WOUND HEALING AND ANTIFUNGAL PROPERTIES OF LEMON GRASS

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FAJJAR AZHAR HUSSAIN

ABSTRACT

Medicinal Plants have the potential to heal wounds. Lemon grass is one of several plants that have antifungal and wound healing properties. The purpose of this study is to confirm the antifungal and wound healing properties of lemon grass extracts in albino rats with acid-burned injuries. The antifungal potential of lemon grass extracts (20 µl and 40 µl) on Candida albicans showed remarkable positive results by indicating the inhibition zone. Lemon grass extracts demonstrated best antifungal results at 20 µl. At 40 µl, it also showed excellent antifungal results. The wound healing potential of lemon grass extracts was estimated using the wound index measurement, antioxidant estimation, sandwich enzyme-linked immunosorbent assay (ELISA), and histological test. The results obtained were highly significant. Lemon grass demonstrated remarkable wound contraction potential by increasing vascular endothelial growth factor (VEGF) level at different doses (50 and 100 mg/ml). Lemon grass extracts demonstrated the greatest wound healing potential at 100 mg/ml (the VEGF marker increased, while apoptosis decreased) whereas at 50 mg/ml it also demonstrated great wound healing ability. In addition, the histopathological tests also confirmed the wound healing potential of lemon grass extracts. Based on the above results, the extraction, recognition, and purification of phytochemicals from lemon grass extracts is strongly recommended as a viable extension for future research.

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LIST OF ABBREVIATIONS

No.	Abbreviations	Full Form
1	VEGF	Vascular Endothelial Growth Factor
2	HIF	Hypoxia-Inducible Factor
3	PlGF	Placental growth factor
4	PDGF	Platelet-derived growth factor
5	ECM	Extracellular matrix
6	AT	Antiangiogenic
7	bFGF	Basic Fibroblast Growth Factor
8	FDA	Food and Drug Administration
9	Hsp90	Heat Shock Protein 90
10	EOs	Essential oils
11	ELISA	Enzyme-Linked Immunosorbent Assay
12	TBS	Tris Buffered Saline
13	BSA	Bovine Serum Albumin
14	HRP	Horseradish Peroxidase
15	TMB	Tetramethylbenzidine
16	HCL	Hydrochloric Acid
17	NADH	nicotinamide adenine dinucleotide
18	PMS	phenazine methosulphate
19	CAT	Catalase
20	GSH	Glutathione
21	SOD	Superoxide dismutase
22	SEM	Standard Error of the Mean
23	ANOVA	Analysis of variance
24	DTNB	5, 50-dithiobis-(2-nitrobenzoic acid)

CHAPTER 1

INTRODUCTION

The wound healing process, in general, consists of hemostasis, inflammation, proliferation, and remodeling. The cell types engaged in this process include fibro blasts, neutrophils, macrophages, lymphocytes, endothelial cells, and keratinocytes (1). The most apparent aspect of typical wound healing is the formation of a new capillary network through angiogenesis (2). Angiogenesis first develops as a thick but disorganized capillary network in skin wounds and is later reduced to its initial density and structure. A recent study has called into question the long-held belief that increased capillary growth is vital for healing (2). According to new research, usually healed wounds have an abnormally vigorous and mostly ineffective angiogenic response, which could have a negative impact on repair outcomes (3, 4).

The growth of new blood vessels is known as angiogenesis. The endothelial cells, which are present inside the blood vessel walls, move, multiply, and differentiate. The body's chemical signals have an impact on angiogenesis. The receptors on the functional endothelial cells surface are able to bind to some of these signals, particularly VEGF (5). In wound healing, angiogenesis plays an important role because it allows the restoration of normal blood flow, which allows for proper oxygen and nutrient exchange as well as metabolic waste disposal (6). The creation of blood vessels is considered to necessitate the majority of cellular components, extracellular matrix elements, and growth factors that have been identified during wound healing and skin regeneration (7). A protein called VEGF, which is produced in response to injury, is released by a variety of cell types, such as macrophages, fibroblasts, and keratinocytes (8). Hypoxia triggers the transcription factor Hypoxia-Inducible Factor (HIF), which boosts VEGF synthesis (9). Wound healing is delayed by antibodies that reduce VEGF activity or result in conditional gene deletion (10).

