Constituents [74] :

Table 2: Chemical Constituents of Hemidesmus indicus

Sr. No.	Part Used	Constituents
01.	Roots	Hemidesmol, Resin, Glucoside, Tannin, Luperol, α and β amyrins, β-sitosterol, Hemidesmine, Hemidesmine-1, Hemidesmine-2, Glucose, Hemidesterol, Steroid.
02.	Stem	Indicine, Hemidine, Lactone, Lupanone, Glycosides, Hemidescine, Emidine, Indicusin, Medidesmine,
03.	Leaves	Tannins, Hemidesmine, Hemidesmine-1, Hemidesmine-2, Flavonides (Hyperoside, Rutin)
04.	Flowers	Flavonoids glycosides (Hyperoside, Isoquercitin, Rutin)

Pharmacological Activities [75] :

Antioxidant and Free Radical Scavenger Activity:

Ravishankara et al. (2002) found that Methanolic extract (50%) demonstrated antioxidant properties by several in vitro and ex vivo models. Mary et al. (2003) found out that methanolic extract of H. indicus roots inhibited lipid peroxidation and scavenges hydroxyl and superoxide radicals in vitro.

Antibacterial Activity :

Naovi et al (1991) was reported that Ethanolic extract (95%) was effective against Corynebacterium diphtheria, Diplococcus pneumonia, Staphylococcus aureus, Streptococcus pyogenes and Streptococcus viridians.

Antinociceptive Activity :

Verma et al. (2005) revealed that alcoholic extract of H. indicus possesses a dose-dependent antinociceptive effect from 25-100 mg/kg orally, in all the models viz, acetic acid induced writhing, hot plate and tail flick method of antinociception and it blocked both the neurogenic and inflammatory pain and its activity is due to the presence of triterpenes, flavonoids and sterols.

Anti-inflammatory Activity :

Dutta et al. (1982) found that the ethyl acetate extract of roots of H. indicus exhibited significant anti-inflammatory activity in both acute and subacute inflammation as revealed by significant inhibition of inflammation induced by carageenin, bradykinin and cotton pellet implantation methods in rats.

Antiulcer Activity:

A. Austin (2003) found out the antiulcer activity of H. indicus. It acts through mucoprotective action selectively inhibiting prostaglandin $PGF_{2\alpha}$.

Antidiarrhoeal Activity :

Das et al. (2003) proved that methanolic extract at a dose of 500-1500 mg/kg body weight elicited antidiarrhoeal activity which was effective than the standard antidiarrhoeal drug, Lomotil and the activity was due to inhibition of intestinal motility and its bactericidal activity.

Antivenom Activity :

Alam et al. (1996) proved that the methanolic extract of H. indicus significantly neutralized by viper-venom induced lethality and hemorrhagic activity in rats.

Antileprotic Activity :

Gupta et al (1981) found that the aqueous extract of H. indicus was given orally at a concentration of 2% in mice was active against Mycobacterium leprae.

Figure : 9 - Cissus quadrangularis plant

2.18.2.2. Cissus quadrangularis [76] :

One of the many plants which are being evaluated for their therapeutic efficacies is *Cissus quadrangularis* which is commonly known as Hadjod (Bengali) and Edible Stemmed Vine (English). It is an annual or perennial herb, entire leaves, buff colored with greenish ting and requires warm tropical climate and propagated by stem cuttings in months of June and July. The whole plant used specially leaves, roots and stem.

Trees with simple leaves (entire leaves), Leaves are simple or lobed, cordate, broadly ovate or reniform, serrate, dentate, sometimes 3-foliate and glabrous, Stem is buff colored with greenish ting, dichotomously, branched, sub-angular, glabrous, fibrous and smooth. Internode measures 4-5 cm long and 1-2 cm thick, a tendril occasionally present at nodes. Flowers are small, greenish white, bisexual, tetramerous, in umbellate cymes, opposite to the leaves Petals are 4-5, imbricate. Fruit are globose or obovoid fleshy berries, succulent, very acrid, dark purple to black .

Scientific Classification [77] :

Kingdom: Plantae

Order: Rhamnales

Family: Vitaceae

Genus: *Cissus* L.

Species: *Cissus quadrangularis* L.

Vernacular Names [78] :

Table 3 : Vernacular Names of Cissus quadrangularis

Sr. No.	**Language**	**Name**
01.	Sanskrit	Asthisamdhani, Asthishrinkhala
02.	Hindi	Hadjod, Hadjora
03.	English	Edible Stemmed Vine, Bonesetter
04.	Marathi	Kandvel
05.	Gujrati	Hadsankal
06.	Bengali	Harbhanga
07.	Malyalam	Peranta
08.	Tamil	Vajjravalli
09.	Telgu	Vajravalli

Phytochemical Constituents [79] :

Table: 4 - Chemical Constituents of Cissus quadrangularis

Sr. No.	Part Used	Constituents
01.	Stem	Calcium ions and phosphorus, Calcium oxalate, 31 methyl tritiacontanoic acid, taraxeryl acetate, taraxerol and iso-pentadecanoic acid, A andβ - amyrins, β-sitosterol, Ketosetosterol, Phenols, Tannins, Vitamin, Carotene, Saponins and Phenol
02.	Aerial Parts	7Oxo-Onocer8-ene-3 β 21 α diol
03.	Root Powder	Potassium, Calcium, Zinc, Sodium, Iron, Lead Cadmium, Copper and Magnesium
04.	Leaves	Resveratrol, Piceatanon Pallidol, Parthenocissus, Alicyclic lipids

Pharmacological Activities [80] :

Antioxidant and Free Radical Scavenging Activity:

Methanol extract of Cissus quadrangularis exhibits strong antioxidant and free radical scavenging activity in vitro and in vivo systems mainly due to the presence of β-carotene.

Antimicrobial and Antibacterial Activity:

Methanol extract (90%) and dichloromethane extract of stems possess antibacterial activity against S. aureus, E. coli, and P. aeruginosa and mutagenicity against Salmonella microsome. Antimicrobial activity has also been reported from stem and root extract. The alcoholic extract of aerial part was found to possess antiprotozoal activity against Entamoeba histolytica. Alcoholic extract of the stem showed activity against E. coli.

Bone Healing Activity:

Paste of alcoholic extract of the plant was locally as well as intramuscularly facilitates rapid healing of fracture in albino rats. Ethanol extract (95%) enhances the development of cortical bone and trabeculae in fetal fumor, which may be related to rich content of calcium, phosphorous and phytoestrogenic steroids and shown to influence early regeneration and quick mineralization of bone fracture healing process.

Anti-ulcer Activity:

Methanol extract showed significant antiulcer activity in experimentally induced ulcer in rat model by decreasing gastric secretions and by enhancing glycoprotein levels. Methanol extract produce healing effect on aspirin induced gastric mucosal damage in rats through its antioxidative mechanism. .

Analgesic, Anti-inflammatory and Stimulatory Activity:

Methanol extract possess analgesic, anti-inflammatory and venotonic effects associated with hemorrhoids, anti-inflammatory activity is due to flavonoids especially luteolin and by β-sitosterol. β-sitosterol present in methanol extract has ability to reduce the enzymes MPO indicating a reduction of neutrophils influx in the inflamed tissue. Calcium oxalate, carotene, tetraterpenoids, β-sitosterol, amyrin and anabolic ketosteroids, which are responsible for acceleration of healing and possess anti-inflammatory and analgesic activity. Ethanol extract exhibit protective effect on neutrophils mediated tissue injury induced by aspirin in rats. Methanol extract (90%) and dichloromethane extract of stems possess anti-inflammatory activity against COX-2. The stimulatory effect of extract is probably due to vitamins and is greater than that of the anabolic hormone durabolin.

Central Nervous System Activity:

The root extract possess central nervous system depressant activity indicated by decrease in exploratory behavior. Methanol extract of roots contains saponins which show potent sedative activity and also inhibit spontaneous motor activity in mice.

Figure 10: Leaves of **Azadirachta indica**

2.18.2.3 Azadirachta indica:

Scientific classification [81]

Binomial name: Azadirachta indica

Kingdom: Plantae

Division: Magnoliophyta

Order: Sapindales

Family: Meliaceae

Subfamily: Melioideae

Genus: Azadirachta

Species: indica

Synonyms: Antelaea azadirachta (L).

Vernacular names[82]

English: Neem, Indian lilac Margosa tree

Hindi: Nim, Nimb, Neem

Marathi: Kadu-Limba

Sanskrit: Arishtha, Nimba

Tamil:	Vembu
Punjabi:	Nimm
Malayalam:	Arya Veppu
Oriya:	Nimba
Gujarati:	Limdo
Telugu:	Vepa
Kannada:	Bevu
Konkani:	Kodu nimb

Ayurvedic properties [83]

Rasa:	Madhur (sweet),
Tikta :	bitter
Guna:	Guru (heavy),
Snigdha :	oily
Virya :	Shita (cold)
Vipaka :	Madhur (sweet)
Doshakarma :	Tridoshashamak (alleviate all three Dosha- Vata, Pitta, Kapha)

Medicinal uses of Azadirachta indica in Ayurveda [84]

Leaf: Leprosy, eye problem, epistaxis, intestinal worms, anorexia, ulcers.

Bark: Analgesic, alternative and curative of fever.

Flower: Bile suppression, elimination of intestinal worms and phlegm.

Fruit: Relieves piles, intestinal worms, urinary disorder, epistaxis, phlegm, eye Problem, diabetes, wounds and leprosy.

Twig: Relieves cough, asthma, piles, phantom tumour, intestinal worms, spermatorrhoea, obstinate urinary disorder, diabetes.

Gum: Effective against skin diseases like ringworms, Scabies, Wounds and Ulcers.

Seed: Leprosy and intestinal worms.

Oil: Leprosy and Intestinal worms.

Root, bark, leaf, flower and fruit together: Blood morbidity, Biliary Afflictions Itching, Skin Ulcer, Burning sensation and Leprosy.

Botanical description[85]

Stem: A medium to large tree, 15–20 m in height

Bark: Grayish to dark grey.

Leaves: The opposite, pinnate leaves are 20–40 centimetres (7.9–16 in) long, with 20 to 31 medium to dark green leaflets about 3–8 centimetres (1.2–3.1 in) long. The terminal leaflet is often missing. The petioles are short. These leaves are also used in many Indian festivals by making them into garlands

Flowers: Azadirachta indica flowers are white and fragrant and arranged axillary, normally in more-or-less drooping panicles which are up to 25 cm long. The inflorescences, which branch up to the third degree, bear from 150 to 250 flowers. An individual flower is 5–6 millimeters long and 8–11 millimeters wide. Female flowers and male flowers exist on the same individual.

Fruits: Neem fruits are bitter taste.The fruit is a smooth glabrous olive-like drupe which varies in shape from elongate oval to nearly roundish. When riped are 1.4–2.8 cm. The fruit skin (exocarp) is thin and the bitter-sweet pulp (mesocarp) is yellowish-white and very fibrous. The mesocarp is 0.3–0.5 cm thick. The white, hard inner shell (endocarp) of the fruit encloses one, rarely two or three, elongated seeds (kernels) having a brown seed coat.

Seeds: Azadirachta indica seeds are bitter in taste.

Chemical profile of Azadirachta indica[86]

A. indica has been the subject of extensive phytochemical studies because of the varied biological effects of its medicinal purposes. The various Ingredients obtained from different parts of A. indica plant based on their chemical nature are as under:

From leaves:

Tannin, β-sitosterol and its glucoside,24-methylene-cycloartenol, 4,14, α-dimethyl-5-α-ergosta-8,24(28)-dien-3β-o1, 4α-methyl-5α-ergosta-8, 24(28)-dien-3β-o1, nimatone, nimbinene, 6-desacetyl nimbinene, nimbolins , vanilic acid, catechol,camprsterol, stigmasterol, sitosterol, β-amyrin, lupeol, nimbin, nimbidin, nimbinin, sugiol, kulinone, kulactone, kulolactone methyl kulonate, 6β-hydroxy-4-stigmasten-3-one and 6β-hydroxy-4-campesten-3-one. Sulphurous compounds - A number of cyclic tri and tetrasulfides have been isolated from the leaves.

From flowers:

Azadirachtin, azadirachtanin, azadirone, azadiradione and epoxyazadiradione, isoazadirolide, nimbandiol, nimbinene, 6-desacetylnimbinene, nimbin, nimbocinolide, nimbolide, nimocinolide, isonimocinolide, nimocinone, 2',3' – hydrosalannol,kaempferol-3-0-β-glucoside, myricetin and 3-L-arabinoside (melictrin), 3-0-α- L- rhanmoside and 3-0-rutinoside, quercetin, its 3-galactoside, 3-0-L rhamnoside and 3-0-rutinoside, nimbaflavones, scopoletin, β-sistosterol and

its β-D-glucoside, amino acids, β-carotene, carbohydrates, n-hexacosanol, nonacosanol, protein and vitamins

From fruits:

Azadirachtin, azadirachtol, azadirachnol, deacetyl – azadirachtinol (= 3 tigloylazadirachtol), azadiradione, an isomer of epoxya zadiradione,17 epi and 17-B hydroxyazadiradione, gedunin, 7-hydroxygedunin, melianone, nimbiol, nimboeinol (7-acetoxy-7-hydroxyazadiradione), nimocin, 7-deacetoxy nimolicinol, nimolinone, nimbocalcin and nimbocetin, 21,23:24,25 diepoxytirucall-7-en-21-0l salannin

Seeds : benzyl alcohol, β-sitosterol, thioamyl alcohol, arachidic, behenic, linoleik, oleic, palmitic and stearic acids, kaempferol and its 3-glucoside, quercetin-3 galactoside and myricetin-3-L-arabinoside, azadirachtin and margosene

From seed oil:

Tocopherol, arachidic, linoleik, margosic, myristic, oleic, palmitic and stearic acids, azadirone azadiradione, epoxyazadiradione (nimbinin), gedunin,meldenin, meliatriol, nimbine, nibinene, 6-desacetyl nimbinene, nimbidin, nimbidiol, 6-0-acetylnimbandiol, nimbidic acid, salanin, 3-desacetylsalannin, salannol and its acetate, salannolide, vepinine, vilasinin 1,3-diacetylvilasinin, 1-tigloyl-3-acetyl-vilasinin, and tiglic acid

in addition, azadirachtin, 22-23-dihydro-23-β-methoxy-azadirachtin (vepao)and its C-23epimer (isovepaol), 7-desacetyl-7-benzoyl derivatives of azadirone,

azadiradione, epoxyazadiradione, 2-dihydro epoxyazadiradione, 1β, 2β-diepoxyazadiradione, 7-desacetyl-7-benzoyl-gedunin, acetylneotrichilenone, nimbidin, nimbidinin,, salannic

From stem bark:

Gedunin, 7- deacetoxy-7-oxogedunin, fraxinellone, nimbolin cycloeucalenone,melianin

From wood:

Margosinolide, isomargosinolide, desacetylnimbinolide and desacetyl isonimbinolide , cycloeucalenol, 24-methylene cycloartenol and β-sitosterol

From root:

Nimbin and Nimbidin

From root bark:

Besids aesculetin, campesterol, 6-hydroxy-7-methoxy-coumarin, 4 α-6α-dihydroxy-A-homoazadirone, isomeldenin, meldenindiol, 17-acetoxy-meliacin,6-0-acetylnimbandiol,desacetylnimbin, nimocinol, isonimolicinolide and nimolinolic Products and preparations of almost every part of the tree are used as soil fertilizer, insect repellant, insecticide, animal fodder, dye was, fuel, lubricant, soap, mouth hygiene products and in traditional medicine.

Biological effects

Antioxidant Activity

The study conducted by Lok Ranjan evaluates the ROS scavenging activity of crude extractof Azadirach indica and different solvent fractions of Azadirach indica. The chemical investigations of leaves of Azadirachta indica resulted in isolation of several putative pharmacophores viz. nimbidin, azadirachtin, flavonoids like quercetin and rutin, phytosterol like β- sitosterol. Quercetin is a bioflavonoid with potent antioxidant property Azadirachta indica showed higher activity than that of α-tocopherol and BHA at all tested concentrations and aqueous/methanol fraction was the most active. [87]

In Another study showed that leaf and bark fraction extracts of Azadirachta indica evaluated for their antioxidant activity, total phenolic (TP) and total flavonid (TF) contents. HPLC method was employed to quantify the amount of Azadirachtin and nimbin present in the seed, leaf and the bark extracts of neem. The result showed that the highest azadirachtin content was found in the methanolic extract of the seed (3300 μg/g dw). Similarly, the hexane fraction of bark showed the highest nimbin content (271 μg/g dw) followed by the methanolic extract (260 μg/g dw). [88]

Gayatri Nahak assessed A.indica to see the antioxidant activity and it was compared with that of Melia azedarach leaves by in vitro method DPPH.The study showed that both the plants have antioxidant activity by DPPH scavenging

assay leaf Melia Azedarach contains highest amount of phenolic compounds exhibited the greatest anti-oxidant activity in comparison to Azadirachta indica Neem. The high scavenging property may be due to hydroxyl groups existingin the Phenolic compounds. [89]

Antimicrobial:

Varying concentrations of methanol extracts of A.indica(200mg/ml, 150 mg/ml, 100mg/ml, 50mg/ml, 25mg/ml) prepared by using disc diffusion method showed maximum inhibition on Bacillus pumillus, Pseudomonas aeruginosa and Staphylococcus aureus in an ascending order when compared With Gentamycin 200mg.[90]

Saradhajyothi Koona showed that antibacterial potential, the agar well diffusion assay was used against Gram-negative and Gram-positive bacteria. Penicillin was used as positive and negative controls, respectively. Methanol extract showed the highest and chloroform extract showed moderate antibacterial activity. [91]

Antifungal activity

Azadirachta indica extract at a concentration of 15µg/ml (below MIC) was observed to distort the growth pattern of the organisms tested. This finding supports the use of Azadirachta indica seed oil in the treatment of various skin infections. [92]

Hypoglycemic activity:

Akbar Waheed found that Azadirachta indica has significant hypoglycemic activity in high dose and can be successfully combined with oral hypoglycemic agent in type -2 diabetic patient. [93]

Antinociceptive activity

Tail flick reaction time was significantly increased in rats with both leaf extract and seed oil. Naloxone pretreatment partially reversed the antinociceptive effect of leaf extract and seed oil. Glaysial acitic acid (GAA)induced writhing was reduced with both leaf extract and seed oil. Pretreatment with Naloxone partially reversed the inhibitory effect of leaf extract and seed oil on GAA induced writhing. They showed that both the preparations of Azadirachta indica leaf extract and seed oil possess antinociceptive activity in rats, leaf extract being more potent than seed oil. [94]

Antiulcer activity:

Azadirachta indica significantly reduced ulcer index in a dose dependent manner supporting its cytoprotective effect, which may be mediated by prostaglandins. The ulcer healing effect of Azadirachta indica could be attributed to its predominant effect on the mucosal defensive factors. [95]

Hepatoprotective activity:

Yanpallewar studied hepatoprotective role of fresh juice of tender leaves of Azadirachta indica in the dose of 200 mg/kg body wt. p.o. inhibited Paracetamol - induced lipid peroxidation and prevented depletion of sulfhydryl groups in liver cells after Paracetamol administration There was an increase in serum marker enzymes of hepatic damage (aspartate transaminase, alanine transaminase and alkaline phosphatase). Azadirachta indica pretreatment stabilized the serum levels of these enzymes. Histopathological observations of liver tissues corroborated these findings. [96]

The aqueous extract of Azadirachta indica leaves was found to offer hepatoprotective against paracetamol induced liver necrosis in rats. The elevated levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transpeptidase (GGT) indicative of liver damage were found to be significantly reduced on administration of the Azadirachta indica aqueous leaf extract. [97]

Antifertility activity:

Azadirachta indica leaf extract was found to possess anti-fertility properties after oral administration in male mice.

The effect of aqueous leaf extract of Azadirachta indica on reproduction was studied on male albino rats with the aqueous leaf extract adminstratin daily at 250mg/kg body wt., and 350mg/kg body wt., respectively for a period of 30days.

Significant decreases in the weights of testis, epididymes and seminal vesicle were observed. A dose related reduction in the testicular sperm count, epididymal sperm count and motility and abnormal sperm count were observed. The results showed that Azadirachta indica has effects on male rat reproduction, affecting the sexual behavior and epididymal sperm concentration. [98]

Subhash chander reported that A.indica has a chemopreventive effect against induced for stomach tumors in murine model. Because of lack of toxicity and ubiquitous bioavailbility, A indica may play a promising role in future drug discovery and development as far as chemoprevention of cancer is concerned. Azadirachta indica aqueous extractof leaf effectively suppressed oral squamous cell carcinoma induced by 7,12-dimethylbenz anthracene , as revealed by reduced incidence of neoplasm. Azadirachta indica leaf extract showed chemopreventive effect in the oral mucosa by modulation of glutathione and its metabolizing enzymes. Protective effect of Azadirachta indica leaf extract exerts its protective effect in Nmethyl- N-nitro-N-nitrosoguanidine (MNNG) (a carcinogenic material)-induced oxidative stress has also been demonstrated by the reduced formation of lipid peroxides and enhanced level of antioxidants and detoxifying enzymes in the stomach. [99]

Figure 11 : Plant and seeds of Linum usitatissmum L

2.18.2.4. Linum usitatissmum

Binomial name:	Linum usitatissmum
Kingdom:	Plantae
Division:	Magnoliophyta
Order:	Mangoliopsida
Family:	Malvales
Subfamily:	Dipterocarpacee
Genus:	Linum
Species:	usitatissmum

Vernacular names[100]

English	Linseed,
Hindi	Alsi
Marathi	Jawas

Description :

Flax, *Linum usitatissimum*, is an upright annual plant growing to 1.2 m (3 ft 11 in) tall, with slender stems. The leaves are glaucous green, slender lanceolate, 20–40 mm long and 3 mm broad.

The flowers are pure pale blue, 15–25 mm diameter, with five petals; they can also be bright red. The fruit is a round, dry capsule 5–9 mm diameter, containing several glossy brown seeds shaped like an apple pip, 4–7 mm long.

In addition to referring to the plant itself, the word "flax" may refer to the unspun fibers of the flax plant. New Zealand flax is not related to flax but was named after it, as both plants are used to produce fibers

Habitat:

Cultivated throughout India.

Parts used in the preparation :

Oil.

Chemical constituents:

Oil contains moisture, protein, fats, mineral matters as calcium, phosphorus, carotine , thiamine, riboflavin, niacin, pantothenic acid choline and Vit E.

Flax seed[101]

Nutritional value per 100 g (3.5 oz)

Energy	2,234 kJ (534 kcal)
Carbohydrates	28.88 g
Sugars	1.55 g
Dietary fiber	27.3 g
Fat	42.16 g
Saturated	3.663 g
monounsaturated	7.527 g
polyunsaturated	28.730 g
Protein	18.29 g
Thiamine (vit. B_1)	1.644 mg (143%)
Riboflavin (vit. B_2)	0.161 mg (13%)
Niacin (vit. B_3)	3.08 mg (21%)
Pantothenic acid (B_5)	0.985 mg (20%)

Vitamin B_6	0.473 mg (36%)
Folate (vit. B_9)	0 µg (0%)
Vitamin C	0.6 mg (1%)
Calcium	255 mg (26%)
Iron	5.73 mg (44%)
Phosphorus	642 mg (92%)
Potassium	813 mg (17%)
Zinc	4.34 mg (46%)

Uses[102]

Oil is used for external application it is a common base for liniments.Crushed linseed is applied in the form of poultice for the relief of local inflammations, ulcers, boils and carbuncles.

Figure 12 : Shorea robusta plant and resin

2.18.2.5. Shorea robusta (103)

Binomial name:	Shorea robusta
Kingdom:	Plantae
Division:	Magnoliophyta
Order:	Mangoliopsida
Family:	Malvales
Subfamily:	Dipterocarpacee
Genus:	Shorea
Species:	Robusta

Vernacular name:

English: Sal

Hindi: Sal

Marathi : Sal

Other names: Shala, Sarai, Sargi, Salwa, Sakhu, Sakher, Shal, Kandar and Sakwa

Description [104]

Large desiduous tree 18-30 m in ht. With smooth or longitudinally fissured reddish brown or gray bark, leaves simple, ovate, oblong, acuminate, tough, coriacious, base cordate or rounded, lateral nerves 12-15 pairs. Flowers yellowish.

Habitat:

North east and central India.

Chemical constituents [105]

Presence of 2-(2-iminoacetic acid) – 3- (2H)- benzo- furanone glucoside of 4 hydroxy choline, leuconathocyanidin, hopcaphenol, triterpenoids and a terpene alcohol, furfural , monomethylene and dimethyl ether of homocatechol alkyl benzene derivatives, pentosans, linan, tannin, amino acids and fatty acids.

Parts used in the preparation:

Resin

Medicinal uses:

The resin is used in the indigenous system of medicine as an astringent and detergent and is given in diarrhea and dysentery. It is also used as an ingredient of ointments for skin diseases and in the ear troubles. It is also used in the foot care cream. The fruits of the Sal tree are used in the treatment of excessive salivation, epilepsy, and chlorosis. The powered seeds have insecticides properties. The

powered seeds are even used to treat dental problems. It cleanses the skin of oily secretion and is used as the cleanser for washing hair.

Other uses:

The leaves of the Sal tree are used by the tribal people for preparing rice cakes and for smoking. The leaves are used to make platters, bowls, small baskets and many more. Distilled leaves produce an oil which is used in perfumery. It is also used in flavouing chewing gums and tobacco. Its dried and fallen leaves are used as fertilizers. It is used for caulking ships and boats. The oil that comes out from its seed is edible and is known as Sal butter. It is often used in cooking and for the burning in the oil lamps. The seeds of the Sal tree are used for fat extraction. Its oil is even used for adulterating ghee. Tribal people give marriage invitation in the form of folded Sal leaves, with the little bit of turmeric and rice inside it.[105]

In the view of the morbidity and mortality caused by burn injury and presently available treatment and potential of herbal medicine it was considered worthwhile to develop a herbal ointment and compare its efficacy with Silver sulfadiazine ointment with and without systemic administration of antioxidants.