The growth of new capillaries during angiogenesis, which results in the production of granulation tissue, is a vital step in the healing of wounds. New capillaries develop in the wound site as granulation tissue three to five days after tissue damage, serving as a matrix for expanding blood vessels, migrating fibroblasts, and developing collagen (11). Wound angiogenesis is affected by growth factors, hypoxia, and inflammation. Chronic wounds are a result of angiogenesis defects that have been identified at the molecular and cellular levels (12).

Angiogenesis, which is the process by which new blood vessels are produced through pre-existing capillaries, is essential for wound healing because it enters the wounded clot and arranges itself into a microvascular network (13). Angiogenesis in wounds is influenced by angiopoietin, fibroblast growth factor, vascular endothelial growth factor, and angiogenic cytokines (14). For the first time, VEGF was found to be an essential growth factor for vascular endothelial cells. Many VEGFs play similar but distinct roles in the formation of new blood vessels (15).

VEGF is one of the most significant growth and survival agents for the endothelium (15). VEGF is involved in vasculogenesis regulation and stimulates angiogenesis and endothelial cell proliferation (16). Endothelial cells do not produce VEGF, which is released by a variety of cells. The first VEGF, VEGF-A, was named the vascular permeability factor because it increased vascular permeability. VEGF, the most essential proangiogenic factor, promotes endothelial cell proliferation, movement, division, and survival, allowing new blood vessels to form. VEGF possesses anti-apoptotic characteristics in addition to boosting cell migration and preventing apoptosis (17).

By enhancing angiogenesis, VEGF influences wound contraction and epidermal repair, the development of granulation tissue, the strength of the healed wound, and the quantity of scar tissue that is formed (14). Even though VEGF has been proven in multiple studies to affect a wide range of wound repair, the overwhelming of VEGF's impacts on wound repair have been associated to its proangiogenic activity (8). Recent findings indicate that cells with VEGFRs, like keratinocytes and macrophages, can interact directly with

VEGF. Normal wound healing produces high quantities of VEGF, which triggers a potent angiogenic response (18).

Lemon grass (Cymbopogon citratus) is a tall, fragrant perennial plant with rhizomes and a fiber base that is thickly tufted. Short underground stems with ringed segments and thick clusters of coarse, green, leathery leaves (19). In several regions of the world, it is used to treat digestive problems, mental disorders, fevers, menstrual disorders, rheumatism, and other joint issues. Lemon grass aerial components are commonly utilized in traditional medicine as an infusion or stew (20). The plant's primary phytochemicals are flavonoids, essential oils, and phenolic substances. Saponins, steroids, alkaloids, tannins, and anthraquinones are also found in the plant (21). Lemon grass gets its name from the essential oil in the shoot, which has a lemon-like aroma. The leaves of lemon grass can be used to make paper and cardboard as a cellulose source. In soil supplied with C. flexuosus leaves, the root-knot nematode disease was minimized (22).

Lemon grass is a popular fever-relieving plant in the Caribbean. It is applied to the skin as an ointment to treat arthritis and discomfort. An herbal paste made from leaves is used to cure ringworm in India (19). Lemon grass oil is among the essential oils that are most often used in the manufacture of citral. Only the Indian state of Kerala produces and exports lemon grass oil. Lemon grass oil is produced in tiny quantities all over the world, with an annual production of about 1,000 t from a 16,000-hectare farm. It is grown on about 4000 acres in India, with an annual output of around 250 t. The crop is widely grown as live mulch in low, marginal, and waste sites, as well as along bunds. Citral is the beginning ingredient for ionone production (23). Flavors, cosmetics, and perfumes all include ionone. Vitamin A is synthesized with the help of B-ionone. The most common component of oil, citral b, may be a robust b-glucuronidase inhibitor. Bactericides, insect repellants, and medical uses are among the other uses for the oil (21).

Lemon grass has a lot of antioxidants in the form of flavonoids and phenolic chemicals. It has antibacterial and antifungal activities, as well as anti-inflammatory and antioxidant qualities (24). Quercetin, a flavonoid having anti-inflammatory and antioxidant effects, is found in lemon grass (25). In Africa, lemon grass is utilized to treat coronary heart disease (26). When applied topically, lemon grass essential oil possesses antifungal and anti-inflammatory properties (27). Medicine is made from the leaves and oil. Lemon grass can aid in the treatment of fatigue, fever, the common cold, convulsions, nausea, high blood pressure, soreness, and achy joints (rheumatism).It is also used as a mild astringent and to kill bacteria (28).

Lemon grass (Cymbopogon citratus) is an important herb that is commonly used in tropical countries, particularly Southeast Asia, in human diets (29). Lemon grass essential oil contains citral, which is needed to produce vitamin A. Lemon grass may contain antifungal qualities, making it a promising choice for future plant disease control programmes targeted at reducing the spread of fungi. Citral and lemongrass oils show potent anti-Candida spp. action in vitro (30). Alkaloids, cardiac glycosides, and tannins are some of the phytochemical components of lemon grass oil that are believed to be responsible for its antibacterial and preservation qualities. The activities of lemon grass aqueous extract support its use as an active food supplement, and further research has shown that when subjected to heat during extraction, evaporation, spray drying, and storage, it has minimal cytotoxicity activity (31). Lemon grass powder is also used for wound healing and regeneration because it improves the immune system. It effectively detoxifies body organs and eliminates toxins (32).

This study aims to evaluate the antifungal properties of lemon grass extracts against Candida albicans and to determine the potential of lemon grass for skin regeneration of wounds in rats.

RATIONALE

To study the potent antifungal properties of lemon grass and to identify whether it is beneficial in the angiogenesis process or wound healing in acid-burn rats. In preclinical investigations, animal models are utilized to develop cures for various diseases. The whole world prefers neutraceutical remedies to drugs that do significant harm to animals rather than treating them. As a result, the entire globe needs to adopt neutraceutical plants that are more effective, have fewer side effects, and are reasonably inexpensive.

AIM

To evaluate the potency of lemon grass for wound healing and antifungal properties.

OBJECTIVES

1. Preparation of aqueous extract of lemon grass.
2. Evaluation of wound healing in acid burn rats.
3. Evaluation of antifungal Properties of lemon grass.

CHAPTER 2

LITERATURE REVIEW

2.1 Wound healing

A wound is a break in the natural skin epithelial barrier that may change the shape and function of the surrounding normal tissue (33). A contusion, haemorrhage, laceration, or abrasion can all result in a wound (1). The various mechanisms involved in wound healing are complex, vigorous, and well-coordinated. A wound is considered healed when the connective tissues have regenerated and the tissue has been fully re-epithelialized by regrowth, restoring it to its normal structural form and function (34). Chronic, non-healing wounds ensure sustained care because some wounds do not heal promptly (35). Acute wound healing, a closely regulated, systemic series of overlapping events, requires the coordinated performance of a number of cellular activities, including chemotaxis, extracellular matrix production, mitogenesis, and phagocytosis (36). The processes, which are set off by tissue damage, can be categorized into four stages: haemostasis, inflammation, proliferation, and remodeling (37).

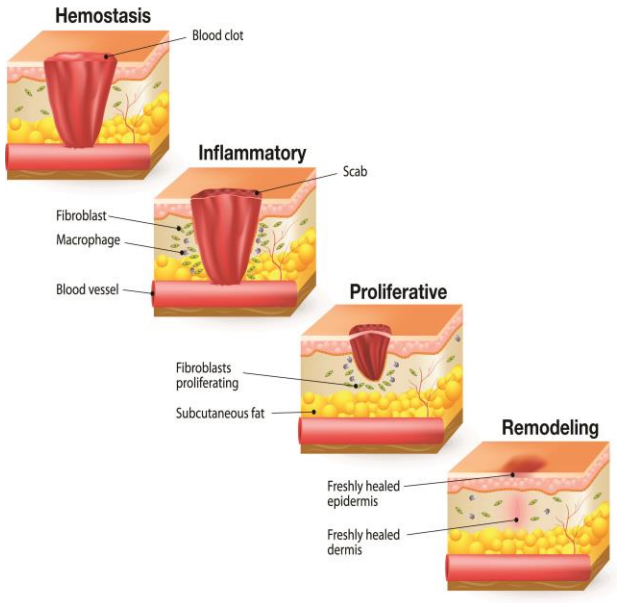


Figure 2.1: Wound healing Process (38)

2.2 Angiogenesis and VEGF Overview

In recent writings, British surgeon John Hunter frequently mentions the initial appearance of the term "angiogenesis" (39). According to Rogers et al, Van de Kolk got several intriguing results: When Schroeder Van der Kolk performed the first injection tests in neoplastic tissue in 1826, he remarked on the differences in blood supply between spontaneous and transplanted tumours (40). With only 40 papers published in 1980 and about 6000 in 2010, the field of angiogenesis has changed dramatically in the course of recent years. According to the Angiogenesis Foundation, angiogenic therapy has the potential to enhance the lives of at least 1 billion people around the world (5).