After reviewing the literature following research question was formulated using the PICOT format

Which of the following is the best treatment out of the following for healing of partial thickness thermal burns in New Zealand white rabbits in comparison with the untreated control group when the treatment is given for 28 days?

1. Ointment silver sulfadiazine alone.
2. New Herbal Ointment alone.
3. Silver sulfadiazine in combination with Hemidesmus indicus.
4. Silver sulfadiazine in combination with Cissus quadrangularis.
5. New Herbal Ointment in combination with Hemidesmus indicus.
6. New Herbal Ointment in combination with Cissus quadrangularis.

4. MATERIAL AND METHODS

4.1 Locus of study

The present study was undertaken in Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha. All the Biochemical procedures were carried out in Central Research Laboratory, DMIMS, Sawangi (M), Wardha.

4.2 Duration of Study

Duration of the study is from October 2011 to October 2013.

4.3 Approval from Institutional Ethics Committee (IEC)

The approval of Institutional Ethical Committee was obtained vide their letter no .**DMIMS (DU)/IEC/2010-11/77 dated 24/11/2010**. The approval of Institutional Animal Ethical Committee was obtained vide their letter no. **DMIMSU/IAEC/2011-12/006 dated 22/06/2011**.

Figure 13: a) Central research Laboratory DMIMS

b) Spectrophotometer

4.4. Chemicals

Malonaldehyde bis (diethyl-acetal): (Merck Schuchardt OHG 85662 Hohenbrum, Germany).

Trichloroacetic acid: (Merck Specialities pvt. Limited).

Thiobarbituric acid: (Loba Chemie pvt. Limited).

Sulphuric acid: (S.D fine chem. Limited).

Sodium sulphate solution: (S.D fine chem. Limited).

n- Butyl alcohol: (S.D. fine chem. Limited).

Superoxide dismutase: (Sigma Cemie pvt. Limited).

Tris buffer: (Loba Chemie pvt. Limited).

Sod.Cacodylate: (HiMedia laboratory pvt. Limited).

Dethyl triamine penta acetic acid (DTPA): (S.D. fine chem. Limited).

Pyrogallol: (S.D. fine chem. Limited).

Paracetamol :(Nicholas Piramal Research center Goregaon (E), Mumbai-400063

Vitamin C: (GSK)Manufactured by- GlaxoSmithKline (gsk),10,MIDC, Ambad, Pathari block, Nashik. 422010.

Drabkin's reagent:

Cyanmethemoglobin standard:

Both were manufactured by - Pathozyme Diagnostics, Plot No. A-115, Kagal Hatkanangale Five Star, MIDC,Tq. Kagal, Dist. Kolhapur.

4.5 Methodology:

4.5.1 Study design (Type of study)

Experimental Randomized Open Study

4.5.2. Animals required

Male rabbits (New Zealand white) procured from the animal house of JNMC Sawangi

Total Number of animals required – 104 (one hundred four)

Inclusion criterion: male rabbits which were disease free with weight between 1-2.5 kg and age 1 to 1 and half years.

Exclusion criterion : female rabbits , diseased animals , animals with the weight less than 1 kg and more than 2.5 kg , animals with age less than one year and more than 1 and half years.

The study did not involve the sacrificing of the animals. The animals in the study were rehabilitated and reused after completion of study and wash out period as judged by biochemical parameters.

4.5.3. Herbal formulations required

The Plant material of Hemidesmus indicus (Root Powder) and Cissus quadrangularis (Stem Powder), Linum usitatissmum (seed oil), Shorea robusta (resin) was obtained from "Rasa-shala" of Mahatma Gandhi Ayurved College, Hospital and Research Centre, Salod (H), Wardha. These preparations were ready to use for medicinal purpose.

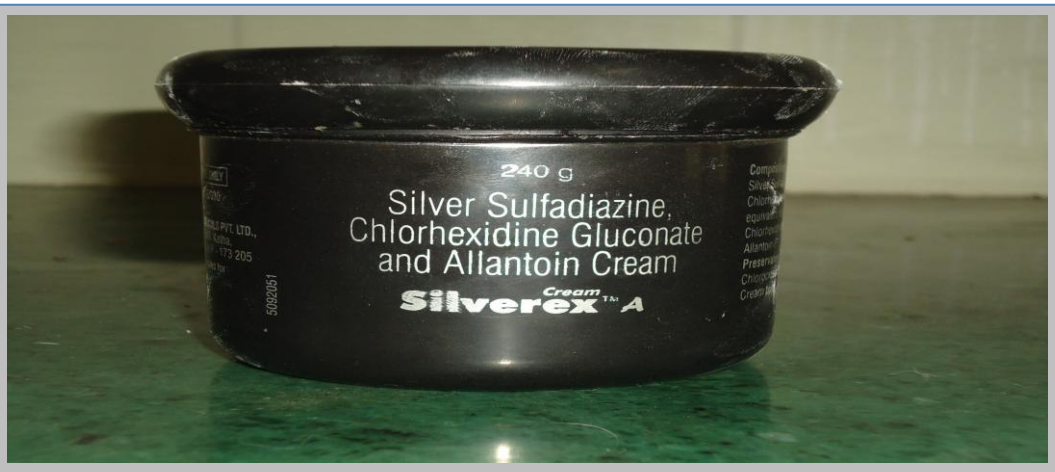
4.5.4 Method of preparation of New Herbal Ointment

New Herbal Formulation was prepared in the Central Research Laboratory DMIMS under sterile conditions. 20 gm of Neem leaves were boiled in the linseed oil 500 ml till the colour of the oil becomes greenish. It was then allowed to cool at the room temperature. It was then triturated with Powdered resin of Shorea robusta 100 gm. The contents were washed 100 times with distilled water to obtain pure form of New Herbal Formulation. The preparation was done as per the previous study by Pathak SS, Patel SS, Borkar MA published in the journal of JNMC [106]

Figure 14: Ointments under Study

(a) New Harbal Ointment (b) Silver sulfadiazine ointment

(a)



STANDARD DRUG : SILVER SULFADIAZINE

4.6. Experimental protocol:

4.6.1 Preparation of Animals:

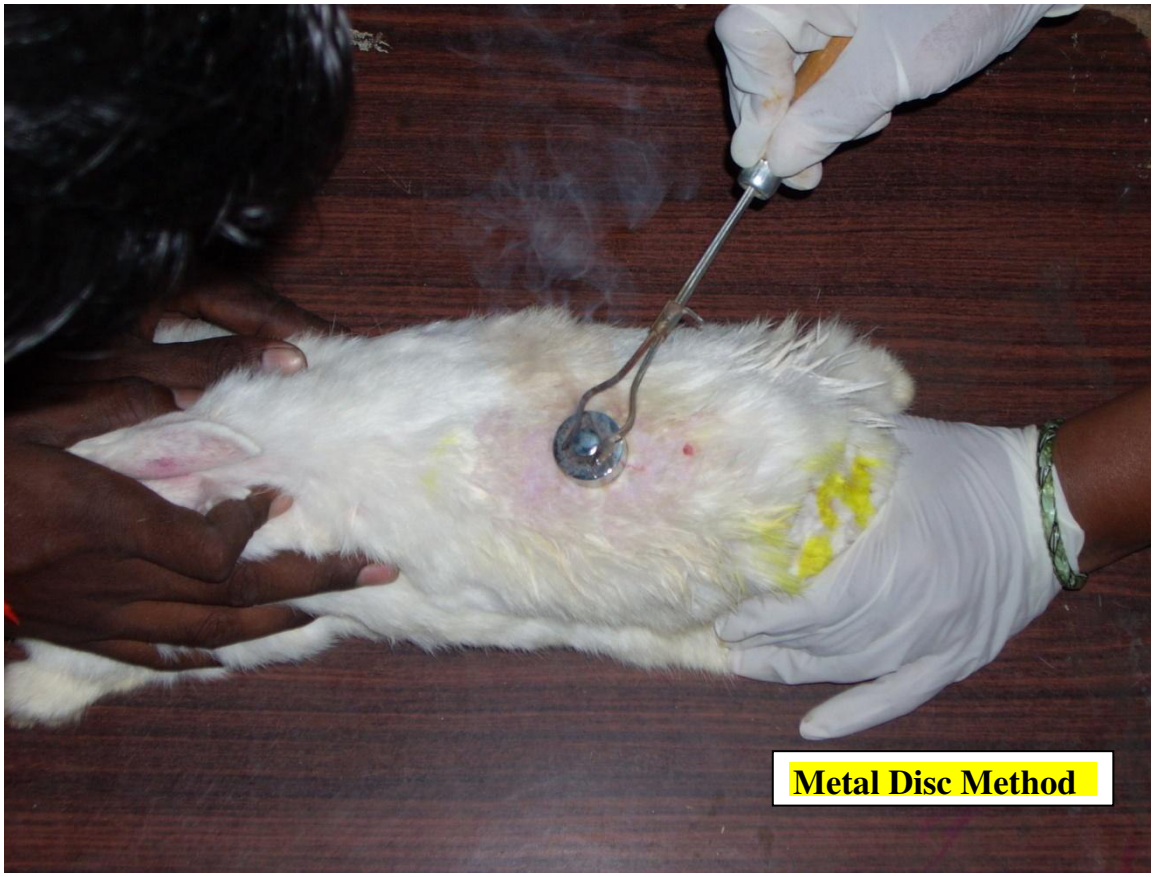
Animals were acclimatized for 8 days in the Central Animal House before experiment. Animals were housed in separate cages under standard condition of light, temperature and humidity. They were fed with standard laboratory chow and provided with water ad libitum.

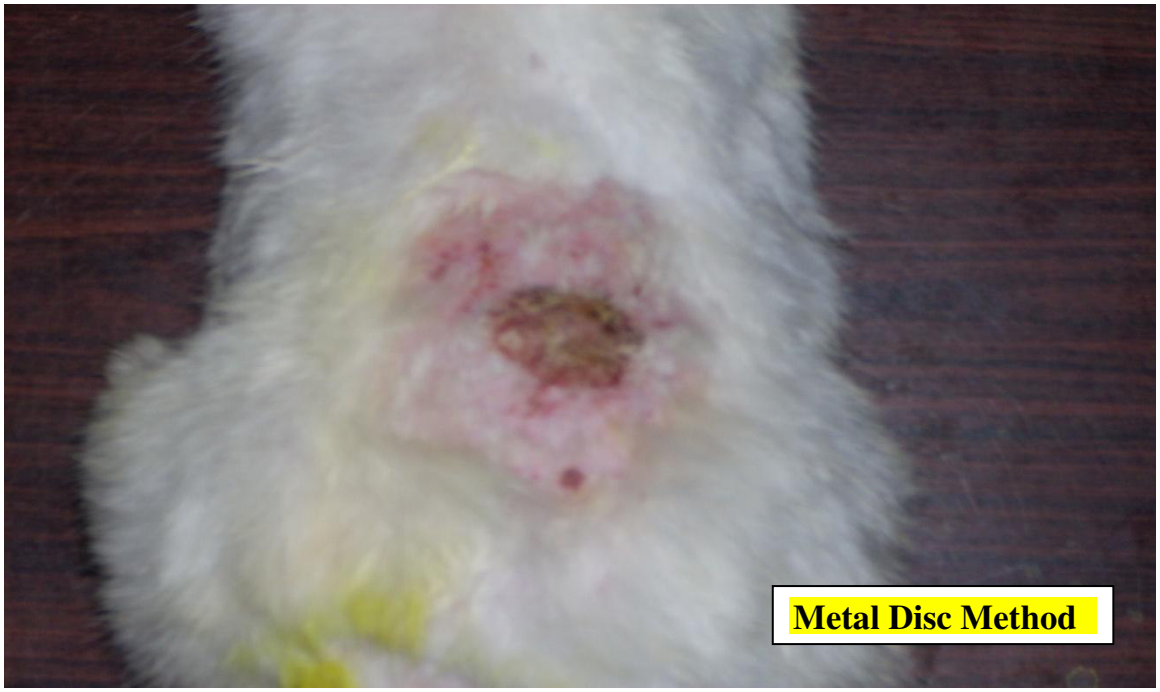
4.6.2 Infliction of Burn Wound:

For producing partial thickness burn in rabbits, the method developed by, Pathak S.S., Patel S.S., Borkar M.A. of Depatment of Pharmacology, Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha , was used as follows [107]

The area on the back of the rabbit was shaved and animal kept for fasting overnight. The next day the animal were anaesthetized using Ketamine in the dose of 50 mg/Kg of body weight I.M. (1 ml/kg of body weight) .A metal disc of wt 50 gm, diameter 2.5 cm (25mm), thickness 1.1cm (11mm) and area 4.910 sq. cm (491.07 sq. mm) was heated in the blue portion of the flame of spirit lamp for 5 minute and then immediately kept on the shaved part for 30 seconds with minimal pressure. **This method was found to be more accurate and convenient in producing the second degree burns in comparison with the molten wax method. The scientific paper in this regard was presented in 56th annual national conference of physiologist and pharmacologist of India, APPICON 24th December 2010, JNMC, Sawangi, Maharashtra.**

Figure 15: Infliction of Burn Wound





4.6.3 Administration and Application of Drugs:

Standard Ointment Silver Sulfadiazine was applied daily on the burn wound. The New Herbal Ointment was applied daily on the burn wound. H. indicus and C. quadrangularis were administered orally in the form of distilled water suspension in the dose of 500 mg/Kg body weight once daily.

4.6.4 Estimation of Wound Contraction:

Wound contraction was monitored by measuring the progressive changes by tracing the raw wound area on a transparent paper on day 1st, 7th, 14th, 21st, and 28th post burn days. The tracing was then transferred to 1 mm^2 graph sheet, from which the wound surface area was calculated. The calculated surface area was then employed to calculate the percentage of wound contraction, taking the initial size of wound as 100% by using the following equation [108]

$$\text{Wound Contraction (\%)} = \frac{\text{Initial Wound Size} - \text{Specific Day Wound Size}}{\text{Initial Wound Size}} \times 100$$

4.6.5 Microbiological Examination:

Wound swabs were taken for the culture and sensitivity reporting on the seventh day of creation of wound to determine the presence of any infection in the wound.

4.6.6 Re-epithelization:

Falling of eschar leaving no raw wound area was considered as end point of complete reepithelization and the days required for this was taken as period of epithelization.

4.6.7 Histopathological Examination:

Histopathology was done for assessing the grading of epithelial regeneration. Samples were collected by punch biopsy on 14th day in accordance with the study conducted by Lemo N et[109] Sample was fixed in 10% neutral buffered Formalin. Histological examination was performed by staining with Haemotoxylin and Eosin stain and Masson's Trichrome stain.

Figure 16: Evaluation Procedures

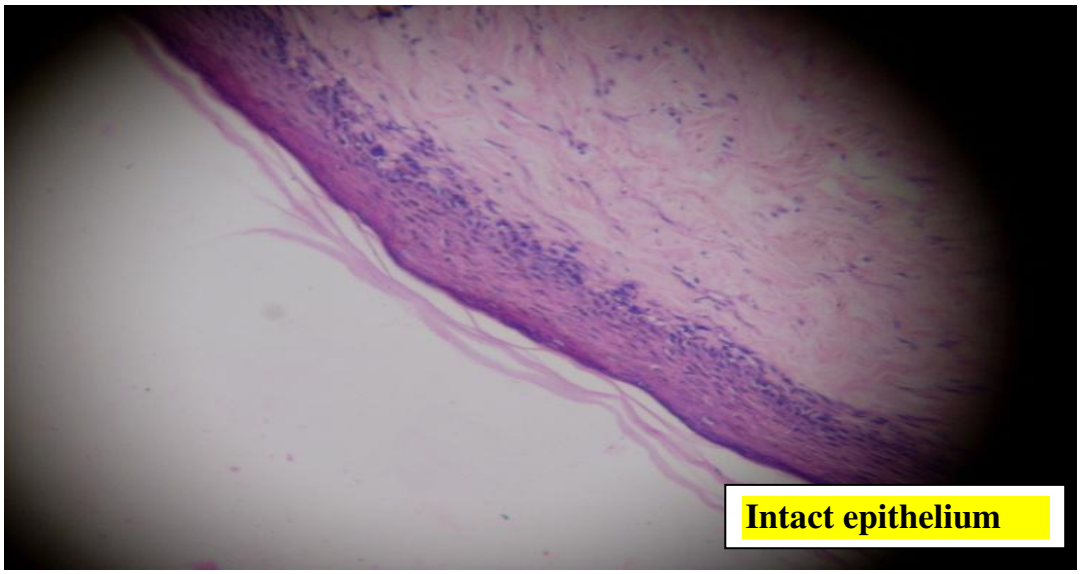
BLOOD COLLECTION FOR MDA AND SOD ESTIMATION

MEASUREMENT OF WOUND CONTRACTION ON TRANSPARENT SHEET

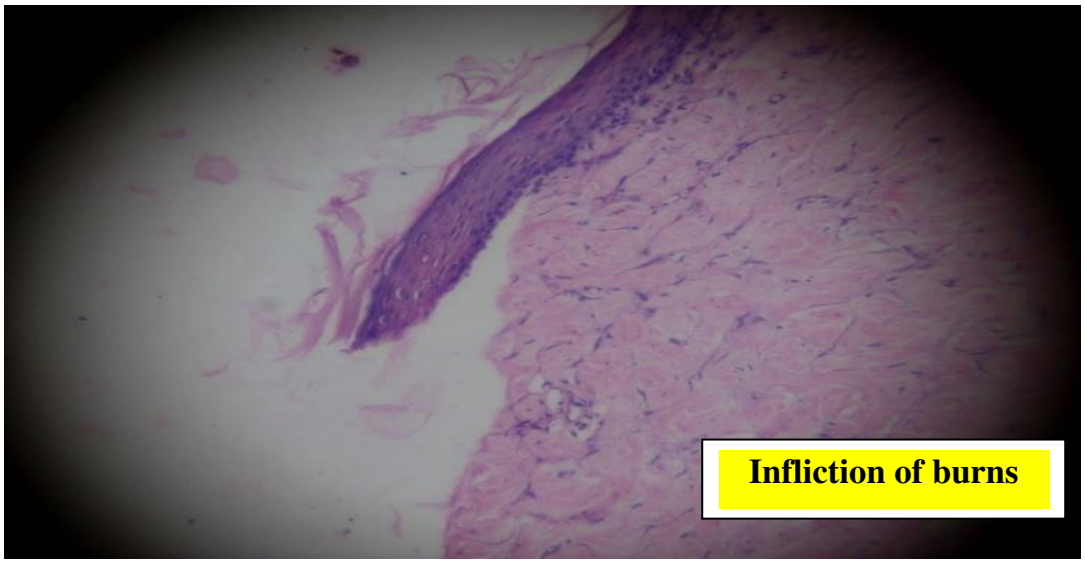
SAMPLE COLLECTION FOR MICROBIOLOGICAL EXAMINATION

SAMPLE COLLECTION FOR HISTOPATHOLOGICAL EXAMINATION

Figure 17: Histopathology



(a)



(b)

Healing status of wound was assessed by following Scoring Criteria:

Table 5 : Criteria for Scoring of Wound Healing Status by Sultana et al. [110]

Sr. No.	Histological Findings	Score
1.	Amount of granulation tissue	Profound – 1 Moderate - 2 Scanty - 3 Absent - 4.
2.	Inflammatory infiltrate	Profound – 1 Moderate – 2 Few – 3
3.	Collagen fiber orientation	Vertical – 1 Mixed- 2 Horizontal - 3
4.	Pattern of Collagen	Reticular – 1 Mixed – 2 Fascicle - 3
5.	Amount of early collagen	Profound – 1 Moderate – 2 Minimal – 3 Absent – 4
6.	Amount of mature collagen	Profound – 1 Moderate – 2 Minimal - 3

Total healing score of each case was calculated by adding the score of individual criteria. Lower scores indicated poorer wound healing while higher scores were indicating better healing process.

Healing status was graded as follows:

(a). Good : 16 – 19 (b). Fair: 12 – 15 (c). Poor: 08 – 11

Grading of epithelial regeneration was done by using following parameters:

Table 6: Criteria for Grading of Epithelial Regeneration[110]

Sr. No.	PARAMETER	GRADING
1.	Inflammatory Response	Mild
2.	Granulation Tissue Formation and/or Angiogenesis	Moderate
3.	Repair of Connective Tissue and Epithelium and/or Remodelling	Prominent

4.6.8 Estimation of MDA and SOD

Markers of oxidative stress Malondialdehyde (MDA) and Superoxide Dismutase (SOD) were carried out spectrophotometrically on day zero (a day before the production of the burn wound) and then after 24 hours .The subsequent readings were taken on each 7th day till the levels return to the MDA and SOD levels of day zero respectively. Both the methods were standardized in the Central Research Laboratory of D.M.I.M.S. (Deemed University), Sawangi (Meghe), Wardha using the following standard operating procedures.

4.6.8.1 Estimation of Malondialdehyde (MDA) in serum [111]

(Method of Marklund S & Marklund : 1974 was used)

Principle:

Thio-barbituric acid reacts with Malonaldehyde, one of the Aldehyde products of lipid peroxidation to give a colored product which is extracted in Butanol and absorbance measured Spectrophotometrically at 530 nM.

Reagents:

Malonaldehyde bis (diethyl-acetal):

Trichloroacetic acid (TCA) – 20% .

Thiobarbituric acid (TBA) -0.67 gm%.

Sulphuric acid - 0.05 M.

Sodium sulphate solution:

n- Butyl alcohol:

Standard solution: Malonaldehyde bis (diethyl-acetal) was dissolved in 0.05M sulphuric acid to prepare 10 uM solution. This 10uM solution was further diluted to obtain standard MDA of different concentration like 1 nmole/ml, 2 nmole/ml, 3 nmole/ml, and 4nmole/ml ---------- upto 10 mole/ml.Before starting with the samples a standard graph of concentration against absorbance was plotted. A straight line graph indicates persistency and accuracy of the procedure.

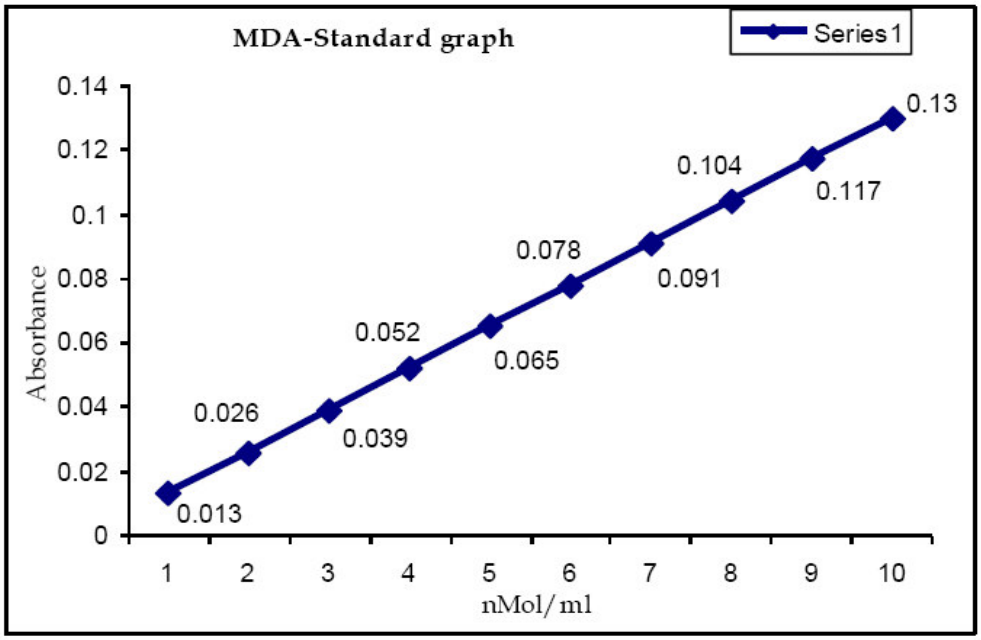
Table 7: Addition of reagents for estimation of MDA.

	Standard (ml.)	Test (ml.)
Standard sol.	0.200	---
Sample	---	0.200
TCA (20%)	2.00	2.00
TBA (0.67%)	0.800	0.800

2.00 ml of TCA was added to each test tube, (0.200) ml of standard or sample was added to standard and test tubes respectively. 0.800ml of TBA (0.67%) was also was added to each test tube. It was mixed well and then kept in boiling water bath for 30 min. After30 min. the test tubes were cooled under tap water. 4.00ml of n-Butyl alcohol was added to each test tube. All the tubes were centrifuged at 3000 rpm for 10 min. The absorbance of supernatant was read at 530nM using n-Butyl alcohol as blank.

Calculations: MDA

Conc. (nMol/ml) test = Abs. of test/Abs. of std. X Conc of std. (nMol).



Graph 1: Standard Graph MDA

4.6.8.2. Estimation of Superoxide dismutase (SOD)[111]

(Marklund S & Marklund : 1974)

Principle:

Tris oxidises pyrogallol. SOD prevents the oxidation of pyrogallol this prevention is measured by spectrophotometrically 420nM.

Superoxide dismutase enzyme.

Reagents:

Superoxide dismutase.

Tris cacodylic acid buffer (p^H 8.2).

Tris - 3.025 gm.

Sodium cacodylate - 4.00 gm.

Dethyl triamine penta acetic acid (DTPA) – 0.200gm.

Pyrogallol reagent (2.5 mM).

All dissolved in 450.00 ml of distilled water. The p^H of the solution was adjusted to 8.2. The volume was made upto 500.00 ml with distilled water.

Procedure:

The assay mixture in a 3.00 ml volume consisted of 300 ul of Pyrogallol (0.2mM).

Table 8: Addition of reagents for estimation of SOD.

Reagents	Control (ml)	Standard (ml)	Test (ml)
Tris buffer	2.7	2.7	2.699
Standard/Haemolysate	---	0.001	0.001
Working Pyrogallol	0.300	0.300	0.300
Total volume	3.000	3.000	3.000

The reaction mixture prepared in three sets of test tubes according to table

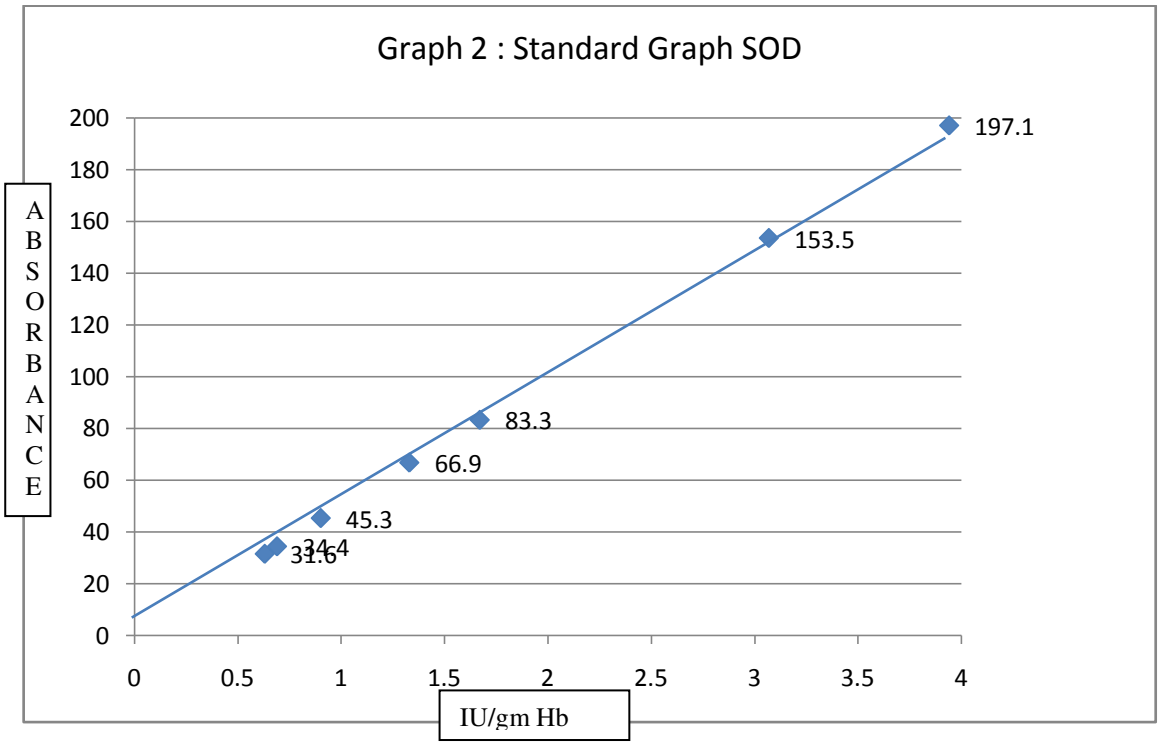
Control – Neither test nor standard was added to assay mixture to obtain uninhibited auto oxidation of Pyrogallol.

Standard – Known amount of SOD units in different concentrations were added to assay mixture to achieve inhibition of Pyrogallol auto-oxidation.

Test – The haemolysate of individual samples were added in place of standard SOD units.

Pyrogallol was added after the addition of all other reagents to reaction. Initial 90 sec. period was considered as induction period of enzyme. So after 90 sec. change in absorbance at 420 nm at 20 sec. interval was recorded for a period of 270 sec (4.5 min.). Change in absorbance per min. was calculated and percentage of inhibition in standard and test was calculated using the formula

$$\% \text{ of inhibition} = 100 - \frac{\text{Diff. Blank} - \text{Diff. STD/ Diff. Sample}}{\text{Diiff.blank}} \times 100$$



For standard graph in place of sample different concentrations of standard were added and absorbance was read at 420nM.

Before starting with the samples a standard graph of concentration against absorbance was plotted. A straight line graph indicates persistency and accuracy of the procedure.

4.6.8.3. Estimation of stress index and protective index

Stress index and Protective index are calculated using the following formula

$$\text{Stress index} = \frac{\text{MDA level on a particular day}}{\text{Corresponding SOD levels on the same day}}$$

$$\text{Protective index} = \frac{\text{SOD level on a particular day}}{\text{Corresponding MDA levels on the same day}}$$

4.7. Process for calculation of sample size:

Assistance of statistician was sought for determination of sample size. The details of the procedure is as under-

Sample size calculation based on pilot study:

For the estimation of sample size a pilot study was conducted with five animals. The partial thickness burns were produced in each animal using the process described above. Animals were provided with the standard diet and water ad libitum, no antibiotic coverage was provided. Baseline readings of MDA and SOD were taken. The MDA and SOD estimation was again performed after 24 hrs,7 days,14 days and 21st day of burn wound production.

Table 9: Sample size calculation based on pilot study

MDA estimation nmol/ml

No	Baseline	24hrs	7days	14days	21days	Difference	Percentage increase
1	1.298	1.363	10	12.14	21.6	20.3	1563
2	1.51	1.61	3.5	16.6	26.6	25.09	1661
3	2.01	2.05	3.6	14.1	25.8	23.79	1183
4	4.4	5.2	4	18.8	30.4	26	590
5	1.165	1.163	7.8	18.9	27.08	25.91	2224
Mean ±sd	2.076±1.3	2.277±1.6	5.78±2.9	16.108±2.0	24.47±1.8	24.21±2.3	1444.2±605

Table 10: Sample size calculation based on pilot study

SOD estimation units/gm Hb

No	Baseline	24hrs	7days	14days	21days	Difference	Percentage decrease
1	0.148	0.15	0.24	0.193	0.06	0.088	59.4
2	0.078	0.089	0.128	0.127	0.025	0.053	67.9
3	0.1	0.11	0.14	0.136	0.028	0.072	72
4	0.125	0.127	0.17	0.188	0.033	0.092	73.6
5	0.14	0.147	0.177	0.194	0.008	0.132	94.2
Mean± sd	0.118±0.02	0.124±0.02	0.171±0.04	0.167±0.03	0.030±0.018	0.087±0.029	73.4±12.8

The sample size was estimated for the expected event rate between 40-50%and protection fold of 60 -50% using the formula described by **Dulao Wang and Ameet Bhakai** [112]

$$n = \frac{\left[z(\alpha/2)\sqrt{2\pi(1-\pi)} + z(\beta)\sqrt{\pi_1(1-\pi_1)+\pi_2(1-\pi_2)} \right]^2}{\delta^2}$$

where

α = the Type I error rate

β = the Type II error rate

$\pi 1$ = the expected event rate

$\pi 2$ = the expected protection fold

$\pi = (\pi 1 + \pi 2) / 2$

$\delta = \pi 1 - \pi 2$

$z\ (\alpha / 2)$ = constant from the standard normal distribution depending on the value of α

$z\ (\beta)$ = constant from the normal distribution depending on the value of β

For the given study:

<u>Step I : for the event rate of 40% and protection fold of 60%</u>

$\alpha = 5\% = 0.05$ (Type I error)

$\beta = 20\% = 0.209$(Type II error)

$\pi 1$ = the expected event rate = 40

$\pi 2$ = the expected protection fold = 60

$\pi = (\pi 1 + \pi 2) / 2 = 50$

$\delta = \pi 1 - \pi 2 = 20$

$z\ (\alpha / 2)$ = constant from the standard normal distribution depending on the value of $\alpha = 1.96$

$z\ (\beta)$ = constant from the normal distribution depending on the value of β=0.842

Putting the values in the formula

$$n = \frac{\left[z\ (\alpha / 2) \sqrt{2\pi\ (1 - \pi)} + z\ (\beta) \sqrt{\pi_1 (1 - \pi_1) + \pi_2 (1 - \pi_2)} \right]^2}{\delta^2}$$

we get

n = **7.08 per group** for the event rate of 40 and protection fold of 60%

Step II : for the event rate of 60% and protection fold of 40%

$\alpha = 5\% = 0.05$ (Type I error)

$\beta = 20\% = 0.20$(Type II error)

$\pi 1$ = the expected event rate = 60

2 = the expected protection fold = 40

$\pi = (\pi 1 + \pi 2) / 2 = 50$

$\delta = \pi 1 - \pi 2 = -20$

$z\ (\alpha / 2)$ = constant from the standard normal distribution depending on the value of $\alpha = 1.96$

$z\ (\beta)$ = constant from the normal distribution depending on the value of β=0.842

Putting the values in the formula

$$n = \frac{\left[z\ (\alpha / 2) \sqrt{2\pi\ (1 - \pi)} + z\ (\beta) \sqrt{\pi_1 (1 - \pi_1) + \pi_2 (1 - \pi_2)} \right]^2}{\delta^2}$$

we get

n = **11.2 per group** for the event rate of 60 and protection fold of 40%

Table 11: Sample size per group for the protection fold of 40% and 60%

Expected event rate	Expected protection fold	Sample size per group
40%	60%	7.08
60%	40%	11.2

To compensate for any drop outs for unforeseen events.

Final sample size taken = 13 per group

This can give statistical significance with expected protection fold anywhere between 40%-60%.

This sample seize was calculated in consultation with the statistician

4.8 Randomization

Procedure for simple randomization [113]

Step I**:** Healthy rabbits of either sex weighing between 1.5kg-3kg were considered for randomization.

Rabbits were assigned numbers from 1 to 104

Chits having the numbers 1 to 104 were designed.

Step II: A third person not associated with the study was asked to pick up the chit from the set of chits.

For example if the person takes out the chit number 24 then the rabbit with 24 number was taken

Step III: For assigning the rabbit particular group in the study a set of chits from 1 to 8 was prepared

Now a third person not associated with the study was asked to pick up the chit and the rabbit was included in that group

For example if for the 24 number rabbit chosen in the above example the chit of number 4 comes then the rabbit is included in the fourth group of the study

The procedure is repeated till there were 13 rabbits in each group

4.9. Grouping of animals

104 rabbits with age group of 6 -8 months and weight 1.5 to 2 kg were divided into 8 groups of 13 animals each by the method of random allocation

Table 12: Grouping of animals

No.	GROUPS	NUMBER OF ANIMALS
1	**Control** 1) Healthy control without burn wounds	13
2	**Control** 2) Group with burn wound not receiving any treatment	13
3	**Standard** treated with Silver Sulfadiazine	13
4	**Test 1** treated with New Herbal Ointment	13
5	**Test 2** treated with silver sulfadiazine +Hemidesmus indicus	13
6	**Test 3** treated with silver sulfadiazine +Cissus quadrangularis	13
7	**Test 4** treated with New Herbal Ointment + Hemidesmus indicus	13
8	**Test 5** treated with New Herbal Ointment + Cissus quadrangularis	13

4.10. Statistical analysis (Statistical technique used)

Considering the nature and type of data to be generated in the present study following statistical measures were used.

Mean and Standard deviation:

Data relating to various characteristics of animals under study were represented in form of some basic statistical tools like mean and standard deviation (Mean + S.D).

95% Confidence Interval:

The exact value of parameter as per animals

data for different doses of treatment, point estimates were used with 95 % of confidence interval.

One Way ANOVA and Post –Hoc Dunnet's test

To test the effects of various treatments, One Way ANOVA technique was used followed by Post –Hoc Dunnet's test.

Student's t test paired and unpaired:

Two compare between two different groups or between the same groups before and after the treatment

Chi square test:

To compare between the subgroups in same group.

Statistical software SPSS13.0 and SYSTAT 12.0: To handle the large data and reduce the error for several variables, Statistical software SPSS13.0 and SYSTAT 12.0 and statistical functions in MS-Excel 2007 were used.

5. OBSERVATIONS AND RESULTS

Table : 13 - Percentage of Wound Contraction

GROUP	PERCENTAGE OF WOUND CONTRACTION (Mean±S.D.)				
	Day 1	Day 7	Day 14	Day 21	Day 28
UC	0±0	20.63±2.67	35.23±2.55	49.53±4.19	60.37±5.44
SS	0±0	21.17±5.13	38.17±8.58	55.62±4.07	72±3.66
NHO	0±0	14.17±4.11	30.62±6.15	50.10±3.39	75.18±2.37
SS +Hi	0±0	19.48±8.01	36.03±8.14	49.35±7.94	73.97±1.69
SS + Cq	0±0	12.10±3.32	33.02±8.19	53.17±8.80	71.72±3.30
NHO+Hi	0±0	41.75±9.00	73.37±8.51	87.07±4.81	94.17±4.34
NHO+Cq	0±0	41.68±5.77	55.97±7.35	67.62±4.40	81.75±6.62

UC: Untreated Control **SS :** Silver sulfadiazine **NHO :** New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus **SS + Cq :** Silver Sulfadiazine + Cissus quadrangularis

NHO+Hi: New Herbal Ointment+ Hemidesmus indicus

NHO+Cq: New Herbal Ointment+ Cissus quadrangularis

Table : 14 – Comparison of Percentage of Wound Contraction at 28th day

GROUP	COMPARISON OF PERCENTAGE OF WOUND CONTRACTION AT 28TH DAY (BY ONE WAY ANOVA AND DUNNETT'S TEST)			
	Mean Difference	Standard Error	p-Value	Level of Significance*
SS	11.63	2.41	0.0242	p<0.05 Significant
NHO	10.2	2.41	0.0434	p<0.05 Significant
SS +Hi	0.60	2.41	1.000	p>0.05 Not significant
SS + Cq	4.35	2.41	0.327	p>0.05 Not significant
NHO+Hi	32.80	2.41	0.000	p<0.001 HighlySignificant
NHO+Cq	21.38	2.41	0.0455	p<0.05 Significant

*** : as compared to control group.**

SS : Silver sulfadiazine

NHO : New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus

SS + Cq : Silver Sulfadiazine + Cissus quadrangularis

NHO+Hi: New Herbal Ointment+ Hemidesmus indicus

NHO+Cq: New Herbal Ointment+ Cissus quadrangularis

Table : 15 - Period of Re-epithelization

GROUP	PERIOD OF RE-EPITHELIZATION: DAYS (Mean±S.D.)
UC	34±1.26
SS	30.33±1.37
NHO	30.50±1.87
SS +Hi	31.33±1.97
SS + Cq	31.16±1.47
NHO+Hi	26±1.41
NHO+Cq	28.66±1.51

UC: Untreated Control **SS :** Silver sulfadiazine **NHO :** New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus **SS + Cq :** Silver Sulfadiazine + Cissus quadrangularis

NHO+Hi: New Herbal Ointment+ Hemidesmus indicus

NHO+Cq: New Herbal Ointment+ Cissus quadrangularis

Table : 16 – Comparison of Period of Re-epithelization

GROUP	COMPARISON OF PERIOD OF RE-EPITHELIZATION (BY ONE WAY ANOVA AND DUNNETT'S TEST)			
	Mean Difference	Standard Error	p-Value	Level of Significance*
SS	-3.66	0.92	0.002	p<0.05 Significant
NHO	-3.50	0.92	0.003	p<0.05 Significant
SS +Hi	-2.66	0.92	0.034	p<0.05 Significant
SS + Cq	-2.83	0.92	0.021	p<0.05 Significant
NHO+Hi	-8.00	0.92	0.000	p<0.001 Highly Significant
NHO+Cq	-5.33	0.92	0.012	p<0.05 Significant

*** : as compared to control group.**

SS : Silver sulfadiazine

NHO : New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus

SS + Cq : Silver Sulfadiazine + Cissus quadrangularis

NHO+Hi: New Herbal Ointment+ Hemidesmus indicus

NHO+Cq: New Herbal Ointment+ Cissus quadrangularis

Table : 17 - Healing Status of Wound

GROUP	SCORE (Mean ± S.D.)	HEALING STATUS OF WOUND
UC	9.66±5.39	Poor
SS	15.67±4.89	Fair
NHO	13.83±5.04	Fair
SS +Hi	14.00±4.69	Fair
SS + Cq	13.50±4.23	Fair
NHO+Hi	18.83±2.99	Good
NHO+Cq	14.83±3.82	Fair

Table : 18 - Scoring Criteria for Wound Healing Status

Sr. No.	SCORE RANGE	STATUS
01.	16 - 19	Good
02.	12 - 15	Fair
03.	08 - 11	Poor

Table : 19 – Comparison of Healing Status of Wound

GROUP	COMPARISON OF HEALING STATUS OF WOUND (BY ONE WAY ANOVA AND DUNNETT'S TEST)			
	Mean Difference	Standard Error	p-Value	Level of Significance*
SS	6.00	2.52	0.010	p<0.05 Significant
NHO	4.16	2.52	0.416	p<0.05 Significant
SS +Hi	4.33	2.52	0.046	p<0.05 Significant
SS + Cq	3.83	2.52	0.032	p<0.05 Significant
NHO+Hi	9.16	2.52	0.0005	p<0.001 Highly Significant
NHO+Cq	5.16	2.52	0.013	p<0.05 Significant

*** : as compared to control group.**

SS : Silver sulfadiazine

NHO : New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus

SS + Cq : Silver Sulfadiazine + Cissus quadrangularis

NHO+Hi: New Herbal Ointment+ Hemidesmus indicus

NHO+Cq: New Herbal Ointment+ Cissus quadrangularis

Table : 20 - Grading of Epithelial Regeneration

GROUP	GRADING OF EPITHELIAL REGENERATION		
	Mild	Moderate	Prominent
	NUMBER OF ANIMALS		
UC	09	04	00
SS	04	07	02
NHO	02	07	04
SS +Hi	04	06	03
SS + Cq	09	02	02
NHO+Hi	00	06	07
NHO+Cq	04	05	04

UC: Untreated Control **SS :** Silver sulfadiazine **NHO :** New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus **SS + Cq :** Silver Sulfadiazine + Cissus quadrangularis

NHO+Hi: New Herbal Ointment+ Hemidesmus indicus

NHO+Cq: New Herbal Ointment+ Cissus quadrangularis

Table : 21 – Comparison of Grading of Epithelial Regeneration

GROUP	COMPARISON OF GRADING OF EPITHELIAL REGENERATION (BY CHI-SQUARE TEST)		
	χ^2-Value	p-value	Level of Significance*
SS	1.86	0.39	p>0.05 Not significant
NHO	0.34	0.021	p<0.05 Significant
SS +Hi	0.00	1.00	p>0.05 Not significant
SS + Cq	0.34	0.55	p>0.05 Not significant
NHO+Hi	7.20	0.02	p<0.05 Significant
NHO+Cq	1.33	0.24	p>0.05 Not significant

*** : as compared to control group.**

SS : Silver sulfadiazine

NHO : New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus

SS + Cq : Silver Sulfadiazine + Cissus quadrangularis

NHO+Hi: New Herbal Ointment+ Hemidesmus indicus

NHO+Cq: New Herbal Ointment+ Cissus quadrangularis

Table : 22 - Microbiological Examination

GROUP	FINDINGS OF CULTURE AND SENSITIVITY TEST	
	Growth of Micro-organism	No Growth
	NUMBER OF ANIMALS	
UC	06	07
SS	00	13
NHO	00	13
SS +Hi	00	13
SS + Cq	00	13
NHO+Hi	00	13
NHO+Cq	00	13

UC: Untreated Control **SS :** Silver sulfadiazine **NHO :** New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus **SS + Cq :** Silver Sulfadiazine + Cissus quadrangularis

NHO+Hi: New Herbal Ointment+ Hemidesmus indicus

NHO+Cq: New Herbal Ointment+ Cissus quadrangularis

Table : 23 – Comparison of Microbiological Examination Findings

GROUP	COMPARISON OF MICROBIOLOGICAL FINDINGS (BY CHI-SQUARE TEST)		
	$\aleph^2$-Value	p-value	Level of Significance*
SS	4.00	0.04	p>0.05 Significant
NHO	4.00	0.04	p>0.05 Significant
SS +Hi	4.00	0.04	p>0.05 Significant
SS + Cq	4.00	0.04	p>0.05 Significant
NHO+Hi	4.00	0.04	p>0.05 Significant
NHO+Cq	4.00	0.04	p>0.05 Significant

*** : as compared to control group.**

SS : Silver sulfadiazine

NHO : New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus

SS + Cq : Silver Sulfadiazine + Cissus quadrangularis

NHO+Hi: New Herbal Ointment+ Hemidesmus indicus

NHO+Cq: New Herbal Ointment+ Cissus quadrangularis

Table : 24 - Estimation of MDA

GROUP	MDA (In nmol/ml) (Mean±S.D.)					
	0 Day	1st Day	7th Day	14th Day	21st Day	28th Day
HC	0.28±0.03	0.29±0.02	0.27±0.04	0.29±0.01	0.28±0.02	0.29±0.01
UC	0.27±0.02	0.48±0.03	0.46±0.02	0.44±0.04	0.40±0.03	0.40±0.01
SS	0.29±0.01	0.46±0.01	0.43±0.01	0.41±0.02	0.40±0.02	0.39±0.02
NHO	0.27±0.01	0.42±0.01	0.39±0.02	0.38±0.02	0.37±0.02	0.32±0.02
SS +Hi	0.27±0.01	0.42±0.01	0.39±0.02	0.38±0.01	0.38±0.01	0.33±0.01
SS + Cq	0.27±0.01	0.42±0.02	0.39±0.02	0.38±0.01	0.38±0.01	0.38±0.01
NHO+Hi	0.28±0.02	0.44±0.03	0.40±0.02	0.35±0.02	0.30±0.02	0.29±0.01
NHO+Cq	0.28±0.02	0.44±0.03	0.41±0.02	0.40±0.02	0.40±0.02	0.33±0.02

HC: Healthy control **UC:** Untreated Control **SS :** Silver sulfadiazine **NHO :** New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus **SS + Cq :** Silver Sulfadiazine + Cissus quadrangularis

NHO+Hi: New Herbal Ointment+ Hemidesmus indicus **NHO+Cq:** New Herbal Ointment+ Cissus q.

Table : 25 - Comparison of MDA in each group between day 0 and 28th

GROUP	COMPARISON OF MDA LEVEL BETWEEN 1st AND 28th DAY (BY STUDENT'S PAIRED 't' TEST)				
	Day 0	Day 28	t-value	p-value	Level of Significant Difference
HC	0.28±0.03	0.29±0.01	2.51	0.978	Not Significant
UC	0.27±0.02	0.39±0.02	25.03	0.000	Significant
SS	0.29±0.01	0.39±0.02	16.05	0.000	Significant
NHO	0.27±0.01	0.32±0.02	10.43	0.000	Significant
SS +Hi	0.27±0.01	0.33±0.01	14.37	0.000	Significant
SS + Cq	0.27±0.01	0.38±0.01	23.04	0.000	Significant
NHO+Hi	0.28±0.02	0.29±0.01	2.62	0.878	Not Significant
NHO+Cq	0.28±0.02	0.33±0.02	6.48	0.001	Significant

HC: Healthy control UC: Untreated Control **SS :** Silver sulfadiazine **NHO :** New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus **SS + Cq :** Silver Sulfadiazine + Cissus quadrangularis

NHO+Hi: New Herbal Ointment+ Hemidesmus indicus

NHO+Cq: New Herbal Ointment+ Cissus quadrangularis

Table : 26 - Comparison of MDA level in different groups at 28th day

GROUP	COMPARISON OF MDA LEVEL IN DIFFERENT GROUPS AT 28th DAY (BY ONE WAY ANOVA AND DUNNETT'S TEST)			
	Mean Difference	Standard Error	p-Value	Level of Significance*
SS	-0.0008	0.009	1.000	p>0.05 Not significant
NHO	-0.03	0.009	0.116	p<0.05 Significant
SS +Hi	-0.02	0.009	0.084	p>0.05 Not significant
SS + Cq	-0.02	0.009	0.204	p>0.05 Not significant
NHO+Hi	-0.13	0.009	0.000	p<0.001 Highly Significant
NHO+Cq	-0.005	0.009	0.990	p>0.05 Not significant

*** : as compared to control group.**

SS : Silver sulfadiazine

NHO : New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus

SS + Cq : Silver Sulfadiazine + Cissus quadrangularis

NHO+Hi: New Herbal Ointment+ Hemidesmus indicus

NHO+Cq: New Herbal Ointment+ Cissus quadrangularis

Table : 27 - Estimation of SOD

GROUP	SOD (In IU/gm of Hb) (Mean±S.D.)					
	0 Day	1st Day	7th Day	14th Day	21st Day	28th Day
HC	0.0049± 0.0005	0.0048± 0.0004	0.0046± 0.0003	0.0048± 0.0005	0.0048± 0.0006	0.0048± 0.0001
UC	0.0048± 0.0005	0.0036± 0.0004	0.0039± 0.0006	0.0041± 0.0003	0.0041± 0.0007	0.0043± 0.0004
SS	0.0050± 0.0006	0.0038± 0.0004	0.0039± 0.0004	0.0040± 0.0005	0.0041± 0.0004	0.0042± 0.0005
NHO	0.0047± 0.0005	0.0036± 0.0005	0.0036± 0.0005	0.0036± 0.0005	0.0037± 0.0005	0.0039± 0.0005
SS +Hi	0.0049± 0.0005	0.0037± 0.0004	0.0038± 0.0005	0.0039± 0.0005	0.0039± 0.0005	0.0040± 0.0005
SS + Cq	0.0050± 0.0002	0.0037± 0.0003	0.0037± 0.0003	0.0038± 0.0002	0.0038± 0.0003	0.0040± 0.0003
NHO+Hi	0.0049± 0.0001	0.0038± 0.0002	0.0040± 0.0002	0.0043± 0.0002	0.0045± 0.0002	0.0048± 0.0001
NHO+Cq	0.0048± 0.0003	0.0036± 0.0002	0.0036± 0.0003	0.0038± 0.0003	0.0039± 0.0003	0.0040± 0.0003

HC: healthy control **UC:** Untreated Control **SS :** Silver sulfadiazine **NHO :** New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus **SS + Cq :** Silver Sulfadiazine + Cissus quadrangularis

NHO+Hi: New Herbal Ointment+ Hemidesmus indicus

NHO+Cq: New Herbal Ointment+ Cissus quadrangularis

Table : 28 - Comparison of SOD in each group between 1st and 28th day

GROUP	COMPARISON OF SOD LEVEL BETWEEN DAY 0 AND 28th DAY (BY STUDENT'S PAIRED 't' TEST)			
	Day 0	Day 28	t-value	p-value Level of Significant Difference
CH	0.0049± 0.0005	0.0048± 0.0001	02.80	0.833 Not Significant
UC	0.0048± 0.0005	0.0038±0.0004	14.54	0.006 Significant
SS	0.0050± 0.0006	0.0042±0.0005	16.93	0.001 Significant
NHO	0.0047± 0.0005	0.0043±0.0005	17.88	0.001 Significant
SS +Hi	0.0049± 0.0005	0.0040±0.0005	16.32	0.001 Significant
SS + Cq	0.0050± 0.0002	0.0040±0.0003	15.83	0.002 Significant
NHO+Hi	0.0049± 0.0001	0.0048±0.0001	02.81	0.821 Not Significant
NHO+Cq	0.0048± 0.0003	0.0040±0.0003	17.59	0.001 Significant