Angiogenesis refers to the mechanical and chemical processes that result in the formation of new blood vessels from existing ones (41-43). A systematic study by Bonanno and Iurlaro et al. confirmed the extracellular matrix (ECM) is required for angiogenesis at all stages (44). Angiogenesis is the term used by Adair and Montani to denote the development of new blood vessels. Numerous physiological and pathological events depend on this process (tumor growth, arthritis, diabetic retinopathy) (45). Folkman et al. were the first to isolate an angiogenic factor from Walker 256 tumour cells in the ascites form (46). An angiogenic factor generated by activated macrophages was described by Polverini et al. (47). Castellot (48) discovered a substance that promotes capillary endothelial cell proliferation in vitro. Luttuna and Autiero et al. suggest that other molecules, such as PlGF (VEGF homologue), contribute to angiogenesis in diseases while having no effect on quiescent capiesary in healthy organs. As a result, therapeutic prospects for the development of safe anti-angiogenic medicines have emerged (5). Shweiki et al. proposed the finding that hypoxia, which occurs during tumour progression and ischaemia, regulates VEGF expression through transcription (49). The importance of vascular endothelial growth factor in the development of blood vessels is emphasized by the aberrant blood vessel formation and neonatal deaths that result from the deletion of a single VEGF-A allele (15, 50).

Many pathologic situations, including wound healing, chronic inflammation, restenosis, atherosclerosis, and malignancies cause angiogenesis. The VEGF protein family is

universally recognized as being the most significant proteins involved in the growth of the vasculature; they have roles in vasculogenesis, lymphangiogenesis, and angiogenesis in both normal and malignant situations (51-54).

Recent research has shown a previously unreported link between the nervous system and angiogenesis (55). For instance, Mukouyama et al. (56) discovered that in the epidermis of the embryonic mouse limb, the pattern of arterial blood vessel formation and branching is regulated by the local VEGF-A release by sensory neurons (57). The neurotransmitter dopamine inhibits VEGF-A induced angiogenesis, (58) whereas Netrin-1, an angiogenic molecule that magnifies VEGF-A effects, is the axonal attractant (59).

Recent studies have shown that tissue factor can influence angiogenesis through non-clotting routes. First, tissue factor increases VEGF-A synthesis while decreasing thrombospondin-2 transcription, which prevents angiogenesis (60-62). Since it increases the synthesis of tissue factors, tissue factor-induced overexpression of VEGF-A has the potential to promote a self-sustaining feedback mechanism (63, 64). Along with its capacity to produce fibrin and aid in clotting, thrombin has been shown to have a substantial impact on angiogenesis (65). Antiangiogenic effects are also found in thrombin (65). It degrades antithrombin (AT) in vitro and in vivo to create a powerful inhibitor of angiogenesis, bFGF-induced endothelial cell proliferation, and VEGF-A (66). Antiangiogenic AT, domain 5 of the high-molecular-weight kininogen, and prothrombin fragments 1 and 2 are cryptic antiangiogenic fragment-containing proteins that function in the hemostatic system (67).

2.3 Wound healing and Angiogenesis

Wound repair and regeneration rely heavily on angiogenesis. An increase in interest in angiogenesis research has resulted in the understanding required to generate the first anti-angiogenic drugs (12). In 1972, the concept of “antiangiogenesis” as a possible treatment method was proposed (68). Antiangiogenic therapy preclinical investigations and clinical trials, such as the treatment of hemangioma with interferon alfa-2a, indicate guidelines for such a potential treatment in the future care of cancer patients (69). According to

Bauer, the ability of several recombinant angiogenic growth factors to speed healing has been investigated (11). According to Crovetti and Martinelli, only PDGF is accessible as a topical wound ointment that has been authorised by the FDA (70). Falanga claims that VEGF-A works against the benefits of the VEGF-A gene by speeding up healing and angiogenesis in ischemic ulcers. Oral bFGF stimulated angiogenesis and accelerated healing of duodenal ulcers in mice in pre-test trials (71). Studies on VEGF's role in wound healing began soon after it was discovered and described as a proangiogenic agent (18, 72).