HC: healthy control **UC:** Untreated Control **SS :** Silver sulfadiazine **NHO :** New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus **SS + Cq :** Silver Sulfadiazine + Cissus quadrangularis

NHO+Hi: New Herbal Ointment+ Hemidesmus indicus

NHO+Cq: New Herbal Ointment+ Cissus quadrangularis

Table : 29 - Comparison of SOD level in different groups at 28th day

GROUP	COMPARISON OF SOD LEVEL IN DIFFERENT GROUPS AT 28th DAY (BY ONE WAY ANOVA AND DUNNETT'S TEST)			
	Mean Difference	Standard Error	p-Value	Level of Significance*
SS	0.0002	0.0002	0.419	p>0.05 Not significant
NHO	0.00003	0.0002	0.548	p>0.05 Not significant
SS +Hi	0.0001	0.0002	0.939	p>0.05 Not significant
SS + Cq	0.0001	0.0002	0.992	p>0.05 Not significant
NHO+Hi	0.0009	0.0002	0.000	p<0.001 Highly Significant
NHO+Cq	0.0002	0.0002	0.828	p>0.05 Not significant

*** : as compared to control group without treatment**

SS : Silver sulfadiazine

NHO : New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus

SS + Cq : Silver Sulfadiazine + Cissus quadrangularis

NHO+Hi: New Herbal Ointment+ Hemidesmus indicus

NHO+Cq: New Herbal Ointment+ Cissus quadrangularis

Table : 30 - Estimation of Stress Index

GROUP	Stress Index (Mean±S.D.)					
	0 Day	1st Day	7th Day	14th Day	21st Day	28th Day
HC	57.1± 7.88	60.4±10.2	58.6±6.89	60.4±8.88	58.3±9.00	60.4±7.21
UC	56.25±5.54	133.3±10.00	117± 8.90	107.3±8.99	97.5±9.54	93±8.96
SS	58±8.90	121± 7.33	110±7.78	102.5±8.98	97.5±10.90	92.8±10.07
NHO	57.4± 6.78	116.6±7.40	108±10.11	105.5±8.90	100±8.99	82±8.13
SS +Hi	55.1±9.11	113.5±8.90	102± 8.34	97.4±9.99	97.4±8.13	82.5±9.99
SS + Cq	54±8.99	113.5±8.13	105±11.0	100±8.99	100±8.88	95±11.10
NHO+Hi	57.1±10.00	115.7±9.99	100±9.90	81.3±8.90	66.6±9.99	61.4±7.55
NHO+Cq	58.3±9.99	122.2±8.99	113±8.13	105.2±11.10	102.2±8.90	82.5± 9.11

HC: healthy control **UC:** Untreated Control **SS :** Silver sulfadiazine **AO :** New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus **SS + Cq :** Silver Sulfadiazine + Cissus quadrangularis

AO+Hi: New Herbal Ointment+ Hemidesmus indicus **AO+Cq:** New Herbal Ointment+ Cissus

Table : 31 - Estimation of Protective Index

GROUP	Protective Index (Mean±S.D.)					
	0 Day	1st Day	7th Day	14th Day	21st Day	28th Day
HC	0.0175± 0.0004	0.0165± 0.0001	0.017± 0.0003	0.0165± 0.0004	0.017± 0.0002	0.016± 0.0002
UC	0.0177± 0.0006	0.0075± 0.0001	0.008± 0.0002	0.009± 0.0004	0.010± 0.0003	0.010± 0.0001
SS	0.0172± 0.0006	0.008± 0.0004	0.009± 0.0003	0.009± 0.0002	0.010±0 .0003	0.010± 0.0003
NHO	0.0174± 0.0003	0.008± 0.0003	0.009± 0.0001	0.009± 0.0002	0.010± 0.0001	0.0121± 0.0002
SS +Hi	0.0181± 0.0004	0.0088± 0.0004	0.009± 0.0006	0.010± 0.0003	0.010± 0.0003	0.0121± 0.0001
SS + Cq	0.0185± 0.0003	0.0088± 0.0005	0.009± 0.0005	0.010± 0.0002	0.010± 0.0003	0.0105± 0.0005
NHO+Hi	0.0175± 0.0001	0.0086± 0.0003	0.010± 0.0004	0.012± 0.0004	0.015± 0.0003	0.0165± 0.0003
NHO+Cq	0.0171± 0.0002	0.0081± 0.0005	0.008± 0.0005	0.0095± 0.0001	0.009± 0.0002	0.0121± 0.0005

HC: healthy control **UC:** Untreated Control **SS :** Silver sulfadiazine **AO :** New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus **SS + Cq :** Silver Sulfadiazine + Cissus quadrangularis

AO+Hi: New Herbal Ointment+ Hemidesmus indicus **AO+Cq:** New Herbal Ointment+ Cissus quadrangularis

Table: 32 - Comparison of PROTECTIVE INDEX between day 0 and day 28

GROUP	COMPARISON OF PROTECTIVE INDEX BETWEEN DAY 0 AND DAY 28 (BY STUDENT'S PAIRED 't' TEST)			
	Day 0	Day 28	t-value	p-value Level of Significant Difference
HC	0.0175± 0.0004	0.016± 0.0002	02.80	0.833 Not Significant
UC	0.0177± 0.0006	0.010± 0.0001	14.54	0.006 Significant
SS	0.0172± 0.0006	0.010± 0.0003	16.93	0.001 Significant
NHO	0.0174± 0.0003	0.0121± 0.0002	17.88	0.001 Significant
SS +Hi	0.0181± 0.0004	0.0121± 0.0001	16.32	0.001 Significant
SS + Cq	0.0185± 0.0003	0.0105± 0.0005	15.83	0.002 Significant
NHO+Hi	0.0175± 0.0001	0.0165± 0.0003	02.81	0.821 Not Significant
NHO+Cq	0.0171± 0.0002	0.0121± 0.0005	17.59	0.001 Significant

HC: healthy control **UC:** Untreated Control **SS :** Silver sulfadiazine **AO :** New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus **SS + Cq :** Silver Sulfadiazine + Cissus quadrangularis

AO+Hi: New Herbal Ointment+ Hemidesmus indicus **AO+Cq:** New Herbal Ointment+ Cissus quadrangularis

Table: 33 - Comparison of PROTECTIVE INDEX in different groups at 28th day

As compared to the Untreated Control with value 0.010± **0.0001**

GROUP	COMPARISON OF PROTECTIVE INDEX IN DIFFERENT GROUPS AT 28th DAY (BY ONE WAY ANOVA AND DUNNETT'S TEST)			
	Mean Difference	Standard Error	p-Value	Level of Significance*
SS	0.0002	0.0002	0.419	p>0.05 Not significant
NHO	0.0001	0.0002	0.992	p>0.05 Not significant
SS +Hi	0.0001	0.0002	0.939	p>0.05 Not significant
SS + Cq	0.0001	0.0002	0.992	p>0.05 Not significant
NHO+Hi	0.0009	0.0002	0.000	p<0.001 Highly Significant
NHO+Cq	0.0002	0.0002	0.828	p>0.05 Not significant

HC: healthy control **UC:** Untreated Control **SS :** Silver sulfadiazine **AO :** New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus **SS + Cq :** Silver Sulfadiazine + Cissus quadrangularis

AO+Hi: New Herbal Ointment+ Hemidesmus indicus **AO+Cq:** New Herbal Ointment+ Cissus

Table 34: Comparison of STRESS INDEX between day 0 and day 28

GROUP	COMPARISON OF STRESS INDEX BETWEEN DAY 0 AND DAY 28 (BY STUDENT'S PAIRED 't' TEST)			
	Day 0	Day 28	t-value	p-value Level of Significant Difference
HC	57.1± 7.88	60.4±7.21	02.76	0.833 Not Significant
UC	56.25±5.54	93±8.96	15.54	0.006 Significant
SS	58±8.90	92.8±10.07	18.93	0.001 Significant
NHO	57.4± 6.78	82±8.13	17.88	0.001 Significant
SS +Hi	55.1±9.11	82.5±9.99	16.30	0.001 Significant
SS + Cq	54±8.99	95±11.10	14.89	0.002 Significant
NHO+Hi	57.1±10.00	61.4±7.55	02.71	0.821 Not Significant
NHO+Cq	58.3±9.99	82.5± 9.11	16.66	0.001 Significant

HC: healthy control **UC:** Untreated Control **SS :** Silver sulfadiazine **AO :** New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus **SS + Cq :** Silver Sulfadiazine + Cissus quadrangularis

AO+Hi: New Herbal Ointment+ Hemidesmus indicus **AO+Cq:** New Herbal Ointment+ Cissus

Table 35 : Comparison of STRESS INDEX in different groups at 28th day

As compared to the Untreated Control with value 93±8.96

GROUP	COMPARISON OF STRESS INDEX IN DIFFERENT GROUPS AT 28th DAY (BY ONE WAY ANOVA AND DUNNETT'S TEST)			
	Mean Difference	Standard Error	p-Value	Level of Significance*
SS	3.09	2.41	0.0642	p>0.05 Not significant
NHO	10.22	2.41	0.0834	p>0.05 Not significant
SS +Hi	10.02	2.41	1.000	p>0.05 Not significant
SS + Cq	2.99	2.41	0.927	p>0.05 Not significant
NHO+Hi	33.13	2.41	0.000	p<0.001 HighlySignificant
NHO+Cq	10.86	2.41	0.0555	p>0.05 Not significant

HC: healthy control **UC:** Untreated Control **SS :** Silver sulfadiazine **AO :** New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus **SS + Cq :** Silver Sulfadiazine + Cissus quadrangularis

AO+Hi: New Herbal Ointment+ Hemidesmus indicus **AO+Cq:** New Herbal Ointment+ Cissus

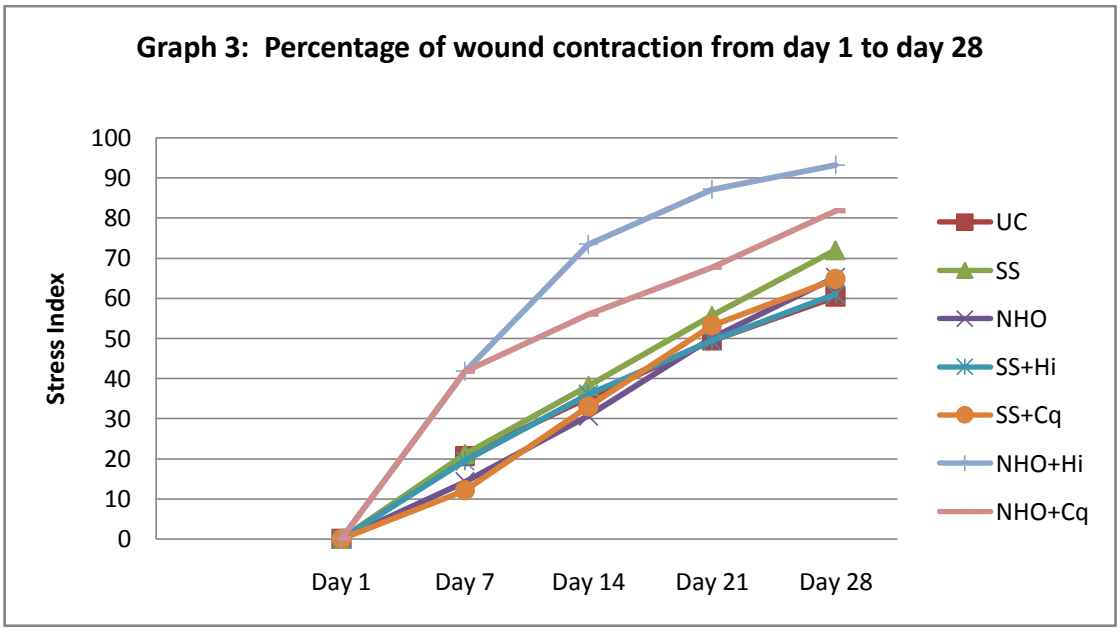
Table 36 : Comparison of treatment costs

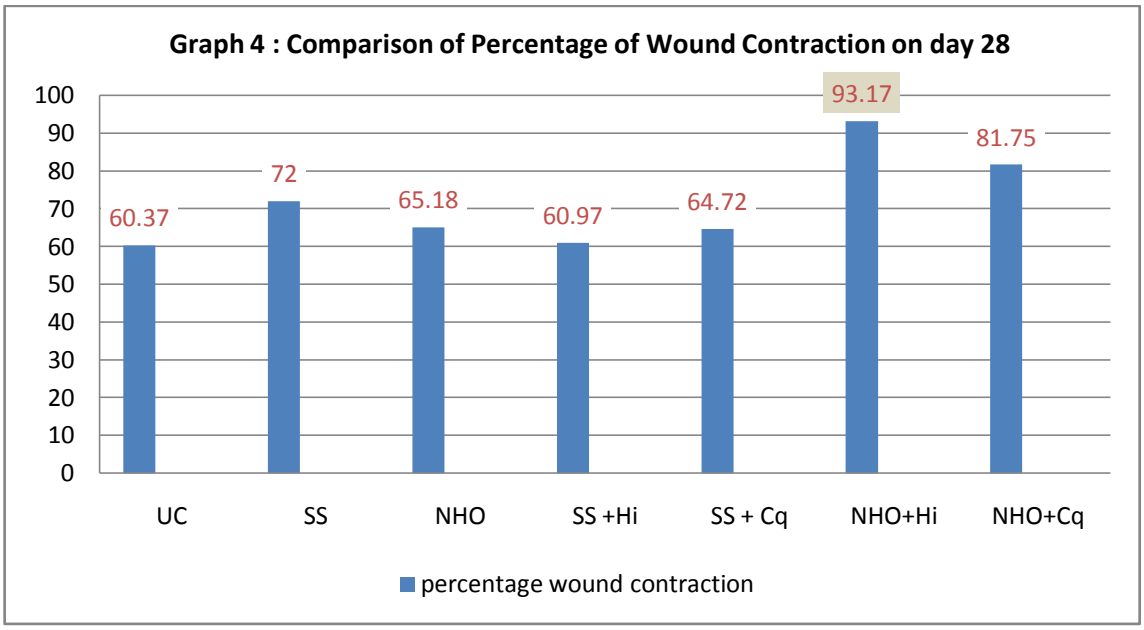
No	Drug / Drugs	Per dose cost	Cost for 28 days therapy
1	Silver sulfadiazine	₹ 2. 45	₹ 68.8
2	New Herbal Ointment	₹ 0. 50	₹ 14
3	Silver Sulfadiazine + Hemidesmus indicus	₹ 2.65	₹ 74.2
4	Silver Sulfadiazine + Cissus quadrangularis	₹ 2.75	₹ 77
5	New Herbal Ointment+ Hemidesmus indicus	₹ 0. 70	₹ 19.60
6	New Herbal Ointment+ Cissus quadrangularis	₹ 0. 80	₹ 22.40

Table: 37 Cost Effectiveness analysis of various treatments

No	Drug / Drugs	Cost effectiveness per unit effect
1	Silver sulfadiazine	₹ 0.951
2	New Herbal Ointment	₹ 0.224
3	Silver Sulfadiazine + Hemidesmus indicus	₹ 1.211
4	Silver Sulfadiazine + Cissus quadrangularis	₹ 1.183
5	New Herbal Ointment+ Hemidesmus indicus	₹ 0.210
6	New Herbal Ointment+ Cissus quadrangularis	₹ . 0.279

6. DISCUSSION





UC: untreated control **SS :** Silver sulfadiazine **NHO :** New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus , **SS + Cq :** Silver Sulfadiazine + Cissus quadrangularis

NHO+Hi: New Herbal Ointment+ Hemidesmus indicus,

NHO+Cq: New Herbal Ointment+ Cissus quadrangularis

6.1 Wound contraction

6.1.1 Wound contraction from day 1 to day 28

Wound contraction was monitored by measuring the progressive changes by tracing the raw wound area on a transparency on day 1st, 7th, 14th, 21st, and 28th post burn days. The tracing was then transferred to 1 mm^2 graph sheet, from which the wound surface area was calculated taking the initial size of wound as 100%. For calculation following equation was used

$$\text{Wound Contraction (\%)} = \frac{\text{Initial Wound Size} - \text{Specific Day Wound Size}}{\text{Initial Wound Size}} \times 100$$

On Day 0, that is, twenty four hours after the infliction of the burn wound the percentage of the wound contraction in all the groups was zero percent

On Day 7, Untreated Control group showed 20.63±2.67 percent wound contraction, Standard group treated with Ointment Silver Sulfadiazine showed 21.17±5.13 percent wound contraction, group treated with New Herbal Ointmentshowed 14.17±4.11 percent wound contraction, group treated with Ointment Silver Sulfadiazine + Hemidesmus indicus showed 19.48±8.01 percent

wound contraction, group treated with Ointment Silver Sulfadiazine + Cissus quadrangularis showed 12.10±3.32 percent wound contraction, group treated with **New Herbal Ointment+ Hemidesmus indicus showed 41.75±9.00 percent wound contraction**, group treated with New Herbal Ointment+ Cissus quadrangularis showed 41.68±5.77 percent wound contraction

On Day 14, Untreated Control showed 35.23±2.55 percent wound contraction, Standard group treated with Ointment Silver Sulfadiazine showed 38.17±8.58 percent wound contraction, group treated with New Herbal Ointmentshowed 30.62±6.15 percent wound contraction, group treated with Ointment Silver Sulfadiazine + Hemidesmus indicus showed 36.03±8.14 percent wound contraction, group treated with Ointment Silver Sulfadiazine + Cissus quadrangularis showed 33.02±8.19 percent wound contraction, group treated with **New Herbal Ointment+ Hemidesmus indicus showed 73.37±8.51 percent wound contraction**, group treated with New Herbal Ointment+ Cissus quadrangularis showed 55.97±7.35 percent wound contraction

On Day 21, Untreated Control showed 49.53±4.19 percent wound contraction, Standard group treated with Ointment Silver Sulfadiazine showed 55.62±4.07 percent wound contraction, group treated with New Herbal Ointmentshowed 50.10±3.39 percent wound contraction, group treated with Ointment Silver

Sulfadiazine + Hemidesmus indicus showed 49.35±7.94 percent wound contraction, group treated with Ointment Silver Sulfadiazine + Cissus quadrangularis showed 53.17±8.80 percent wound contraction, group treated with **New Herbal Ointment+ Hemidesmus indicus showed 87.07±4.81 percent wound contraction**, group treated with New Herbal Ointment+ Cissus quadrangularis showed 67.62±4.40 percent wound contraction.

On Day 28, Untreated Control showed 60.37±5.44 percent wound contraction, Standard group treated with Ointment Silver Sulfadiazine showed 72±3.66 percent wound contraction, group treated with New Herbal Ointmentshowed 75.18±2.37 percent wound contraction, group treated with Ointment Silver Sulfadiazine + Hemidesmus indicus showed 73.97±1.69 percent wound contraction, group treated with Ointment Silver Sulfadiazine + Cissus quadrangularis showed 71.72±3.30 percent wound contraction, group treated with **New Herbal Ointment+ Hemidesmus indicus showed 94.17±4.34 percent wound contraction**, group treated with New Herbal Ointment+ Cissus quadrangularis showed 81.75±6.62 percent wound contraction.

6.1.2 Comparison of Percentage of Wound Contraction on day 28

Percentage of wound contraction analysis on day 28 shows that the maximum percentage of wound contraction was achieved in group treated with New Herbal Ointment+ Hemidesmus indicus (94.17%) followed by a group treated with ayurvedic New Herbal Ointment+ Cissus quadrangularis (81.87%) . Group treated with Silver sulfadiazine showed only 72% of wound contraction. Combining either Hemidesmus indicus or Cissus quadrangularis with Silver Sulfadiazine did not enhance the wound contraction produced by silver sulfadiazine alone. On application of test of significance it was observed that in comparison with untreated control the groups treated with Silver sulfadiazine, New Herbal Ointment, New Herbal Ointment+ Cissus quadrangularis showed significant wound contraction while the group treated with New Herbal Ointment+ Hemidesmus indicus showed highly significant wound contraction.

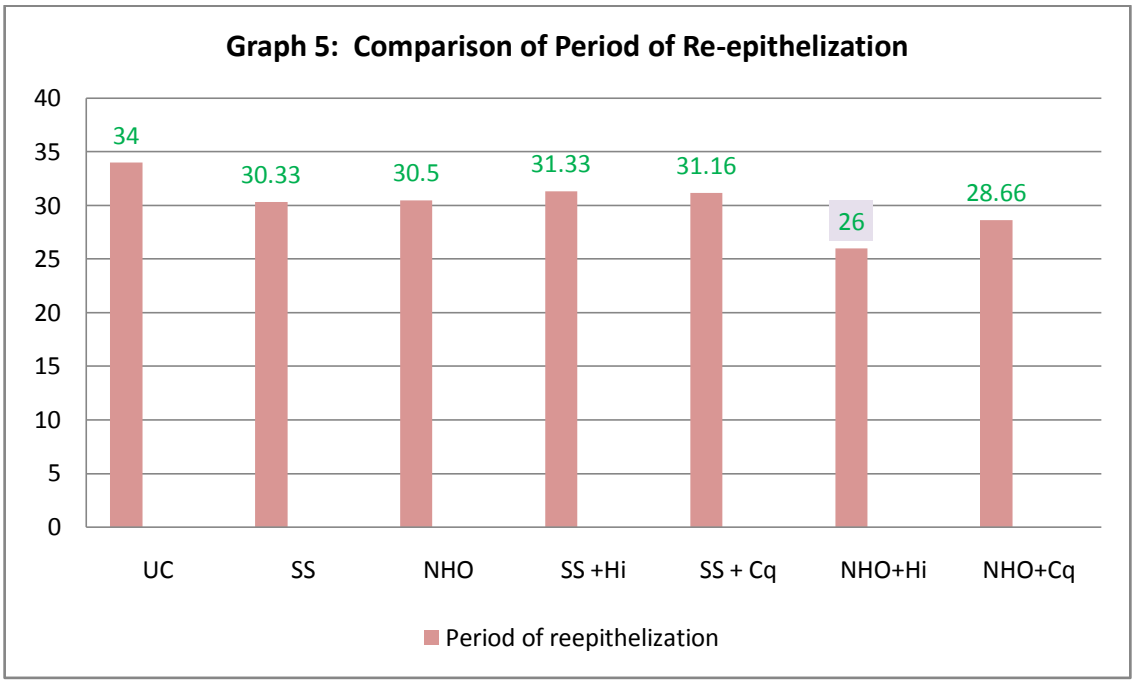
The burn wound healing property of Silver sulfadiazine is well documented [11,12,63,64,65,66]The burn wound healing property of New Herbal Ointmentunder study was also reported in rat models by Pathak SS, Patel SS and Borkar MA.[106] Antioxidant property of Hemidesmus indicus and Cissus quadrangularis are reported by Jainu, et al. [20]

Combination of New Herbal Ointment with Hemidesmus indicus showed synergistic action. This synergism may be because of the antioxidant constituents in New Herbal Ointment in the form of Azadirachta indica and also in

Hemidesmus indicus. The antioxidants present in Hemidesmus indicus and Azadirachta indica in New Herbal Ointment may be acting on different levels of oxidative stress to provide synergism. The stages (levels) of antioxidant action are described by Panchwat S et al.[39]

The combination of New Herbal Ointment with Cissus quadrangularis also showed a synergistic effect but the effect was less in comparison with that of New Herbal Ointment+ Hemidesmus indicus. This may be due to the possibility that the antioxidant activities of Cissus quadrangularis is lesser as compared with that of Hemidesmus indicus. However combining Silver sulfadiazine with Cissus quadrangularis did not show any synergistic action rather showed the overall effect lesser than silver sulfadiazine alone. It may be due to the fact that heavy metal silver , present in silver sulfadiazine acts as pro oxidant as reported by Cortese – Krott, et al. [114]

As far as the use of combination of Azadirachta indica and Hemidesmus indicus is concerned after searching through the leading search engines on the internet we did not come across any study



UC: untreated control **SS :** Silver sulfadiazine **NHO :** New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus **SS + Cq :** Silver Sulfadiazine + Cissus quadrangularis

NHO+Hi: New Herbal Ointment+ Hemidesmus indicus

NHO+Cq: New Herbal Ointment+ Cissus quadrangularis

6.2 Period of Re-epithelization

Falling of eschar leaving no raw wound area was considered as end point of complete repithelization and the days required for this was taken as period of Re-epithelization.