According to Kumar et al., Honnegowda, angiogenesis is vital for the healing of wounds because it penetrates the wound clot and causes the capillaries there to grow new blood vessels, creating a microvascular network in the granulation tissue. Powerful angiogenic cytokines are all potent in wound angiogenesis, angiopoietin, endothelial growth factor, and fibroblasts (7). The researchers also revealed that extracellular Hsp90 is located in the granulation of damaged skin tissues on blood vessels and in a wound healing model in vivo, it enhances angiogenesis. According to the findings, Hsp90 can be released by endothelial cell activation and is a positive angiogenesis regulator, suggesting Hsp90 can be used as a wound repair stimulator (73).

Studies by Arnold and West show that angiogenesis is a critical step in wound healing (74). Bao, Kodra et al proposed that VEGF promotes collagen deposition and epithelialization in addition to wound healing through angiogenesis. More research into the chemical, whether through proteins or other delivery mechanisms like genetic therapies, will improve its therapeutic potential to speed up the healing of chronic wounds (8). The transport of oxygen from surrounding tissue and the circulation from newly-created blood vessels to the wound bed is a crucial part of effective cure (75). Guerra, Belinha et al. proved that Angiogenesis facilitates the restoration of regular blood flow, adequate nutrient and oxygen exchange, and the removal of waste substances, all of which are essential for cell viability and proliferation (76).

The establishment of a new capillary network by angiogenesis is one of the most noticeable characteristics of normal healing. Angiogenesis begins in skin wounds with

the development of an irregular, thick capillary network that is later thinned to its original thickness (77). The wound on the skin that is regularly healing is an excellent model for both vigorous capillary growth and regulated capillary regression (78, 79). When a wound cools, new capillaries form quickly, forming more connections to the new thick blood vessels than normal tissue (80). A strong and active angiogenic response is required for healing process (2). Due to cellular migration, development, and metabolic activity, injuries do create a need for oxygen and nutrients. Despite this, at least eight distinct investigations have revealed that when angiogenesis is decreased, skin wound closure is fully normal. Many approaches have been utilized in this research to minimize wound angiogenesis, including the use of anti-VEGF antibodies, antiangiogenic drugs, and integrin signaling blockage (81-84). Because of the link between vigorous angiogenesis and scar development, antiangiogenic therapy has been proposed as a way to minimize scar formation (85-88). Higher microvascular content in the production of hypertrophic scars has been associated to this condition (89, 90). Furthermore, keloids, a particularly strong kind of skin scarring, have been shown to have a high amount of angiogenesis (88). In tissues other than the skin, such as the liver and the lungs, fibrosis has been linked to angiogenesis (91, 92).

Animal studies have shown that pharmacologic angiogenesis suppression can improve healing outcomes, even if human trials of antiangiogenic treatments for improved wound healing have not yet been conducted. When VEGF was blocked with an antibody, the peak wound vascularity and scar width in mature skin wounds in mice were both significantly reduced (85).

Skin wounds are healed step by step throughout the acute stages of healing, beginning with hemostasis and inflammation. Then there are phases of increased cellular proliferation, ECM formation and remodeling, and scar formation (93, 94). Angiogenesis plays a significant role in the proliferative phase of the healing process (5). The number of blood vessels at the injured site briefly increases as a result of this process. Since the transfer of oxygen and nutrients through these blood vessels is essential to the healing process, angiogenesis issues are intimately related to a delay in the healing of wounds. A

variety of lipid mediators, growth factors, and cytokines produced in response to injury may cause angiogenesis. The most significant proangiogenic mediator is vascular endothelial growth factor, which is thought to be necessary for an efficient healing process. VEGF promotes angiogenesis, (95) which affects wound contraction, epidermal repair, the formation of granulation tissue, and the efficiency of the repair. The majority of VEGF effects during wound repair have been credited to its proangiogenic activity.