The period of re-epithelization, in Untreated Control group was 34±1.26 days, in Standard group treated with Ointment Silver Sulfadiazine was 30.33±1.37 days,

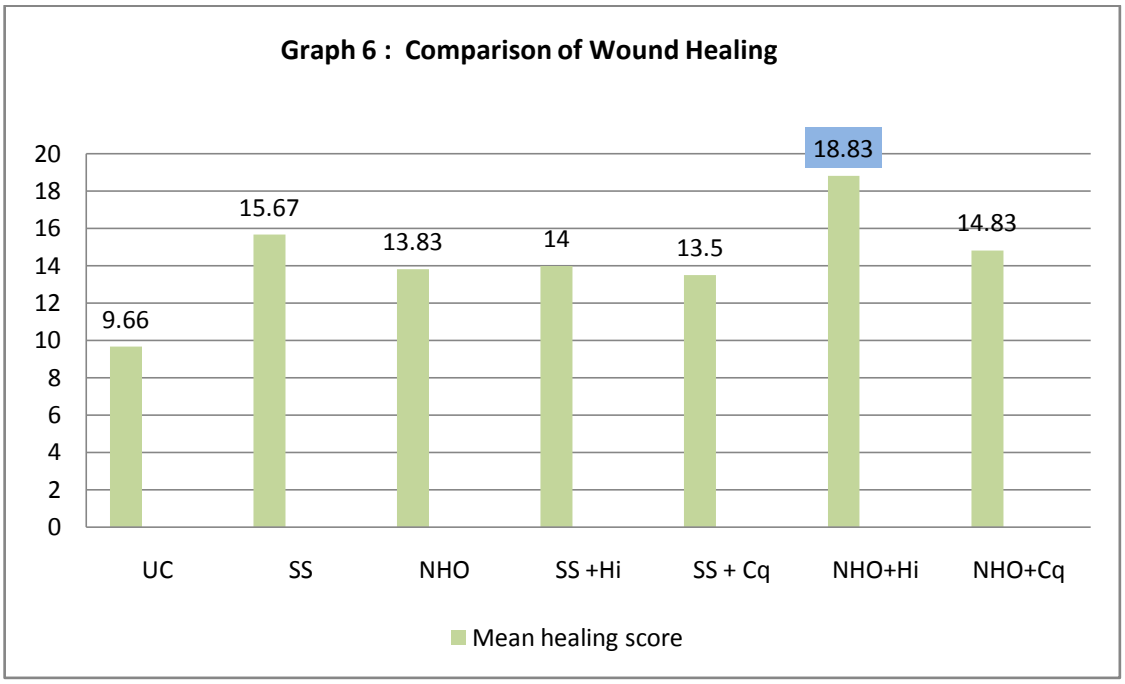
in group treated with New Herbal Ointmentwas 30.50±1.87 days, in group treated with Ointment Silver Sulfadiazine + Hemidesmus indicus was 29.33±1.97 days, in group treated with Ointment Silver Sulfadiazine + Cissus quadrangularis was 30.16±1.47 days, and **in group treated with New Herbal Ointment+ Hemidesmus indicus was 26±1.41 days**, in group treated with New Herbal Ointment+ Cissus quadrangularis was 28.66±1.51 days .

Thus the quickest re-epithelization (26 days) was achieved when the New Herbal Ointment was combined with **Hemidesmus indicus**

It was further observed that untreated control required 34 days for complete reepithelization whereas the average period of reepithelization in New Herbal Ointmentwas shortened to 30.5 days which was further decreased to 26 days when New Herbal Ointment was combined with Hemidesmus indicus. This was followed by the group treated with New Herbal Ointment+ Cissus quadrangularis where in the period of reepithelization comes out to be 28.66 days. This observation demonstrates that combining the New Herbal Ointment with Hemidesmus indicus is more effective as compared to combination of New Herbal Ointment with Cissus quadrangularis. The average period of reepithelization was 30.33 when burn wound were treated with Silver sulfadiazine alone. However reepithelization period was marginally increased to 31.33 and 31.16 respectively when Silver sulfadiazine was combined with Hemidesmus

indicus and Cissus quadrangularis respectively. However the prolongation of this increase in the epithelization was not found to be statistically significant.

We searched literature with leading search engines namely Google, Pub med and Medline plus but did not come across any study combining Hemidesmus indicus or Cissus quadrangularis with the ointments used for the treatment of burn wounds.



UC: untreated control **SS :** Silver sulfadiazine **NHO :** New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus **SS + Cq :** Silver Sulfadiazine + Cissus uadrangularis

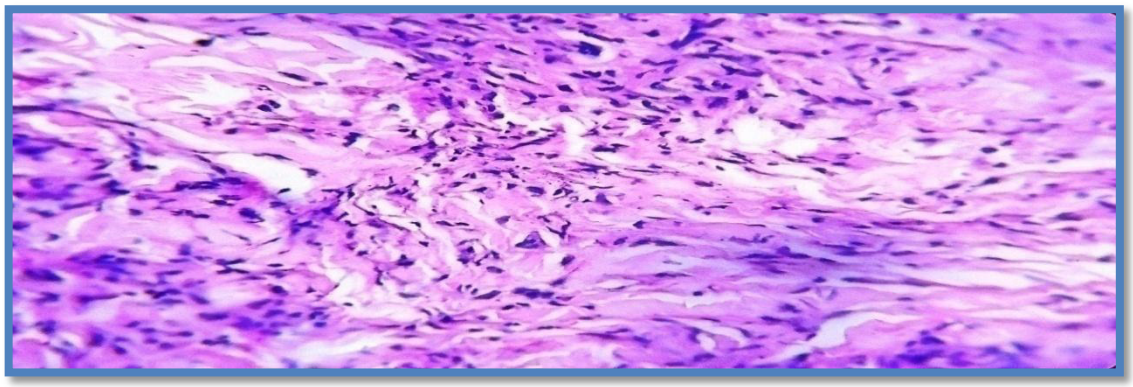
NHO+Hi: New Herbal Ointment+ Hemidesmus indicus

NHO+Cq: New Herbal Ointment+ Cissus quadrangularis

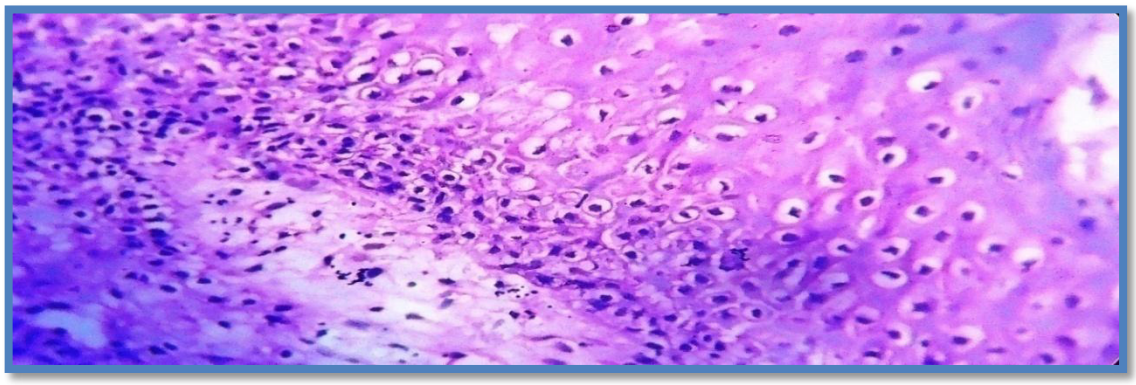
6.3 Histopathological examination

Punch biopsies of the wound were histopathologically examinied assessing the grading of epithelial regeneration and the status of wound healing. Samples were collected on 14th day in accordance with the study conducted by Lemo N et al.[109] Sample was fixed in 10% neutral buffered Formalin. Histological examination was performed by staining with Haemotoxylin and Eosin stain and Masson's Trichrome stain.

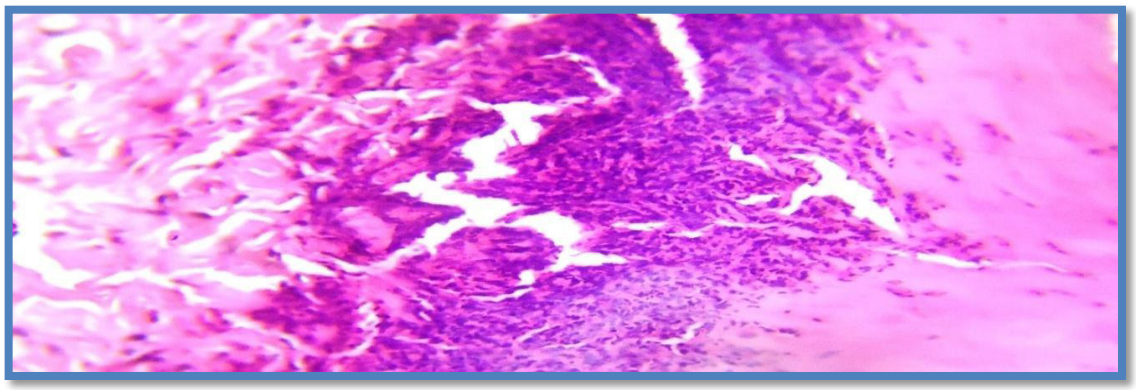
Fig 18 : Microscopic Structure showing (a) Mild Epithelial Regeneration, (b) Moderate Epithelial Regeneration and (c) Prominent Epithelial Regeneration



(a)



(b)



(c)

Healing status of wound was assessed by following Scoring Criteria (Sultana *et al.*)

Sr.N.	Parameter of healing	Observation	Score
1.	Amount of granulation tissue	Profound	1
		Moderate	2
		Scanty	3
		Absent	4
2.	Inflammatory infiltrate	Profound	1
		Moderate	2
		Few	3
3.	Collagen fiber orientation	Vertical	1
		Mixed	2
		Horizontal	3
4.	Pattern of Collagen	Reticular	1
		Mixed	2
		Fascicle	3
5.	Amount of early collagen	Profound	1
		Moderate	2
		Minimal	3
		Absent	4
6.	Amount of mature collagen	Profound	1
		Moderate	2
		Minimal	3

Total healing score of each case was calculated by adding the score of individual criteria. Lower scores indicated poorer wound healing while higher scores indicated better healing process. The wound healing score was calculated on a 20 point scale as used by - Sultana *et al.* [110] where the scores vary from 8 to 19.

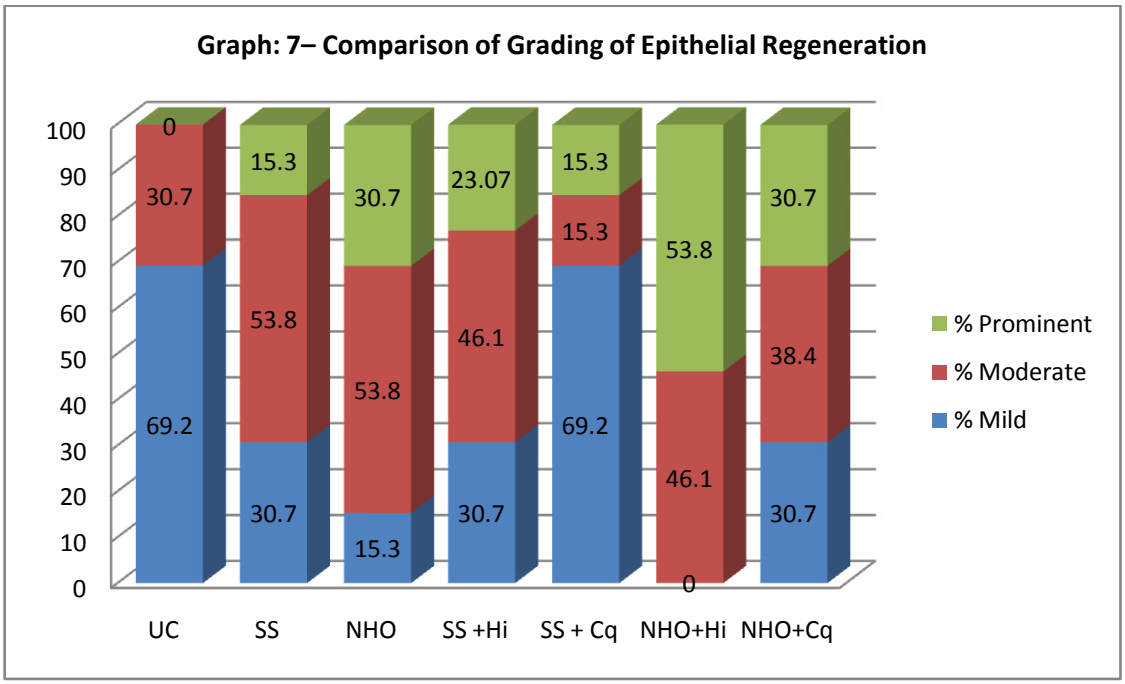
(a). Good : 16 – 19 (b). Fair: 12 – 15 (c). Poor: 08 – 11

On histological examination, the untreated Control group showed healing score of 9.66±5.39 which comes under poor grade of healing of wound

Standard group and groups treated with Ayurvedic Ointment, Ointment Silver Sulfadiazine +Hemidesmus indicus and Ointment Silver Sulfadiazine + Cissus quadrangularis showed score of 15.67±4.89, 13.83±5.04, 14.00±4.69 and 13.50±4.23 respectively, demonstrated fair grade of wound healing.

The Group treated with New Herbal Ointment+ Hemidesmus indicus showed score of 18.83±2.99, this grade denotes good status of wound healing.

Although this criteria of wound healing was used by Sultana et al. for assessing the wound healing however we could not come across any references wherein effect of Hemidesmus indicus , Cissus quadrangularis , Azadarachta indica , Silver sulfadiazine were used to assess their effect on wound healing using the above criteria.



UC: untreated control **SS :** Silver sulfadiazine **NHO :** New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus ,**SS + Cq :** Silver Sulfadiazine + Cissus quadrangularis

NHO+Hi: New Herbal Ointment+ Hemidesmus indicus,

NHO+Cq: New Herbal Ointment+ Cissus quadrangularis

6.4 Epithelial Regeneration

Grading of epithelial regeneration was done by using following parameters used by Sultana *et al*.[110]

Sr. No.	PARAMETER	GRADING
1.	Inflammatory Response	Mild
2.	Granulation Tissue Formation and/or Angiogenesis	Moderate
3.	Repair of Connective Tissue and Epithelium and/or Remodelling	Prominent

The histopathological examination was performed on 14th day. On histopathological examination it was observed that in Untreated Control group, 69.23% animals showed mild regeneration of epithelium and 30.76% animals showed moderate epithelial regeneration, none of the animals showed prominent regeneration.

However in the group treated with Silver sulfadiazine alone, 30.76% animals showed mild regeneration, 53.84% showed moderate regeneration and 15.38% showed prominent regeneration.

While the group treated with New Herbal Ointmentalone, 15.38% showed mild regeneration and 53.84% showed moderate regeneration of epithelium and 30.76% showed prominent regeneration.

Group treated with Ointment Silver Sulfadiazine +Hemidesmus indicus showed mild regeneration in 30.76% animals and moderate regeneration of epithelium 46.14% animals and prominent regeneration in 23.07% and

In group treated with Ointment Silver Sulfadiazine + Cissus quadrangularis, mild regeneration occurred 69.23% animals and 15.38% showed moderate regeneration of epithelium while 2 15.38% animals showed prominent regeneration of epithelium.

New Herbal Ointment+ Cissus quadrangularis treated group showed mild regeneration in 30.76% and moderate regeneration of epithelium occurred in 38.45% animals while prominent regeneration was observed in 30.76% animals

Prominent epithelial regeneration was observed in as high as 53.84% animals and moderate regeneration in 46.14% in the group treated with New Herbal Ointment+ Hemidesmus indicus and none of the animals showed mild regeneration.

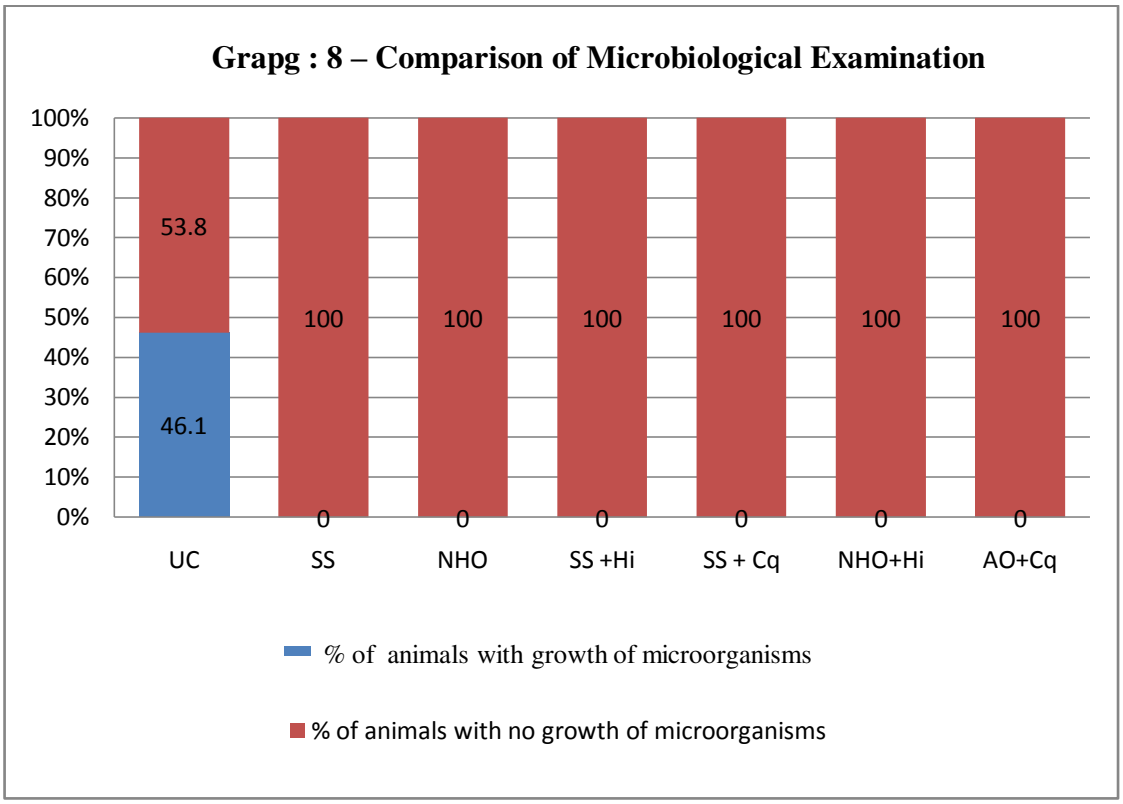
On histopathological examination epithelial regeneration on 14th day was compared it was observed that in the group treated with New Herbal Ointment+ Hemidesmus indicus showed prominent regeneration in 53.84% which is highest amongst all the groups while moderate regeneration in 46.14% , none of the

animals in this group showed mild regeneration confirming that the New Herbal Ointmentin combination with the antioxidant Hemidesmus indicus enhances the ability of the New Herbal Ointmentfor epithelial regeneration.

In the group where silver sufadiazine was used alone it was observed that only 15.38% animals showed the prominent regeneration

However the prominence of the regeneration did not improve significantly when silver sulfadiazie was combined with Hemidesmus indicus and Cissus quadrangularis.

We could not find any literature with respect to Hemidesmus indicus or Cissus quadrangularis where in the epithelial regeneration was studied for the healing of burn wounds.



UC: untreated control **SS :** Silver sulfadiazine **NHO :** New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus ,**SS + Cq :** Silver Sulfadiazine + Cissus quadrangularis

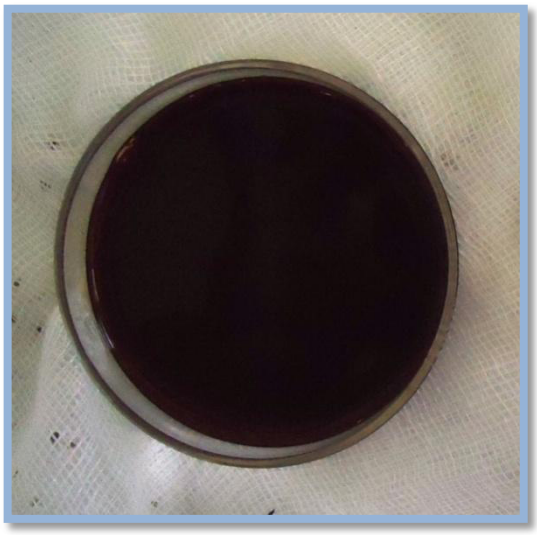
NHO+Hi: New Herbal Ointment+ Hemidesmus indicus ,

NHO+Cq: New Herbal Ointment+ Cissus quadrangularis

6.5 Microbiological Examination

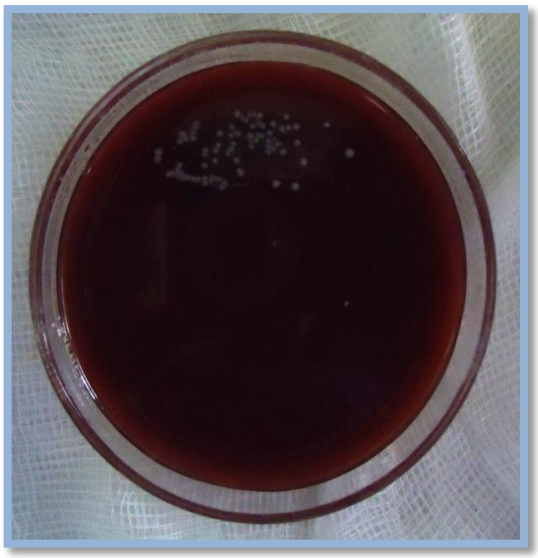
Wound swab was taken for the culture and sensitivity reporting on the seventh day of creation of wound to determine the presence of any infection in the wound.

Figure 19:- Microbiological Examination

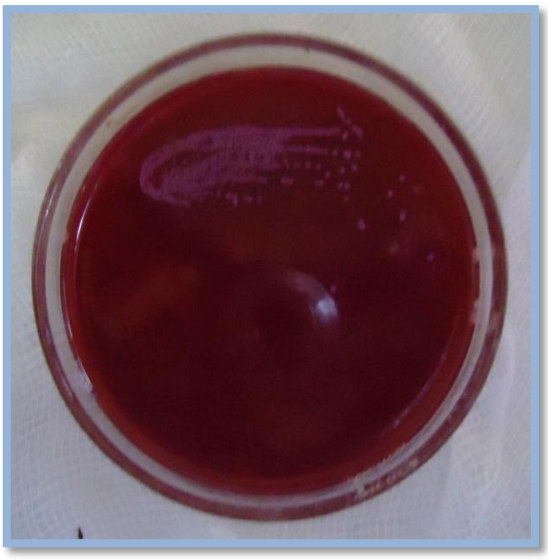


(a)

(b)



(c)



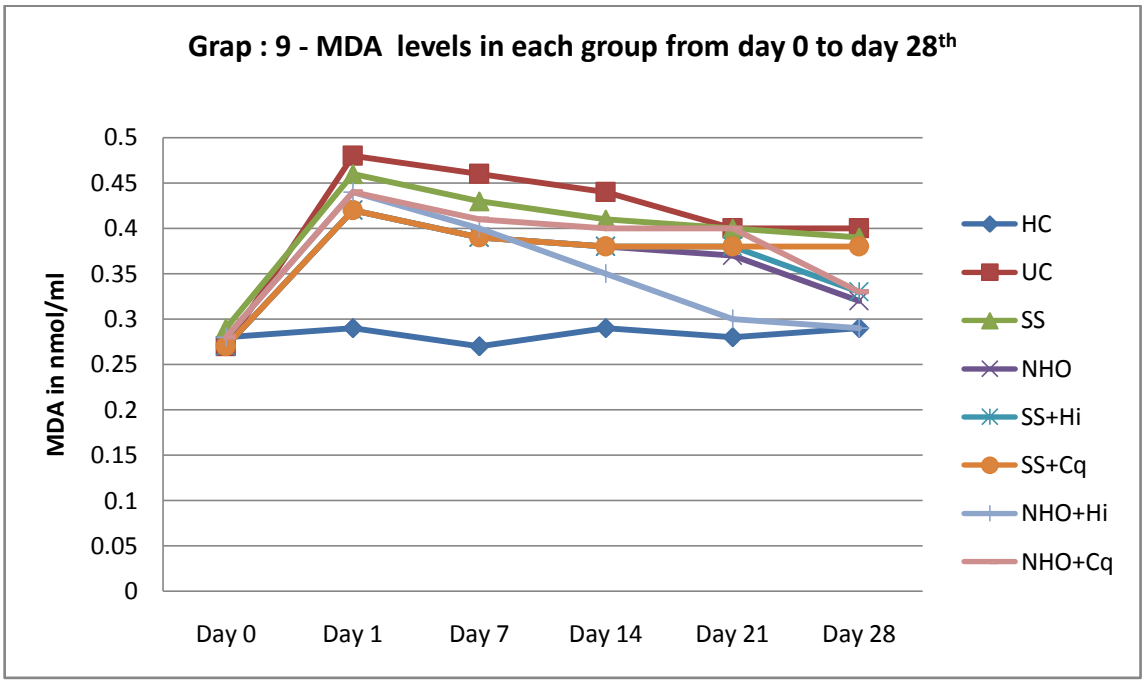
(d)

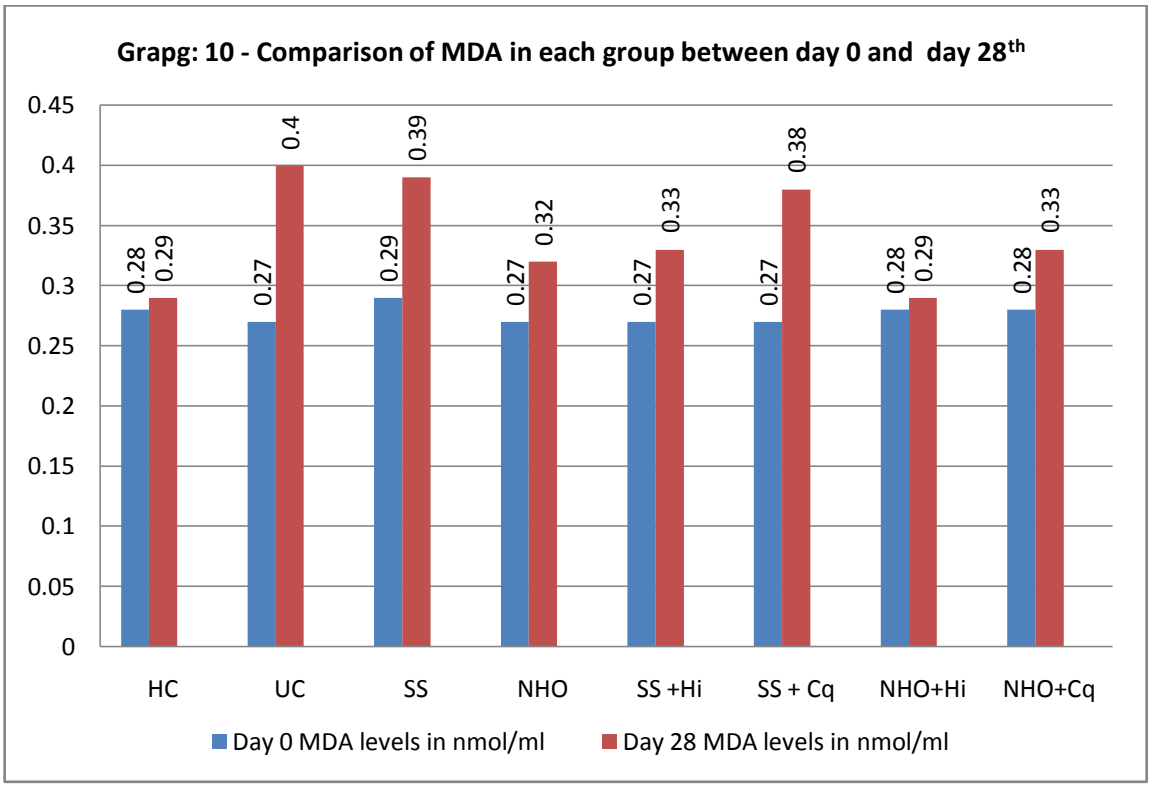
Figure 19: (a)Blood Agar Plate, (b) MacConkey Agar Plate, (c) Growth of Coagulase Negative Staphylococci on Blood Agar Plate and (d) Growth of Coagulase Negative Staphylococci on MacConkey Agar Plate

There was no growth in standard group treated with Ointment Silver Sulfadiazine and in all test groups ,treated with Ayurvedic Ointment, Ointment Silver Sulfadiazine +Hemidesmus indicus, Ointment Silver Sulfadiazine +Cissus quadrangularis, New Herbal Ointment+ Hemidesmus indicus, New Herbal Ointment+ Cissus quadrangularis . However 53.7% animals of untreated control group were affected with coagulase negative staphylococcus. In all other groups in the study no growth of any microorganism was observed.

Antimicrobial properties of Silver Sulfadiazine is has been demonstrated by various researchers. [62,63,64,65]. Azadirachta indica a constituent of the herbal ointment under study has known antimicrobial property as reported by number of researchers [82,84]

Observations of the present study are in accordance to the reports available in scientific literature.





HC: healthy control **UC:** untreated control **SS :** Silver sulfadiazine **NHO :** New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus **SS + Cq :** Silver Sulfadiazine + Cissus quadrangularis

NHO+Hi: New Herbal Ointment+ Hemidesmus indicus

NHO+Cq: New Herbal Ointment+ Cissus quadrangularis

6.7 Oxidative stress in burn wound

6.7.1 MDA levels

One of the markers of oxidative stress Malondialdehyde (MDA) was estimated spectrophotometrically by the method of Murklund and Murklund[111] on day zero (a day before the production of the burn wound) and then after 24 hours .The subsequent readings were taken on each 7th day till 28th day.

On Day 0 that is one day prior to the infliction of the burn wounds MDA levels in all the groups were more or less equal ranging from 0.27 to 0.29 nmol/ml with no statistical significant difference.

On Day 1 , after 24 hours of production of the burn wound, MDA level in all the groups rose but remained in a close range of 0.42 to 0.48 nmol/ml. The levels did not differ significantly from the levels in untreated control group which was 0.42±0.02 nmol/ml

Thereafter the levels started to decline

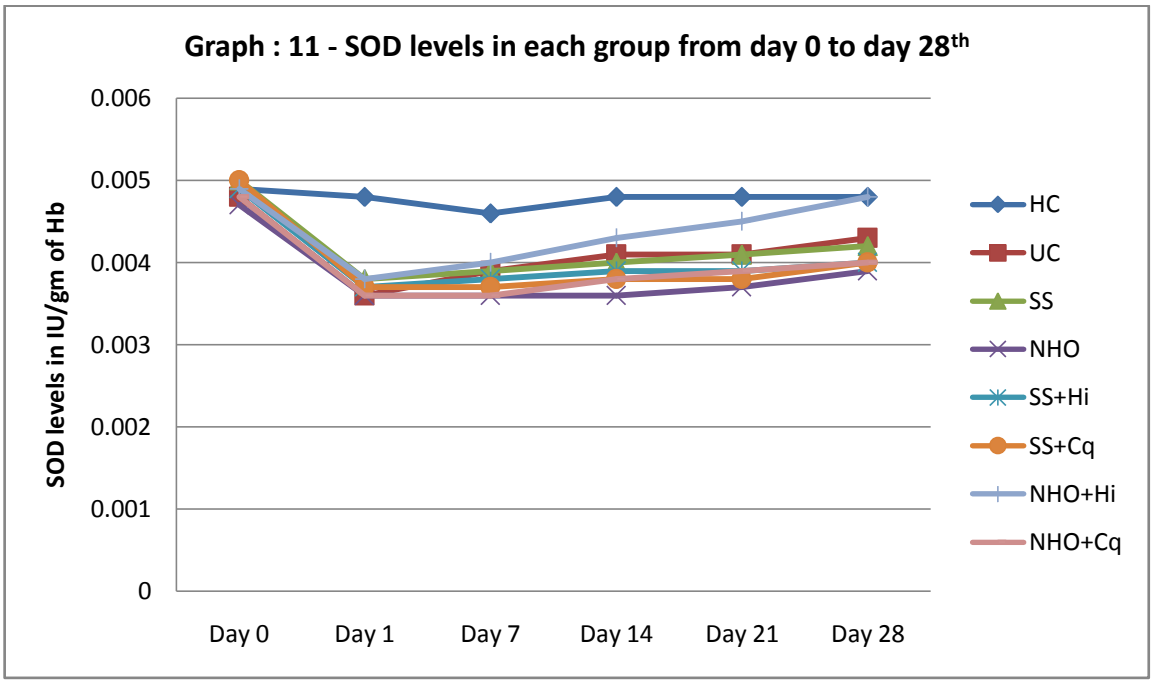
On Day 7, MDA level - in Untreated control was 0.46±0.02 as against 0.48±0.03 on day 1 , in group treated with Silver Sulfadiazine was 0.43±0.01 as against 0.46±0.01 on day 1 , in Group treated with New Herbal Ointmentwas 0.39±0.02 as against 0.42±0.01 on day 1 , in Ointment Silver Sulfadiazine + Hemidesmus indicus treated group was 0.39±0.02 as against 0.42±0.01 on day 1, in Group treated with Ointment Silver Sulfadiazine + Cissus quadrangularis was 0.39±0.02 as against 0.42±0.02 on day 1 , in Group treated with New Herbal Ointment+ Hemidesmus indicus was 0.40±0.02 as against 0.44±0.03 on day 1, in Group treated with New Herbal Ointment+ Cissus quadrangularis was 0.41±0.02 as against 0.44±0.03 on day 1 but the levels in non of the groups differed significantly from the levels in untreated control group.

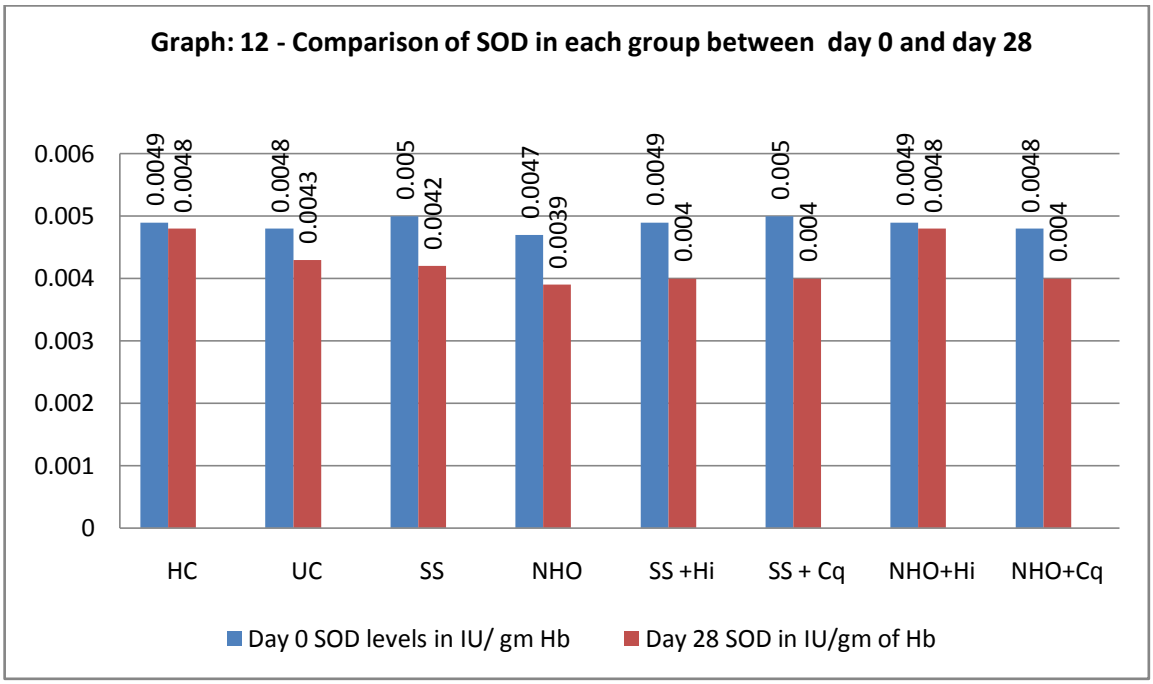
On Day 14, in the group treated with New Herbal Ointment+ Hemidesmus indicus, MDA levels dropped to 0.35± 0.02 which was significantly lower in comparison to that of the untreated control group levels of 0.44±0.04. Though in all the other groups the decline in the levels of MDA was recorded, none of these groups differed statistically significantly from the untreated control group.

On Day 21, MDA level in the group treated with New Herbal Ointment+ Hemidesmus indicus further dropped to 0.30±0.02 which was significantly lower in comparison to the untreated control group levels of 0.40±0.03. In all the other

groups though the decline in the levels of MDA was recorded, none of these groups differed significantly from the untreated control group.

On Day 28, MDA levels in the group treated with New Herbal Ointmentalone was 0.32±0.02 which was significantly lower to the untreated control group which was 0.40±0.01, but the drop in the MDA levels in the group treated with New Herbal Ointment+ Hemidesmus indicus was dropped to 0.29 ±0.01. Difference in this MDA level and the MDA level of untreated control was highly significant. Furthermore this value was same as that of the MDA value of the healthy control group on 28th day meaning thereby that the degree of oxidative stress was brought down to the base levels.





HC: healthy control **UC:** untreated control **SS :** Silver sulfadiazine **NHO :** New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus **SS + Cq :** Silver Sulfadiazine + Cissus quadrangularis

NHO+Hi: New Herbal Ointment+ Hemidesmus indicus

NHO+Cq: New Herbal Ointment+ Cissus quadrangularis

6.7.2 SOD levels

One of the markers of oxidative stress Superoxide Dismutase (SOD) was estimated spectrophotometrically by the method of Murklund and Murklund[111] on day zero (a day before the production of the burn wound) and then after 24 hours .The subsequent readings were taken on each 7th day till 28th day. Superoxide Dismutase (SOD)was expressed in IU/gm of Hb.

On Day 0 that is one day prior to the infliction of the burn wounds SOD levels in all the groups were more or less equal ranging from 0.0047to 0.0050 IU/gm of Hb with no statistical significant difference.

On Day 1 , after one hour of production of the burn wound SOD level in all the groups decreased but remained in a close range of 0.0036to 0.0038 IU/gm of Hb.. The levels did not differ significantly from the levels in untreated control group which was 0.0036±0.0004 IU/gm of Hb

Thereafter the SOD levels began to rise

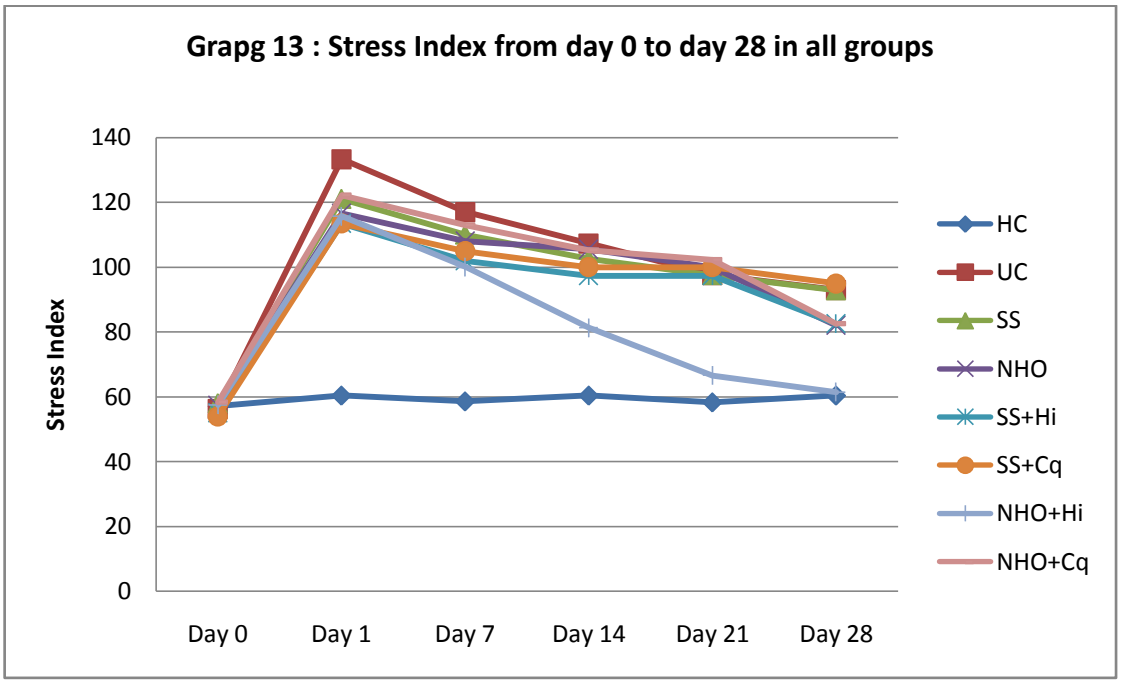
On Day 7, SOD level - in untreated control was group was 0.0039±0.0004 as against 0.0036±0.0004 on day 1 , in group treated with Ointment Silver Sulfadiazine was 0.0039±0.0004 as against 0.0038±0.0004 on day 1, in Group

treated with New Herbal Ointmentwas 0.0036±0.0005 as against 0.0036±0.0005 on day 1, in Ointment Silver Sulfadiazine + Hemidesmus indicus treated group was 0.0038±0.0005 as against 0.0037±0.0004 on day 1, in Group treated with Ointment Silver Sulfadiazine + Cissus quadrangularis was 0.0037±0.0003 as against 0.0037±0.0003 on day 1, in Group treated with New Herbal Ointment+ Hemidesmus indicus was 0.0040±0.0002 as against 0.0038±0.0002 on day 1, in Group treated with New Herbal Ointment+ Cissus quadrangularis was 0.0036±0.0003 as against 0.0036±0.0003 on day 1 but the levels in non of the groups differed significantly from the levels in untreated control group.

On Day 14, SOD level in the group treated with New Herbal Ointment+ Hemidesmus indicus SOD level rose to 0.0043±0.0002 which was not significantly higher in comparison to the untreated control group levels of 0.0041±0.0003. Though in all the other groups the rise in the levels of SOD was recorded, none of these groups differed significantly from the untreated control group.

On Day 21, SOD level in the group treated with New Herbal Ointment+ Hemidesmus indicus further rose to 0.0045±0.0002 which was significantly higher in comparison to the untreated control group levels of 0.0041±0.0007. Though in all the other groups the rise in the levels of SOD was recorded, none of these groups differed significantly from the untreated control group.

On Day 28, SOD levels in the group treated with New Herbal Ointment+ Hemidesmus indicus rose up to 0.0048±0.Differece between this SOD level and the SOD level of untreated control was highly significant. Furthermore this value was same as that of the SOD value of the healthy control group on 28th day meaning thereby that the degree of oxidative stress was brought down to the base levels.



HC: healthy control **UC:** untreated control **SS :** Silver sulfadiazine **NHO :** New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus , **SS + Cq :** Silver Sulfadiazine + Cissus quadrangularis

NHO+Hi: New Herbal Ointment+ Hemidesmus indicus , **NHO+Cq:** New Herbal Ointment+ Cissus q.

6.7.3 Stress Index

The ratio of MDA/SOD, a derived value, is termed as Stress Index (SI). It indicates the level of oxidative stress of an individual.

On Day 0, that is one day prior to the infliction of the burn wounds, Stress index in all the groups were more or less equal ranging from 54 to 58 with no statisticaly significant difference.

On Day 1, that is after 24 hour of production of the burn wound, Stress index in all the groups rose but remained in a range of 122 to 133. The levels did not differ significantly from the levels in untreated control group which was 133.

Thereafter the levels started to decline

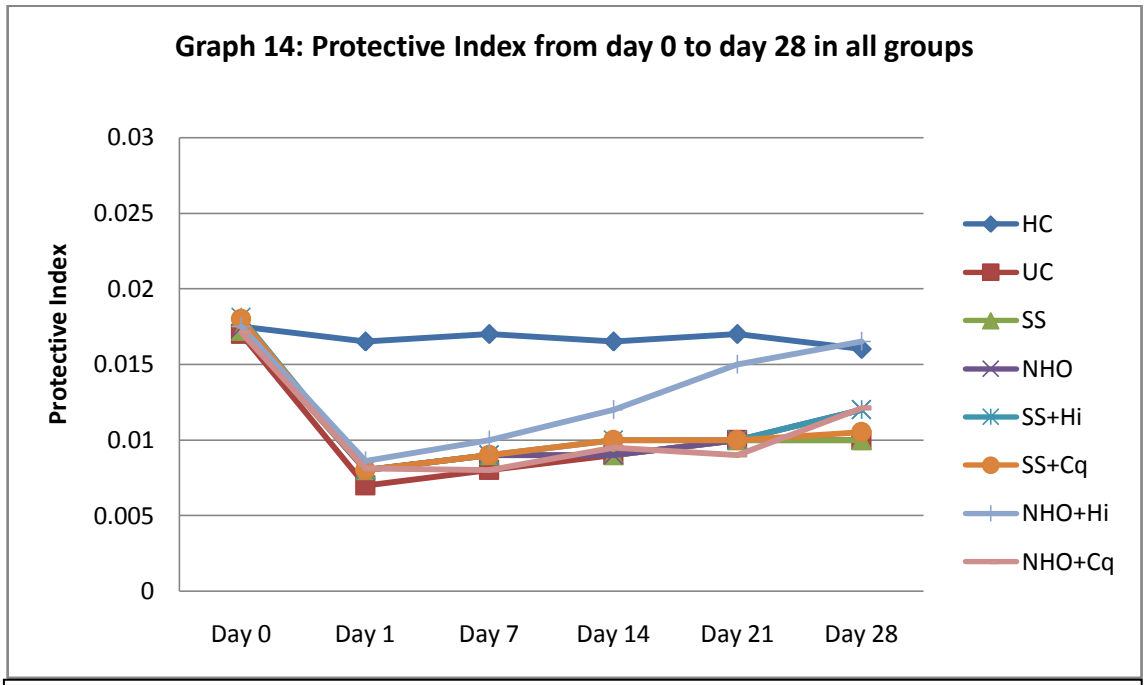
On Day 7, Stress index -in untreated control was 117±8.90 as against 133.3±10 on day 1, in group treated with Ointment Silver Sulfadiazine was 110±7.78 as against 121±7.33 on day 1, in Group treated with New Herbal Ointment was 108±10.11 as against 116±7.40 on day 1, in Ointment Silver Sulfadiazine + Hemidesmus indicus treated group was 102±8.34 as against 113.5±8.90 on day 1, in Group treated with Ointment Silver Sulfadiazine + Cissus quadrangularis was 105±11.00 as against 113.5±8.13 on day 1, in Group treated with New Herbal Ointment+ Hemidesmus indicus was 100±9.90 as against 115.7 ±9.99 on day 1, in Group treated with New Herbal Ointment+ Cissus quadrangularis was 113±8.13 as against 122±8.99 on day 1 but the levels in none of the groups differed significantly from the levels in untreated control group.

On Day 14, in the group treated with New Herbal Ointment+ Hemidesmus indicus, Stress index dropped to 81.3± 8.90 which was significantly lower in comparison to that of untreated control group levels of 107.3±8.99. All the other groups though recorded decline in the levels of Stress index but none of these

groups differed significantly from that of untreated control group. Although in all the other groups the fall in Stress index was recorded, none of these groups differed significantly from the untreated control group.

On Day 21, Stress index in the group treated with New Herbal Ointment+ Hemidesmus indicus further dropped to 66.6±9.99 which was significantly lower in comparison to the untreated control group levels of 97.5±9.54. Though in all the other groups the fall in Stress index was recorded, none of these groups differed significantly from the untreated control group.

On Day 28, Stress index levels in the group treated with New Herbal Ointment+ Hemidesmus indicus came down to 61.4 ±7.55. Difference in this stress index and the Stress index of untreated control 93±8.96 was highly significant. Furthermore this value was very close to 60.4 ±7.1 which was the Stress index value of the healthy control group on 28th day.



HC: healthy control **UC:** untreated control **SS :** Silver sulfadiazine **NHO :** New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus **SS + Cq :** Silver Sulfadiazine + Cissus quadrangularis

NHO+Hi: New Herbal Ointment+ Hemidesmus indicus

NHO+Cq: New Herbal Ointment+ Cissus quadrangularis

6.7.4: Protective Index

The ratio of SOD/MDA, a derived value, is termed as Protective Index (PI). It indicates the antioxidant status of an individual.

On Day 0, that is one day prior to the infliction of the burn wounds, Protective Index in all the groups was more or less equal ranging from 0.0174 to 0.0181 with no statistically significant difference.

On Day 1, that is after 24 hours of production of the burn wound Protective Index in all the groups decreased but remained in a range of 0.0075 to 0.0088. The levels did not differ significantly from the levels in untreated control group which was 0.0075.

Thereafter the levels started to rise

On Day 7, Protective index - in untreated control was 0.008±0.002 as against 0.0075±0.001 on day 1, in group treated with Ointment Silver Sulfadiazine was 0.009±0.003 as against 0.008±0.004 on day 1, in Group treated with New Herbal Ointment was 0.009±0.001 as against 0.008±0.003 on day 1, in Ointment Silver Sulfadiazine + Hemidesmus indicus treated group P.I. was 0.009±0.006 as against 0.0088±0.006 on day 1, in Group treated with Ointment Silver Sulfadiazine + Cissus quadrangularis it was 0.009±0.005 as against 0.0088±0.005 on day 1, in Group treated with New Herbal Ointment+ Hemidesmus indicus P.I. was 0.010±0.004 as against 0.086±0.003 on day 1, in Group treated with New Herbal Ointment+ Cissus quadrangularis it was 0.008±0.005 as against 0.008±0.005 on day 1. But the levels in none of the groups differed significantly from the levels in untreated control group.

On Day 14, in the group treated with New Herbal Ointment+ Hemidesmus indicus Protective Index increased to 0.012±0.004 which was not significantly higher in

comparison to that of the untreated control group levels of 0.009±0.004. Although in all the other groups the rise in Protective Index was recorded, none of these groups differed significantly from the untreated control group.

On Day 21, Protective Index in the group treated with New Herbal Ointment+ Hemidesmus indicus further rose to 0.015±0.003which was significantly higher in comparison to the untreated control group levels of 0.010±0.003. Although in all the other groups the rise in Protective Index was recorded, none of these groups differed significantly from the untreated control group.

On Day 28, Protective Index in the group treated with New Herbal Ointment+ Hemidesmus indicus rose up to 0.0165±0.003. Difference in rise of this Protective Index and the Protective Index of untreated control 0.010±0.001was highly significant. Furthermore this value was very close to 0.0105±0.003which was the Stress index value of the healthy control group on 28th day.

Thus to summarize following trends were observed in the parameters of the oxidative stress in this study

(a) **MDA levels and Stress index** rose in all the groups on day 1 of infliction of burns and thereafter started declining. The levels decreased steadily in New Herbal Ointment+ Hemidesmus indicus group reaching the levels of

healthy control on 28th day .MDA levels and Stress index in all other groups although declined but remained at higher levels as compared to healthy control.

(b) **SOD levels and Protective index** fell in all the groups on day 1 of infliction of burns and thereafter started increasing .The levels increased steadily in New Herbal Ointment+ Hemidesmus indicus group reaching the levels of healthy control on 28th day. SOD levels and Protective index in all other groups although raised but remained at higher levels as compared to healthy control.