2.4 Wound healing and VEGF

During the normal healing process, large concentrations of VEGF are produced, which triggers a potent angiogenic response. (96). People with persistent chronic ulcers, such as diabetics, have very low VEGF protein levels. Inadequate wound vascularization due to low VEGF activity is most likely a factor in these repair delays. Furthermore, medicines that inhibit the function of VEGF, which are used to treat a variety of cancers increase the risk of wound healing problems (97).

VEGF is one of the main proangiogenic mediators in healing process. The increase in VEGF following damage is likely due to multiple cellular sources. Damaged skin produces more VEGF, which is typically expressed at low levels by epidermal keratinocytes (98). Research has shown that keratinocytes release VEGF early in the scar healing process (72, 99). New research reveals that keratinocytes create VEGF during the healing process (100). VEGF is expressed in injured skin by activated fibroblasts, macrophages, and mast cells (99). At various stages after injury, myeloid cells were discovered to be a significant producer of VEGF (100), which is produced by both keratinocytes and myeloid cells. One of the elements that promote the growth of VEGF during the healing of wounds is hypoxia. The VEGF gene is produced as a result of the transcription factor HIF being activated by low oxygen levels in injured skin (101). VEGF production by keratinocytes has been shown to be stimulated by oxidants such as hydrogen peroxide produced in response to injury.

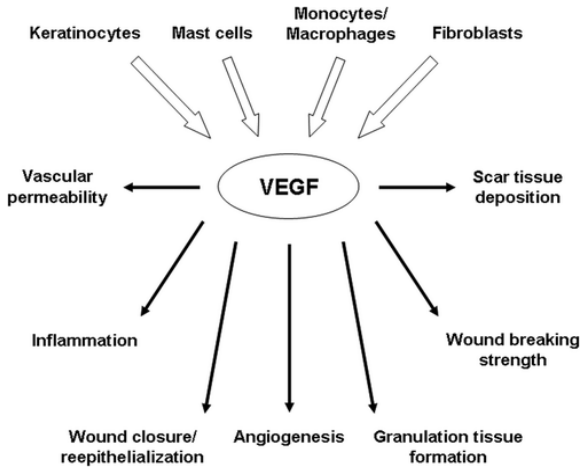


Figure 2.2: Role of vascular endothelial growth factor (VEGF) in the regulation of Wound Repair (18)

2.5 Plant Overview

Lemon grass is a tropical perennial that produces fragrant oil. Lemon grass gets its name from the essential oil in the shoot, which has a lemon-like aroma. The herb is believed to have originated in Asia and Australia. In Pakistan, lemon grass is found in Baltistan, Jammu Kashmir, Hunza and Chitral valley (21, 102). The green leaves of this grass have a pronounced citrus aroma when crushed, earning it the name (103).

Lemon grass (globally known as ***Cymbopogan citratus*****)** belongs to the Poaceae family (24). The height of C. citratus, a tufted, robust perennial grass, reaches around 2 metres. The leaves are lanceolate and linear. It blooms profusely. The inflorescence is a drooping panicle with paired spikes on very large and well-branched tertiary stems. The spikelets come in pairs, one sessile and one pedicellate. The sessile spikelet is an awned bisexual floret, while the staminate floret is awnless (21). At end of its life cycle, this plant produces flowers. Flowering has never been seen during cultivation. The inflorescence is a large spike that is one metre in length. Decompound spatheate panicles with a length of 30 to 60 cm bear flowers. The rhizome, which develops new suckers, rises vertically as tillers to create dense clusters (104, 105).

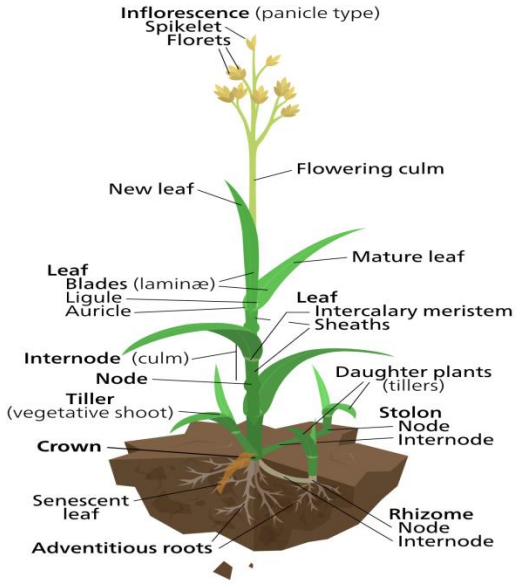


Figure 2.3: Lemon grass Plant (102)