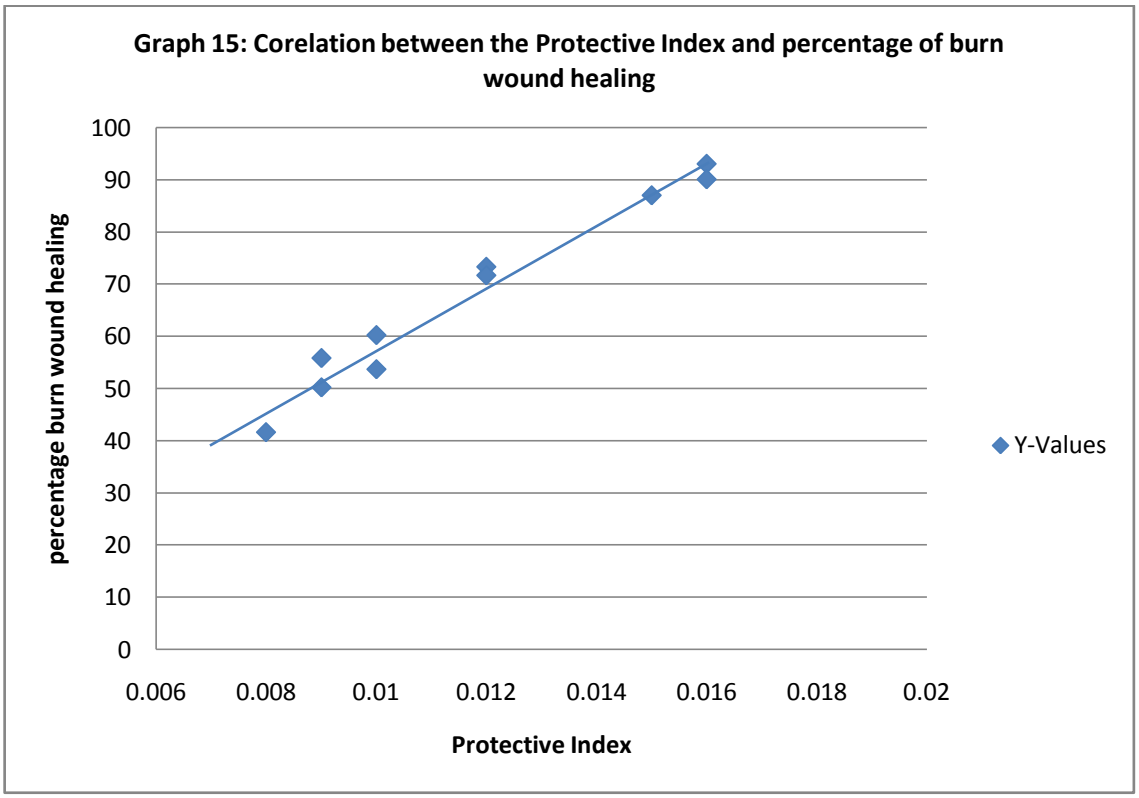
This suggests that there is an increase in the oxidative stress in case of burns which is in accordance with the studies conducted by various researchers [31,32,33,34,35,36,37]

Combination of New Herbal Ointment with Hemidesmus indicus the combination showed synergistic action. This synergism may be due to the antioxidant constituents in New Herbal Ointment in the form of Azadirachta indica and also in Hemidesmus indicus. The antioxidants present in Hemidesmus indicus and Azadirachta indica in New Herbal Ointment may be acting on different levels of

oxidative stress to provide synergism. The stages (levels) of antioxidant action are described by Panchwat S et al.[39]

The combination of New Herbal Ointment with Cissus quadrangularis also showed a additive effect but the effect was less in comparison with that of New Herbal Ointment+ Hemidesmus indicus. This may be due to the possibility that The antioxidants present in Cissus quadrangularis and Azadirachta indica in New Herbal Ointmentmay be acting on same level of oxidative stress resulting in infra additive action . However combining Silver sulfadiazine with Cissus quadrangularis did not show any additive action rather showed the overall effect less than silver sulfadiazine alone. It may be due to the heavy metal silver interfering with the antioxidant action of the plants under study. The silver being a heavy metal may itself act as pro oxidant as reported by Cortese – Krott, et al.[114] that silver ions induce oxidative stress when used as topical antimicrobial agents.

We searched literature through leading search engines namely Google, Pub med and Medline plus but did not come across any study combining with therapies of Azadirachta indica, Hemidesmus indicus, Cissus quadrangularis using the parameters of MDA, SOD , stress index and protective index in case of treatment of burns.



6.15Corelation between the Protective Index and percentage of burn wound healing

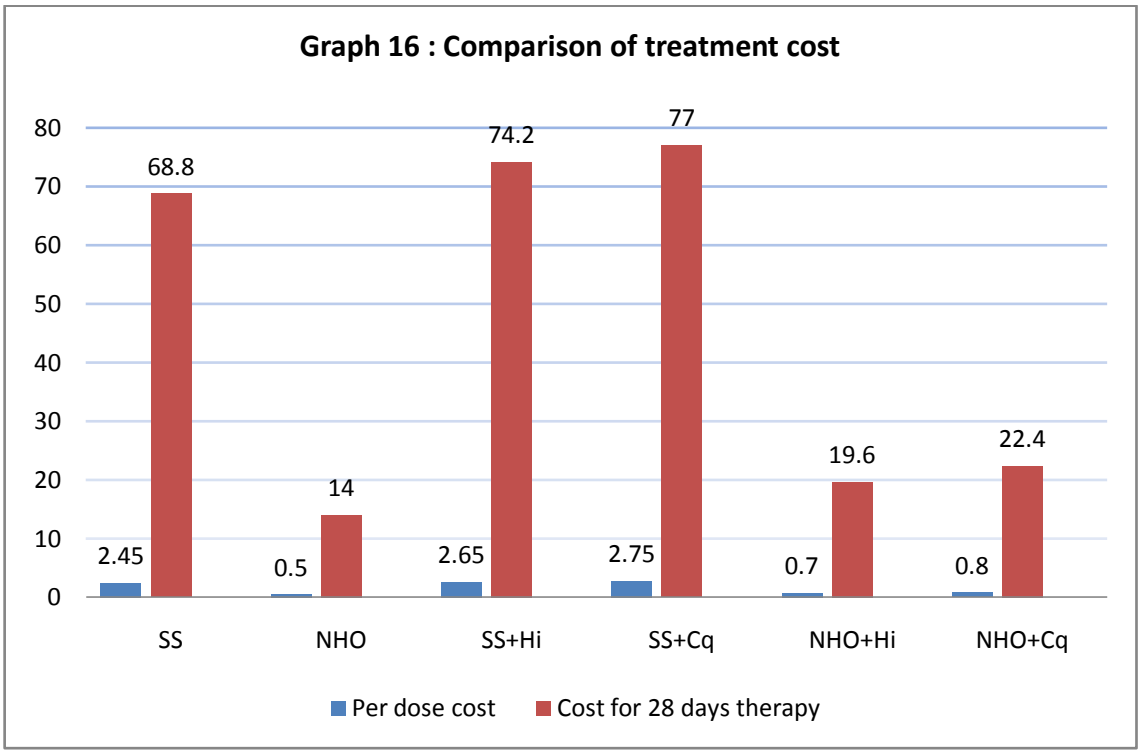
The graph of percentage of burn wound healing plotted against the protective index of corresponding day showed that most of the points on the graph lie on and around the straight line proving the fact that **there is a positive correlation between the burn wound healing and the protective index of the therapy. The correlation coefficient in this case was found to be 0.89 which lie very close to**

one indicating the positive correlation between the burn wound healing and the protective index.

This observation further confirmed the highest healing observed in the group treated with New Herbal Ointment+ Hemidesmus indicus where protective index came back to the levels of protective index in the healthy control after 28 days.

It is worth mentioning that in the group where in the protective index was highest on day 28 that is(0.0165±0.003) , the percentage of wound healing was also highest (93.17%) confirming the role of oxidative stress in the burn wound injury and in the subsequent healing process in experimental animals. However confirmation in clinical setting is desirable.

When we searched the internet using the leading search engines we did not come across any study establishing correlation between the Protective Index and percentage of burn wound healing



SS : Silver sulfadiazine **NHO :** New Herbal Ointment

SS +Hi: Silver Sulfadiazine + Hemidesmus indicus ,**SS + Cq :** Silver Sulfadiazine + Cissus quadrangularis

NHO+Hi: New Herbal Ointment+ Hemidesmus indicus ,

NHO+Cq: New Herbal Ointment+ Cissus quadrangularis

6.13 Treatment cost

On analysis of per dose cost it was observed that the cost per dose of silver sulfadiazine comes out to be ₹. 2.45 , New Herbal Ointment ₹ 0.5 , silver sulfadiazine + Hemidesmus indicus ₹. 2.65, silver sulfadiazine + Cissus

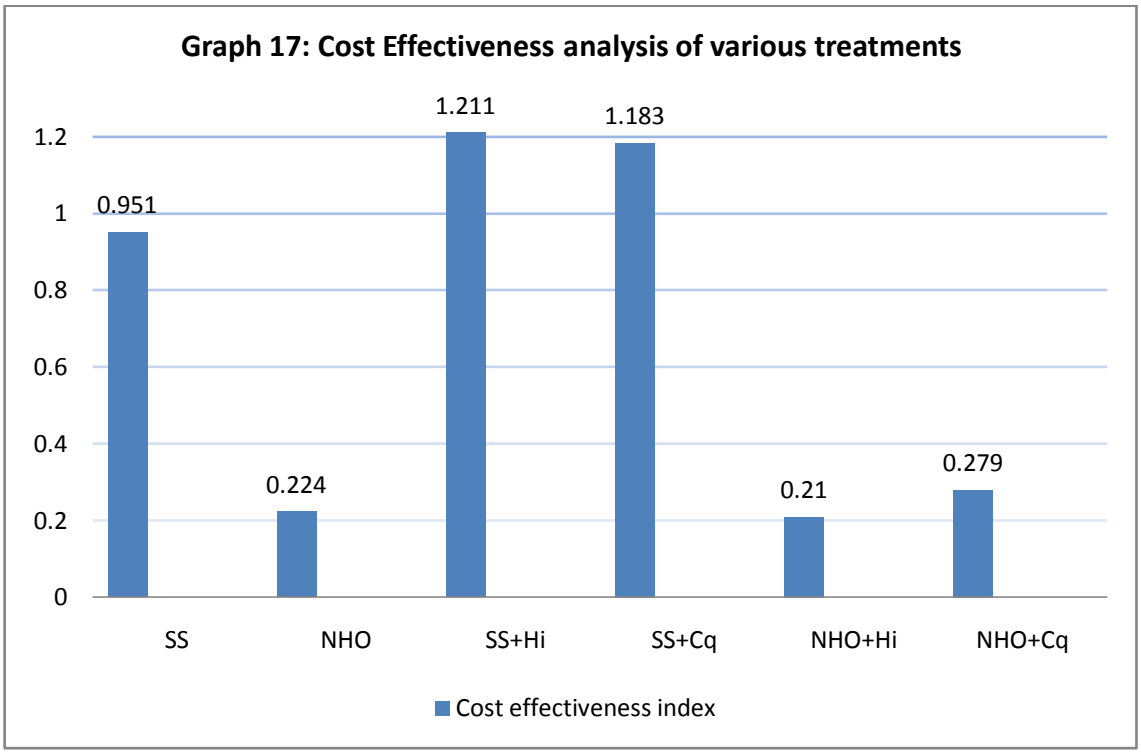
quadrangularis ₹ 2.75 , New Herbal Ointment+ Hemidesmus indicus ₹. 0.7 and New Herbal Ointment+ Cissus quadrangularis ₹ 0.8

Accordingly the total cost of treatment for 28 days comes out to be

- ₹ 68.8 for silver sulfadiazine ,
- ₹ 14 for New Herbal Ointment,
- ₹ 74.2 for silver sulfadiazine + Hemidesmus indicus,
- ₹ . 77 for silver sulfadiazine + Cissus quadrangularis,
- ₹ 19.6 for New Herbal Ointment+ Hemidesmus indicus and
- ₹ . 22.4. for New Herbal Ointment+ Cissus quadrangularis

The cost for 28 days therapy in the group treated with New Herbal Ointment and Hemidesmus indicus was Rs 19.6 as compared to the silver sulfadiazine alone which was Rs 68.8

The New Herbal Ointment alone or in combination with Hemidesmus indicus proves to be more economical (cheaper by 485 % and 308% respectively) that is 4.85 times cheaper and 3.08 times cheaper respectively as compared to the silver sulfadiazine which is supposed to be standard therapy for burn treatment.



SS : Silver sulfadiazine	**NHO :** New Herbal Ointment
SS +Hi: Silver Sulfadiazine + Hemidesmus indicus	**SS + Cq :** Silver Sulfadiazine + Cissus quadrangularis
NHO+Hi: New Herbal Ointment+ Hemidesmus indicus	
NHO+Cq: New Herbal Ointment+ Cissus quadrangularis	

6.14: Cost Effectiveness

Cost effectiveness of the treatments was calculated using the formula [115]

$$\text{Cost effectiveness} = \frac{\text{Total cost of therapy for 28 days treatment in rupees}}{\text{Percentage of wound contraction on } 28^{th} \text{ day}}$$

In other words it gives the cost required for the healing of 1% of the ulcer. Lesser is this ratio more cost effective is the treatment

When cost effectiveness analysis (CEA) was done it was observed that the cost required per unit effect (for 1% healing of the ulcer) is as follows

Silver sulfadiazine alone	₹. 0.951,
New Herbal Ointment alone	₹. 0.224,
Silver sulfadiazine + Hemidesmus indicus	₹ 1.211,
Silver sulfadiazine + Cissus quadrangularis	₹1.183,
New Herbal Ointment+ Hemidesmus indicus	₹. 0.210
New Herbal Ointment+ Cissus quadrangularis	₹. 0.279

Thus the combination of New Herbal Ointment+ Hemidesmus indicus was found to be most cost effective. References are not available regarding the Pharmacoeconomics of Hemidesmus indicus, Cissus quadrangularis, Azadirachta indica and Silver Sulfadiazine in the treatment of burn wounds.

Thus it is evident that the therapy with New Herbal Ointment+ Hemidesmus indicus is the most cost effective treatment amongst all the therapies under present study in experimental animals. However confirmation in the clinical setting is required. Further clinical studies are highly desirable in the view of the findings and observations of this study.

7. CONCLUSION

7.1 A New Herbal Ointment was successfully formulated and an application was filed for the patenting of the formulation.

7.2 The highest percentage of wound contraction (93.17%) was observed in the group treated with New Herbal Ointment+ Hemidesmus indicus.

7.3 The period of complete reepithelization was minimum (26 days) in the group treated with New Herbal Ointment+ Hemidesmus indicus.

7.4 The grade of wound healing was maximum (with score 18.83 on 20 point scale) in the group treated with New Herbal Ointment+ Hemidesmus indicus.

7.5 Most prominent epithelial regeneration was observed in the group treated with New Herbal Ointment+ Hemidesmus indicus.

7.6 No growth of microorganisms was observed in any group under study **except the untreated control group.**

7.7 **MDA levels and Stress index** rose in all the groups on day 1 of infliction of burns and thereafter started declining. The levels decreased steadily in New Herbal Ointment+ Hemidesmus indicus group reaching the levels of healthy control on 28th day while the MDA levels and Stress index in all other groups remained at higher levels as compared to healthy control.

7.8 **SOD levels and Protective index** fell in all the groups on day 1 of infliction of burns and thereafter started increasing .The levels increased steadily in New Herbal Ointment+ Hemidesmus indicus group reaching the levels of healthy control on 28th day while the SOD levels and protective index in all other groups remained at lower levels as compared to healthy control.

7.9 New Herbal Ointment was the cheapest in terms of per dose cost (0.5 rupees) and total cost (14 rupees) for the therapy for 28 days

7.10 New Herbal Ointment+ Hemidesmus indicus was most cost effective therapy as compared to the other treatments costing ₹ 0.21 per unit effect, as against ₹ 0.951 per unit effect for Silver sulfadizine which is considered as the standard therapy for burns.

7.11 There was a strong positive correlation between protective index and percentage of burn wound healing with correlation coefficient of 0.89.

Thus it is deduced that amongst the therapies under consideration the most effectual and cost effective therapy for the second degree burn wounds in New Zealand white rabbits was New Herbal Ointment in combination with Hemidesmus Indicus.

8. SUMMARY

Indian herbal medicine backed with great heritage of Ayurveda and modern studies are receiving tremendous attention from researchers thereby causing exponential rise in research on Indian plants

Burn is defined as tissue damage caused by a variety of agents such as heat, chemicals, electricity, sunlight or nuclear radiation .The most common are burns caused by scalds, building fires and flammable liquids and gases. Thermal burn and related injuries have remained a major cause of death and disability. Although small burns are not usually life threatening, they need the same attention as large burns, in order to achieve functional and cosmetic outcome

Wound healing is the interaction of a complex cascade of cellular and biochemical actions leading to the restoration of structural and functional integrity with regaining of strength of injured tissues. It involves continuous cell-cell interaction and cell-matrix interactions that allow the process to proceed in different overlapping phases and processes including inflammation, wound contraction, reepithelialization, tissue remodelling, and formation of granulation tissue with angiogenesis. The phases of wound healing normally progress in a predictable, timely manner, and if they do not, healing may progress inappropriately to either a

chronic wound such as a venous ulcer or pathological scarring such as a keloid scar [9]

A burn patient who receives the best of treatment is expected to heal without any contractures. The incidence of post-burn contractures is extremely high in our country. An understanding of the burn wound healing is fundamental not only to the management of the acute burn wound, but also for the prevention, minimization and treatment of post-burn scars and scar contractures So, there is a growing need to develop drugs which will decrease the complications and prevent infections more effectively than the presently used drugs

Antioxidants provide protection to living organisms from damage caused by uncontrolled production of ROS and concomitant Lipid peroxidation, Protein damage and DNA stand breaking. Several substances from natural sources have been shown to contain antioxidants and are under study.

The present study was undertaken to compare the second degree burn wound healing property of herbal formulation with Silver sulfadiazine with and without the supportive therapy with antioxidants Hemidesmus indicus and Cissus quadrangularis.

New Herbal Ointment was prepared using Azadirachta indica leaves, Shorea robusta resin and Linum usitatissumum oil. For this a decoction of Azadirachta indica was made by boiling the leaves of Azadirachta indica in Linum usitatissumum oil. This decoction was then triturated with fine powder of Shorea robusta resin and the contents were washed hundred times with distilled water. An application was filed for the patenting of the new formulation.

The study is a preclinical study of randomized open experimental type conducted in New Zealand while rabbits where the second degree burns were inflicted with metal disc method developed by Pathak SS, Patel SS and Borkar MA. The second degree burns thus inflicted were treated with local application of New Herbal Ointment, ointment silver sulfadiazine while Hemidesmus indicus and Cissus quadrangularis were administered orally.

Blood samples were collected from the marginal ear vain of rabbits on day 0, day 1,day 7, day 14 , day 21 and day 28.

The study was conducted over the duration of 3 years using 104 male Rabbits. The animals were randomized in 8 groups of 13 animals each.

The sample size was calculated by using formula

$$n = \frac{\left[z(\alpha / 2)\sqrt{2\pi(1-\pi)} + z(\beta)\sqrt{\pi_1(1-\pi_1) + \pi_2(1-\pi_2)} \right]^2}{\delta^2}$$

The study was undertaken to

1. Compare the efficacy of healing the second degree burn using the parameters of
    - Percentage of wound contraction,
    - Period of repithelization,
    - Wound healing scores based on histopathological examination.
    - Epithelial regeneration and grading based on histopathology.
    - Wound swab culture for presence of infection.

    Wound healing scores based on histopathology and Epithelial regeneration grading were done using the grading given by Sultana et al.

2. Compare the oxidative stress using parameters of
    - MDA,
    - SOD,
    - Stress index,
    - Protective index

    MDA and SOD were estimated in blood using the method of Marklund and Marklund. Stress index is calculated as ratio of MDA/SOD and protective index was calculated as the ratio of SOD/MDA.

3. Analyze the Pharmacoeconomics of therapies using parameters of

- Cost per dose,
- Total cost of the therapy and
- Cost effectiveness

4. Find out correlation between protective index and percentage of burn wound healing

Observations were recorded ,represented in tabular form and graphs. Observation findings were stastistically analyzed using one way ANOVA , Dunnet's post hoc test, paired /unpaired student's t test, chi square test. The results are analyzed and discussed in the light of the available literature. The findings are summarized as under

- The highest percentage of wound contraction (93.17%) was observed in the group treated with New Herbal Ointment + Hemidesmus indicus.
- The period of complete reepithelization was minimum (26 days)in the group treated with New Herbal Ointment + Hemidesmus indicus.
- The grade of wound healing was maximum (with score 18.83 on 20 point scale) in the group treated with New Herbal Ointment + Hemidesmus indicus.
- Most prominent epithelial regeneration was observed in the in the group treated with New Herbal Ointment + Hemidesmus indicus.
- No growth of microorganisms was observed in any group under study except the untreated control group.

- **MDA levels and Stress index** rose in all the groups on day 1 of infliction of burns and thereafter started declining. The levels decreased steadily in New Herbal Ointment + Hemidesmus indicus group reaching the levels of healthy control on 28th day while the MDA levels and Stress index in other groups remained at higher levels as compared to healthy control.
- **SOD levels and Protective index** fell in all the groups on day 1 of infliction of burns and thereafter started increasing .The levels increased steadily in New Herbal Ointment + Hemidesmus indicus group reaching the levels of healthy control on 28th day while the SOD levels and protective index in other groups remained at lower levels as compared to healthy control.
- New Herbal Ointment was the cheapest in terms of per dose cost (0.5 rupees) and total cost (14 rupees) for the therapy for 28 days
- New Herbal Ointment + Hemidesmus indicus was most cost effective therapy as compared to the other treatments with cost effectiveness of 0.21 per unit effect as against 0.951 per unit effect for Silver sulfadiazine which is considered as the standard therapy for burns.
- There was a strong positive correlation between protective index and percentage of burn wound healing with correlation coefficient of 0.89.
- Thus it is deduced that amongst the therapies under consideration the most effectual and cost effective therapy for the second degree burn wounds in New Zealand white rabbits was New Herbal Ointment in combination

with Hemidesmus Indicus.

However confirmation in clinical settings is required. Further clinical studies are highly desirable in the view of the above findings and observations of this study.

BIBLIOGRAPHY

1. Datta HS, Mitra SK, Patwardhan B. Wound Healing Activity of Topical Application Forms Based on Ayurveda . Evidence-Based Complementary and Alternative Medicine.2011; 15-26.
2. Pradhan SL, Pradhan PS. Ayurvedic medicine and anaesthesia. Indian Journal of Anaesthesia. 2011;55(4):334-339.
3. Thakur R, Jain N, Pathak R, Sandhu SS. Evidence-Based Complementary and Alternative Medicine.2011;234-245
4. Shrivastava P, Durgaprasad S. Burn wound healing property of various substances : An Appraisal. Indian Journal of Pharmacology. 2008;40(4):144-146.
5. Pithayanukul P, Lapett B, Bavovada R, Pakmanee N, Suttisri R. Burn wound healing and Ayurveda Pharmaceutical Biology.2007 45(2) : 282-288.
6. Curtis PA, John AM. In ; The Treatment of Burns. Curtis PA, John AM,2nd ed; Philadelphia,WB Saundres Company;1969 :341.
7. Robert HD. Burn and Other Thermal Injuries. In: Current Surgical Diagnosis and Treatment.Lawrence WW, Gerard MD,11th ed;Large Medical Books/McGraw Hill;2003:267
8. Mike T. Burns. In : Baily and Love's Short Practice of Surgery. Russel RC, Normans, Williams,Christopher JK,24th ed,Arnold London;2004:270.
9. Mclachie GR,Leaper DJ. Oxford Handbook of Clinical Surgery.2nd ed;Oxford University Press, Oxford;2002:497.
10. Tabassum N, Agrawal S. Hepatoprotective activity of Eclipta alba Hassk. Importance of herbal medicine in treating burns JK – Practitioner2004; 11(5): 280.

11. Atiyeh BS, Costagliola M, Hayek SN, Dibo SA. Effect of silver on burn wound infection control and healing: Review of the literature. Burns. 2007;33: 139-148.
12. Silver Sulfadiazine (Topical),USP DI Drug Information For The Medical Care Professionals, 2000.
13. Sutter VL, Hurst V. Sources of Pseudomonas aeruginosa Infection in Burns: Study of Wound and Rectal Cultures with Phage Typing. Annals of Surgery. 1966; 163(4): 597-602.
14. Goel A, Shrivastava P. Post-burn scars and scar contractures. Indian Journal of Plastic Surgery. 2010;43 (suppl) :63-71.
15. Warrier PK,Nambiar VP,Ramankutty C.Indian Medicinal Plants Vol:1;1st ed; Orient Longman, Hyderabad,1994:227.
16. Prajapati,Purohit,Sharma,Kumar.A Handbook of Medicinal Plants : A Complete Source Book;1st ed;Agrobios (India),Jodhpur,2003:474.
17. Prajapati,Purohit,Sharma,Kumar.A Handbook of Medicinal Plants : A Complete Source Book;1st ed;Agrobios (India),Jodhpur,2003:318
18. Ojha JK. Chyawanprash. Antiquity of Chyawanprash. Chaukhamba Sanskrit Pratishthan Delhi 1ST edition 2003; 11-17.
19. Borkar MA and Patel SS oxidative stress in infectious and degenerative diseases and its modification by specific and antioxidant therapy. Thesis submitted for the Phd in medical pharmacology , DMIMS, 2011.
20. Jainu, Mallika and Shyamala Devi (2003) "Tent antiulcerogenic activity of methanolic extract of Cissus quadrangularis by antioxidative mechanism"; *ournal of Clinical Biochemistry and Nutrition*, 34; 43-47

21. Goel A, Kumar S, Singh DK, Bhatia AK. Burn the important public issue Indian Journal of Experimental Biology. 2010;48: 402-406.
22. Gayathri M, Kannabiran K. Epidemiology of burns Indian Journal of Experimental Biology. 2010;48: 159-164.
23. Banu J, Varela E, Bahadur AN, Soomro R, Kazi N, Fernandes G Burns in Indian rural population.Evidence-Based Complementary and Alternative Medicine. 2012; 2012:10 pages.
24. Dhande PP, Simpy R, Kureshee NI, Sanghavi DR, Pandit VA. Commonest causes of superficial and deep burns Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2012;3(4):747-754.
25. Robert HD. Burn and Other Thermal Injuries. In: Current Surgical Diagnosis and Treatment. Lawrence WW, Gerard MD,11th ed;Large Medical Books/McGraw Hill;2003:283
26. Mike T. Burns. In : Baily and Love's Short Practice of Surgery. Russel RC, Normans, Williams,Christopher JK,24th ed,Arnold London;2004:370.
27. Meena K, Mohan AV, Sharath B, Somyaji SN, Bairy KL. Classifications of burn wounds and their scientific rationale. Indian Journal of Experimental Biology. 2011;49:56-59.
28. Gupta JL, Makhija LK, Bajaj SP. Burns a review. 2010;43(supp 1):6-10.
29. Chamania S. Healing process and its modification in burns . Indian Journal of Plastic Surgery. 2010;43(supp 1):126-130.
30. Tiwari VK. Burns and the systemic responses Indian Journal of Plastic Surgery. 2012;45(2):364-373.

31. Yoshikawa T, Naito Y. What Is Oxidative Stress?. Japan Medical Association Journal. 2002;45(7):271–276.
32. Mühl D, Woth G, Drenkovics L, Varga A, Ghosh S, Csontos C et al. Comparison of oxidative stress & leukocyte activation in patients with Severe sepsis and burn injury. Indian Journal of Medical Research. 2011;134:69-78.
33. Sahib AS, Al-Jawad FH, Al-Kaisy AA. Burns, Endothelial dysfunction, and Oxidative Stress: The role of Antioxidants. Annals of Burns and Fire Disasters. 2009;22(1):6-11.
34. Irshad M and Chaudhuri Oxidant –antioxidant system: Role and significance in human body. Indian journal of experimental Biology.2002; 40 (9): 1233-1239
35. Kumar, Abbas,Fausto.Robbins Pathological Basis of Disease cellular Responses to Stress and toxic Insults: adaptation, injury and Death. Thomson Press Reprinted. 2007; 22.
36. M.A.R. Bhuiyan, M.Z. Hoque and S.J. Hossain. Free Radical Scavenging Activities of Zizyphus mauritiana. World Journal of Agricultural Sciences.2009; 5 (3): 318-322.
37. Ramadoss S, Kannan K, Balamurugan K, Jeganathan NS, Manavalan R. Oxidative stress a review. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2012; 3 (1): 497-502.
38. Joana Paixão, Teresa C. P. Dinis, and Leonor M. Almeida .Protective Role of Malvidin-3-Glucoside on Peroxynitrite-Induced Damage in Endothelial Cells by Counteracting Reactive Species Formation and Apoptotic Mitochondrial Pathway . Oxidative Medicine and Cellular Longevity. 2012; 4(3):1-30

39. Panchwat S, Rathore KS, Sisodia SS. Review on Herbal antioxidant. International Journal of Pharm Tech Reseearch.2010;2 (1):232-239.
40. Talim Manisha. antioxident and vitamins and their role in diabetes mellitus, heart disease and cancer. Journal of diab Assoc .India 1999; 39(3):69-73.
41. Pattanayak P, Behera P, Das D, Panda SK. Ocimum sanctum Linn. Enzymatic antioxidants: An overview. Pharmacognosy Review. 2010; 4(7): 95-105.
42. Torn M, YardimS, Gonenc A, sargin H, Menevse A and Simsk B. Serum B-carotene, Vitamin E, Vitamin C and Malondialdehyde levels In several types of cancer J. clin. Pharmacol. 1995; 20(2); 259.
43. Flemming Nielsen, Bo Borg Mikkelsen, Jesper Bo Nielsen, Helle Raun Andersen, and Philippe Grandjean. Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors: Clinical Chemistry. 1997; 43(7): 1209–1214.
44. Oberley LW & Oberley TD. The role of Superoxide dismutase and the gene amplification in carcinogenesis. J Theor BioI.1984; 10 (6): 403.
45. Marklund S L, Holme E & Hellner L. Superoxide dismutase in extracellular fluids. Clin Chim Acta. 1982;19(1) 126.
46. Abdel-Aziz M, Kamal, Mohammed El, Khafif, Soher, Koraah, Aly Massoud, Jean-Francois Caillard. Blood superoxide dismutase and plasma Malondialdehyde among workers exposed to asbestos. American Journal of Industrial Medicine. 2007; 21(3): 353-361.
47. Richard W. Strange, Michael A. Hough .Structural Evidence for a Copper-Bound Carbonate Intermediate in the Peroxidase and Dismutase Activities of Superoxide Dismutase. Can. J. Appl. Physiol .2012; 34 (1) 1-24.

48. Ruben Quintana-Cabrera, Seila Fernandez-Fernandez. γ-Glutamylcysteine detoxifies reactive oxygen species by acting as glutathione peroxidase-1 cofactor Nat Commun. 2012 6 (3): 6-25.
49. De Varies J. Catalase and its role in oxidative process Ttansfer Biochem Pharmacol 1981; 30(1) 299-402.
50. Senthil Kumar and K.L. Senthil. Healing of burns in Rats. European Journal of Biological Sciences.2012; 4 (1): 01-04.
51. Girish C, Koner BC , jayanthi S , RaoKR , Rajesh B, and Pradhan SC. Healing process ib burns and interventions Indian J Med Res.2009; 12(9): 569-578
52. Surendra K, Sharma, Mohammad Ali and Gupta Jyoti. Epithelization and healings in burns Phytochemistry and Pharmacolog. 2005;51(2): 267 .
53. Handa SS. Burns and its treatment with ayurvedic plants. Central council of Ayuruveda and Siddha New Delhi.2006; 14.
54. Gibanananda Ray and syed Akhtar Husain. Burn wound behavior . Indian journal of experimental Biology.2002; 40 (9): 1213-1232.
55. Gayatri G, Ramesh Babu J, Sumalatha N, Venkateswarao J and S Vidyadhara. Healing process and its enhancement International Journal of Comprehensive Pharmacy. 2011; 1 (1):1-3
56. Jitendra jena and Ashish kumar Gupta. Management of burns Review. International Journal of Pharmacy and Pharmaceutical Sciences.2012; 4(4):1.17
57. Sunita Dalal, Sudhir Kataria, Sastry K, Rana SVS.Management of burns in practice and herbal formulations. Ethnobotanical Leaflets.2010; 14(2): 248-258.
58. Nilesh Mehta. Steps in management of burns . www.Medscape.com updated. 2012:1-2.

59. Stefan Russmann, Gerd A, Kullak-Ublick and Ignazio Grattagliano. Current Concepts of Management of burns. Current Medicinal Chemistry. 2009; 16(7): 3041-3053.
60. Malik K.K, PAL M.B. Burns in Children , Paediatric reviews 1979; 5: 23.
61. Ramon Rodrigo, Cristian Guichard, Roberto Charles. Neutrition in burns. Fundamental and Clinical Pharmacology.2007;14(2)111-127.
62. Lara HH, Trevino ENG, Turrent LI, Singh DK. Silver nanoparticles are broad-spectrum bactericidal and virucidal compounds. Journal of Nanobiotechnology. 2011; 9: Article 30.
63. Borsuk DE, Gallant M, Richard D, Williams HB. Silver-coated nylon dressings for pediatric burn victims. Canadian Journal of Plastic Surgery. 2007;15(1): 29-31.
64. Hosseinimehr SJ, Khorasani G, Azadbakht M, Zamani P, Ghasemi M, Ahmadi A. Effect of Aloe Cream versus Silver Sulfadiazine for Healing Burn Wounds in Rats. Acta Dermatovenerologica Croatica. 2010;18(1):2-7.
65. V.B. Fulzele, A.t. Shedage, A. Anton Smith, T.V. Gaikwad, S.R. Kirtane. Healing activity of silver sulfadiazine in rats International Journal of Pharmacy and Pharmaceutical Sciences. 2012;4 (8):54-62.
66. K.Raghunathan, Ms. Roma Mitra. Advantages and disadvantages of silver sulfadiazine International Journal of Pharmacy and Pharmaceutical Sciences. 2012;4 (8):54-62.
67. V. M. Jadhav . R. M.Thorat, V.J. Kadam and K.P. Salaskar. Indian medicinal plants and burns Journal of Pharmacy Research. 2009; 2(8):1129-1231
68. Sandeep Majumdar, M. Natvarlal Saraf and R. Yadav Kamble. Herbs in the treatment of burns Iranian journal of Pharmacology & therapeutics.2010; 9(2): 103-105.

69. Nazim Uddin , Atiar Rahman , Nazim Uddin Ahmed , Sohel Rana , Rasheda Akter, Masudul Azad Chowdhury. Hernal remedy of burns Int J Biol Med Res. 2010; 1(4): 341-346.
70. Karthikumar S, Vigneswari K and Jegatheesan K. treatment of burns with some of the medicinal plants of central india Scientific Research and Essay.2007; 4 (2): 101-104.
71. Indian Council of Medical Research Medicinal Plants of India. Volume-2, 1987
72. Austin A. A Review on Indian Sarasparilla, Hemidesmus indicus (L.) R. Br.. Journal of Biological Sciences. 2008; 8(1): 1-12.
73. Sawant S, Issac JC, Narayanan M. Analgesic studies on total alkaloids and alcohol extracts of Hemidesmus indicus Phytotherapy Research.2004; 10(5): 111-112.
74. Arunachalam G, Subramanian N, Pazhani GP, Ravichandran V. Anti-inflammatory activity of methanolic extract of Hemidesmus indicus African Journal of Pharmacy and Pharmacology.2009; 3(7): 97-100.
75. Ohkawa H, Ohishi N, Yagi K. Pharmacological and medicinal activities of Hemidesmus indicus and applications in biochemistry Analytical Biochemistry. 1979; 95:351-358.
76. Sen MK, Dash BK. A review on phytochemical and pharmacological aspects of Cissus quadrangularis L., International Journal of Green Pharmacy. 2012; July-September: 169-173.
77. Shah U. Cissus quadrangularis L.: Phytochemicals, Traditional Uses and Pharmacological Activities – A Review. International Journal of Pharmacy and Pharmaceutical Sciences. 2011; 3(Suppl 4):41-44.

78. G. T. A.Jombo1 and M. N. O. Enenebeaku Profile of Cissus quadrangularis findings from a preliminary analysis Nigerian Journal of Physiological Sciences. 2008; 23 (1): 55-59.

79. Manoj Kumar Pandey, G.N.Singh, Rajeev Kr, Sharma and Sneh Lata. Healing activity of Cissus quadrangularis. Journal of Applied Pharmaceutical Science. 2011;7 (1): 104-107.

80. Bapna S, Asdule S, Shirshat MS , Jadhav S, Patil LS, Deskmukh RA. Cissus quadrangularis a review Journal of Phytopharmacology 2007; 39(1): 91-94.

81. Lok Ranjan Bhatt and Seung Hwa Baek. Antioxidant Activity of Crude Extract and Fractions from Azadirachta indica The Society of Cosmetics and Public Health. 2006 2(1): 5-8.

82. Amal Kumar Ghimeray, Cheng-Wu Jin1, Bimal Kumar Ghimire and Dong Ha Cho. Antioxidant activity and quantitative estimation of Azadirachtin and Nimbin in Azadirachta Indica A. Juss grown in foothills of Nepal. African Journal of Biotechnology.2009; 13 (4): 3084-3091.

83. Gayatri Nahak and R.K. Sahu. In vitro antioxidative acitivity of Azadirachta indica and Melia azedarach Leaves by DPPH scavenging assay. Journal of American Science. 2010; 6(6):123-128.

84. Maragathavalli S, Brindha, SKaviyarasi, NS. Annadurai, B. & Gangwar, S.K. Antimicrobial Activity in leaf extract of Neem (Azadirachta indica Linn.) I.J.S.N.2012; 3(1): 110-113.

85. Saradhajyothi Koona, Subbarao Budida. Antibacterial potential of the Extract of the Leaves Azadirachta indica Sci Bio. 20011; 3(1): 65-69.

86. V natarajan ,PV Venugopaland T Menon. Effect of Azadirachta indica on the growth Pattern of Dermatophyte. Indian Journal of Medical Microbioloy 2003; 21(2):98-101.

87. Chattopadhyay R R and M Bandyopadhyay. Effect of Azadirachta indica leaf extract on serum Lipid profile change in normal and Streptozotocin induced diabetic rats. African Journal of Biomedical research. 2005; 8(10):101–104.

88. Akbar Waheed, Ga Miana and Si Ahmad. Clinical investigation of hypoglycemic effect of seeds of azadirachta indica in type-2 Diabettes Mellitus. Pak J Pharm Sci. 2006; 19 (4): 322-325.

89. Khosla P, Bhanwra Sangeeta, Singh J, R. k Srivastava. Antinociceptive activity of Azadirachta indica (neem) in rats. Indian Journal of Pharmacology 2000; 32(9): 372-374.

90. M. Dorababu, MC. Joshi,Bhawani GM. Mohan Kumar Aditi Chaturvedi and R.K Gole. Effect of Aques Extract of Azadirachta indica Leaves on Offensive and Diffensive Gastric Mocosal Factor in Rats. Indian Journal of Pharmacology. 2006;50(3): 241–249..

91. Yanpallewar SU, Sen S, Tapas S, Kumar Mohan, Raju SS, Acharya, SB. Phytomedicine International Journal of Phytotherapy & Phytopharmacology. 2003; 5(2):234-141.

92. Bhanwra S, Singh J. and Khosla P, Indian J. Some Protective Effects of Aqueous Leaf Extract of Azadirachta indica on Paracetamol- induced Hepatotoxicity in Adult Wistar Rats American Journal of tropical medicine & Public Health. 2011; 1(3): 97-106.

93. Despande V Y, Mendulkar K N and Sadre N L. J. Male antifertility activity of Azadirachta Indica in mice. Postgrad. Med. Bombay 1980; 26(3): 167–170.

94. K. sathiyaraj, A, P.Vinoth, Mar K Devi, B. Senthil Kumar. Antinosiceptive and Spermicidal Activity of Azadirachta indica (Neem) Aqueous Leaf Extract on Male Albino Rats International Journal of Pharm Tech Research.2010; 2(1): 588-591.

95. Subhash Chander, Gangar, Rajat S, Sanddhir ,Durg VijayRai, Ashwani Kou. Modulatory .Effect of Azadirachta indica on benzo(a)pyren-induced forestomach tumorigenesis in mice. World Gastroenterol. 2006; 12 (17): 2749-2755.

96. J. Ananthia A, Prakasam and K.V. Pugalendi. Haepatoprotective activity of Azadirachta indica Yale. journal OF Biology and Medicine .2003; 76(1): 97-102.

97. Tabassum N, Agrawal S. Hepatoprotective activity of Azadirachta indica Against Paracetamol induced hepatocellular damage in mice. JK – Practitioner2004; 11(5): 280.

98. Saxena AK, Singh B, Anand KK. Antifertility effects of Azadirachta indica on subcellular levels in rats. Journal of Ethnopharmacology. 1993; 40(1):155-161.

99. Subhash chander, A.indica: chemopreventive effect against induced for stomach tumors in murine model. Archives of Applied Science Research. 2010; 2 (1): 86-94

100. K.Raghunathan and miss Roma mitra *Linum usitatissimum* Pharmacognosy of indigenous drugs. Central council in Ayurvada and Siddha dehli 2nd reprinted 2005 P 228-335.

101. Adel Kadri, Néji Gharsallah, Mohamed Damak and Radhouane Gdoura. Chemical Composition and in vitro antioxidant properties of essential oil of *Linum usitatissimum* Journal of Medicinal Plants Research.2011; 5(8):1466-1470.

102. Sabina Evan Princea, Parameswarib, Rasool Mahaboob Khanc. Protective Effect of

Linum usitatissimum on various pathological conditions Iranian Journal of Pharmaceutical Sciences Autumn 2011: 7(4): 269-278.

103. Asha B, Nagabhushan A, Shashikala GH. Study of wound healing activity of topical Shorea robusta_in albino rats. Journal of Chemical and Pharmaceutical Research. 2011; 3(6):122-126.
104. Shetty S, Udupa S, Udupa L, Somayaji N. Some of the medicinal propertied of Shorea robusta Indian Journal of Physiology and Pharmacology. 2006; 50:163-168.
105. Udupa SL, Shetty S, Udupa AL, Somayaji SN. Exploring the associated uses of Shorea robusta Indian Journal of Experimental Biology. 2006; 44:49-54.
106. Pathak SS , Patel SS , Borkar MA A comparison on healing effects of an ayurvedic preparatioln and silver sulfadiazine on burn wounds in albino rats , Journal of Dutta Meghe Institute of Medical Sciences, 2006, Vol 2, No (2),81-88
107. Pathak S.S., Patel S.S., Borkar M.A. World Academy of science Engineering and Technology, issue 80 August 2013, Kualla Lumpur , 684.
108. Bairy KL, Satish MC, Savin CG, Kiran KN, Avinash M. Effect of Different Formulations of Silver Sulphadiazine Cream on experimentally Induced Burn Wound Healing. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2012; 3(2):884-889.
109. Lemo Niksa , Genevieve Marignac , E R Gomez et al., cutanious reepithelization and wound contraction after skin biopsies in rabbits : a mathematical model , vetarnarski arhiv 80 95) , 637-652,2010.
110. Sultana J, Molla MR, Kamal M, Shahidullah M, Begum F, Bashar MA. Histological Differences in Wound Healing in Maxillofacial Region in Patients with or without Risk Factors. Bangladesh Journal of Pathology. 2009; 24(1):3-8.

111. Marklund S, Marklund G. Involvement of Superoxide Anion Radical in the Autoxidation of Pyrogallol and a Convenient Assay for Melanoaldehyde and Superoxide Dismutase. European Journal of Biochemistry. 1974; 47:469-474.

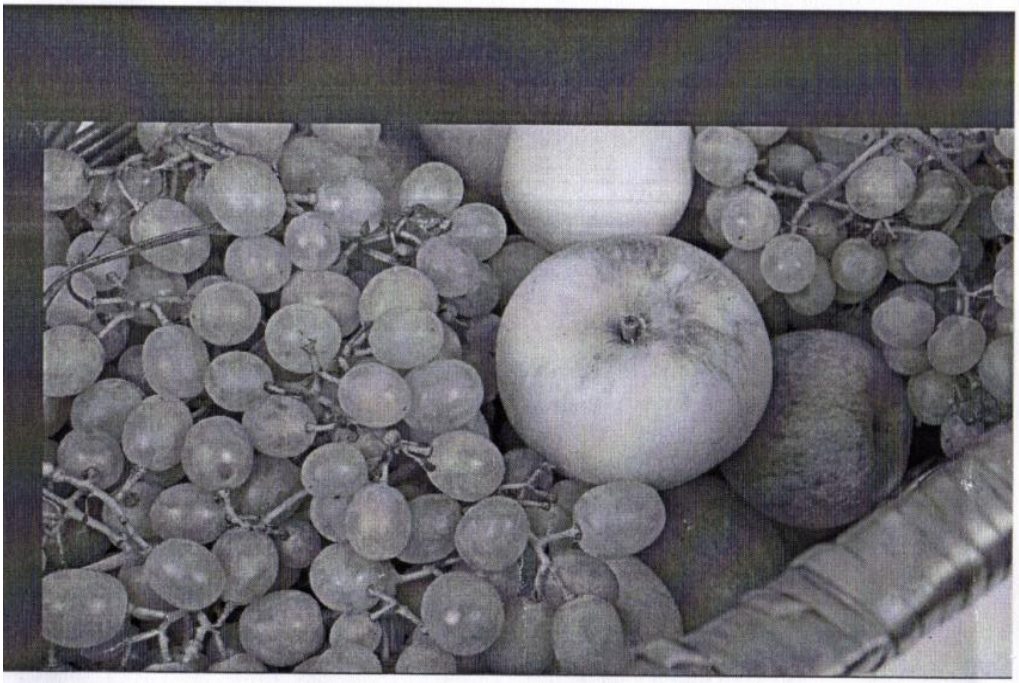
112. Dulao Wang and Ameet Bhakai, calculation of sample size , clinical trials , university press , Singapore , 2001, 234-236.

113. Dulao Wang and Ameet Bhakai, methods of randomization , clinical trials , university press , Singapore , 2001, 333-245.

114. Cortese – Krott MM et al, Silver ions induce oxidative stress and intracellular zink release in human skin fibroblast , freeadbiomed,2009, Epub sept.

115. Berger M.L., Hillman AL,Luce BR et. al. Economic Analysis of health care technology, A report on principles. Ann Intern Med 1995; 122:61-70.

ANNEXURE IV



SWANAND PATHAK
SATYAWANSINGH PATEL
BABASAHEB KALE

ANTIOXIDANTS IN HEALTH AND DISEASE

LAP LAMBERT Academic Publishing

Monograph titled 'Antioxidants in Health and Disease' published by Lambert Academic Press

ANNEXURE I

PATENT OFFICE
INTELLECTUAL PROPERTY BUILDING
S.M. Road, Antop Hill, Mumbai-400 037
Te No. (091)(022) 24137701, 24141026 FAX No. 02 24130987
E-mail : mumbai-patent@nic.in
Web Site : www.ipindia.gov.in

GOVERNMENT OF INDIA

INTELLECTUAL PROPERTY INDIA

Docket NO : 826
To
N. K. GUPTA OF NICHE
38A, NARSINGH AVENUE, NAGER BAZAR, KOLKATA-700 074, INDIA.

Date/Time : 15/01/2013
Agent Number:

Sr. No.	CBR Number	Reference Number /Application Type	Application Number	Title/Remarks	Amount Paid	Amount Computed
1	595	ORDINARY APPLICATION Pages:-16 , Claims:-11	134/MUM/2013	"HERBAL COMPOSITION FOR THE TREATMENT OF THERMAL BURNS".	1200	1200
2		E-101/2461/2013-MUM	134/MUM/2013	Correspondence	0	0
3		E-2/194/2013-MUM	134/MUM/2013	Form2	0	0
4		E-3/147/2013-MUM	134/MUM/2013	Form3	0	0
5		E-45/57/2013-MUM	134/MUM/2013	Form26	0	0
Total Amount					1200	1200

Received a sum of Rs. 1200 (Rupees One Thousand Two Hundred only) as under

Payment Mode	Bank Name	Cheque/Draft Number	Cheque/Draft Date	Amount in Rs
Cheque	HDFC BANK	756258	14/01/2013	1200

Note: This is electronically generated receipt hence no signature required.

* This receipt is issued subject to realization of cheque. In case the amount of fee as mentioned on cheque is not realized, the document so filed shall be deemed to have not been filed at the office under section 142(3) of the Patents Act 1970.

ANNEXURE III

DATTA MEGHE INSTITUTE OF MEDICAL SCIENCES
(DEEMED UNIVERSITY)
NAAC Accredited Grade "A"
Regd. Office : S. D. M. P. Campus, Atrey Layout, Rana Pratap Nagar, Nagpur – 440 022 , Maharashtra (INDIA) * Phone : (0712) 3256552, 3253764
* Fax : (0712) 2245318 * E-mail : info@dmims.org
Camp Office : Sawangi (Meghe), Wardha – 442 004 Maharashtra (INDIA) * Phone : (07152) 287701, 287702, 287703, 287704, 287705, 287706
* Fax : (07152) 287714 / 287719 * E-mail : medical_wda @ sancharnet.in * Web : www.dmims.org

Ref. No. DMIMSU/IAEC/2011-12/ 06 Date: 22.06.2011

INSTITUTIONAL ANIMAL ETHICS COMMITTEE
(I.A.E.C.)
Registration No. 571 / a / CPCSEA

The Institutional Animal Ethics Committee in its meeting held on 21.06.2011 has approved the following Research Work proposed to be carried out at Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha.

This approval has been granted on the assumption that the proposed work will be carried out in accordance with the ethical guidelines prescribed by Indian Council of Medical Research, Medical Council of India and C.P.C.S.E.A., Animal Welfare Division, Ministry of Environment and Forests, Government of India.

The details of the Proposed Research Work approved by the committee are as under :

SR. No.	PH.D CANDIDATE	SUPERVISOR	DEPARTMENT	TITLE OF PROPOSED RESEARCH
01.	DR. SWANAND S. PATHAK	DR. S. S. PATEL	PHARMACOLOGY	COMPARISON OF THE EFFECTS OF SILVER SULFADIAZINE AND HERBAL PREPARATION ON THERMAL INDUCED PARTIAL THICKNESS BURNS IN RABBITS WITH AND WITHOUT SUPPORTIVE THERAPY WITH CISSUS QUADRANGULARIS AND HEMIDESMUS INDICUS

(Dr. Rajesh K. Jha)
Secretary
Institutional Animal Ethics Committee

Copy with compliments to
01. Dr. Swanand S. Pathak
02. Dr. S.S. Patel, Supervisor, Professor of Pharmacology and Chief Co-ordinator , D.M.I.M.S. (D.U.), Sawangi (Meghe)
03. The Head, Department of Pharmacology, J.N.M.C., Sawangi (Meghe)
04. Hon'ble Dean, J.N.M.C., Sawangi (Meghe) for kind information
05. Hon'ble Chairman, Institutional Animal Ethics Committee, D.M.I.M.S. (D.U.)
06. Hon'ble Registrar, D.M.I.M.S. (D.U.), Atrey Layout, Pratap Nagar, Nagpur-440022
07. Hon'ble Prof. Anant P. Hardas, Nominee C.P.C.S.E.A., 52, Madhav Nagar, Nagpur – 440010
08. Hon'ble Dr. (Mrs.) Nilima S. Tankhiwale, Secretary, I.E.C., D.M.I.M.S. (D.U.)

ANNEXURE II

DATTA MEGHE INSTITUTE OF MEDICAL SCIENCES

(Deemed University)

(Established under Section 3 of The UGC Act 1956 vide Notification No F- 9 - 48/2004 - U 3 Govt of India)

NAAC Accredited Grade 'A'

INSTITUTIONAL ETHICS COMMITTEE

Regd. office : Atrey Layout, Pratap Nagar, NAGPUR - 442 022. Maharashtra (India) Ph - 0712- 325652/ 3253764 Fax - 0712-2245318 - E-mail - info@dmims.org website - dmims.org

Comp Office : Sawangi (Meghe), Wardha - 442 004, Maharashtra (India) Ph - 07152- 287701, 287702, 287703 Fax - 07152-287714 - E-mail - medical_wda@sancharnet.in

Ref. No. DMIMS (D U) /IEC/2010-11/97 Date: 24.11.2010

The Institutional Ethics Committee in its meeting held on 29.9.10 has approved the following research work proposed to be carried out at J. N. medical college & A.V.B.R. Hospital, Sawangi (Meghe), Wardha.

This approval has been granted on the assumption that the proposed work will be carried out in accordance with the ethical guidelines prescribed by Central Ethics Committee on Human research (C.E.C.H.R.)

The details of the proposed research work approved by the committee are as under:-

The following research Projects for **Doctoral (Ph. D.)** research was discussed.

Sr. No.	Chief Investigator (Co – Investigator)	Department	Approval	Topic of proposed	Remarks
1	Dr. Sawand S. Pathak (Dr. S. S. Patel)	Pharmacology	Approved	COMPARISON OF THE EFFECTS OF SILVER SULFADIAZINE AND HERBAL PREPARATION ON THERMAL INDUCED PARTIAL THICKNESS BURNS IN RABBITS WITH AND WITHOUT SUPPORTIVE THERAPY WITH CISSUS QUADRANGULARIS AND HEMIDESMUS INDICUS."	Nil

N.S. Tankhiwale

(Dr. N. S. Tankhiwale)
Secretary
Institutional Ethics Committee

Copy W/C to:-

1. Dr. Sawand S. Pathak, Dept. of Pharmacology, JNMC.
2. H.O.D. Dept. of Pharmacology JNMC.
3. Dean, JNMC for Kind information.
4. The Chairman, Institutional Ethics Committee, DMIMS (D U).
5. The Registrar, DMIMS (D U), H.O. Nagpur.