2.5.1 Distribution & Habitat

Lemon grass grows wild in North America, South America, Africa, Australia, Europe, and among other places (22). It is grown in China, Bangladesh, Thailand, and India, and is widely cultivated throughout the tropics. Northern regions including Jammu & Kashmir, Punjab, Rajasthan, Delhi, and Bengal are habitat to native lemongrass plants (106). Lemon grass is grown extensively in the Chinnar wildlife reserve in India's Western Ghats (19).

This grass lovely lemon scent has long been employed in perfumery and associated products, as well as in the culinary industry (102). Thai and Vietnamese cuisine pioneered the use of lemon grass. It is frequently used to flavour tea in nations in Africa and South America. It can be found in both alcoholic and non-alcoholic drinks (107). It also functions as a sedative, diuretic, antipyretic, and anti-inflammatory in Ayurveda (108). Lemon grass is used medicinally by a variety of ethnic groups (103). Brazilians regularly use lemon grass tea as an anti-inflammatory, analgesic, antispasmodic, antipyretic, sedative, and diuretic (109). In Argentina, it is used to treat sore throats. In Cuba, it is used to treat catarrh, rheumatism, and lower blood pressure (110). Lemon

grass also has anti-cancer, anti-carcinogenic, anti-fungal, anti-bacterial, anti-protozoal, antioxidant, antitussive, antiseptic, and anti-rheumatic effects (111).

2.5.2 Ethnobotany

The commercially valuable essential oils produced by the Cymbopogon citratus plant are a common ingredient in traditional medicine and food processing. People are becoming more aware of health issues as a result of arrival of the new ailments. Due to the unfavorable side effects of synthetic medications, treatment with plant-based medicine looks to be an alternative to artificial therapy (112). Lemon grass is used to cure a variety of ailments, including coughs, elephantiasis, the pneumonia, gingivitis, malaria, flu, headaches, leprosy, ophthalmology, and vascular issues. Lemon grass also has antibacterial and antifungal effects. When combined with pepper, it serves as a natural cure for nausea and menstruation issues. Lemon grass is an organic purifier that promotes more detoxification of the liver, kidneys, pancreas, digestive tract, and bladder. It reduces lipids, uric acid, cholesterol, and other body toxins in addition to enhancing lactation, digestion, and blood flow. It also lessens indigestion and gastroenteritis. Lemon grass is also used as a muscle and tissue toner and is thought to help enhance the skin by reducing acne and pimples. Additionally, it may lower blood pressure. The cancer prevention benefits of lemon grass were identified (105, 113).

The biological characteristics of lemon grass and their associations with developments in medicine, gastronomy, cosmetics, and agriculture serve as the basis for most of its uses. The efficient use of lemon grass as a raw material in the production of paper (114), as an adsorbent (115), a fundamental constitute in composites, (116) and in the production of silica has been reported (117).

2.5.3 Chemical Composition

Cellulose (39.5%), hemicellulose (22.6%), and lignin (28.5%) are the three main components of lemon grass (118, 119). Lemon grass is a hydrocarbon that is mostly made up of carbon and oxygen. Hemicellulose is mainly amorphous and has just a small amount of crystalline material, whereas cellulose has a three-dimensional structure with

glossy and amorphous regions. The degree of polymerization, which can range from 100 to 10,000 for cellulose and less than 200 for hemicellulose, is another key distinction between the two (120). Lignin is a nonlinear, amorphous, heterogeneous polymer that binds cellulose components together in three dimensions (121). 1, 4-glucopyranose units are found in cellulose. Xylose, galactose, arabinose, and mannose are subunits of hemicellulose, while guaiacyl, syringyl, and p-hydroxyphenyl are subunits of lignin (122).

Lemon grass oil concentration varies based on genetics, growth zone, culture, and agronomic treatment. 1-2 % of the plant's weight is used to extract essential oils (EOs) (123). On the other hand, the drying process can have an effect on the composition. Despite the fact that dehydrated oil has a high concentration of citral, which is used to evaluate the oil level, oven-drying leaves can produce more EO than sun-drying or shading techniques (124).

Geranial and neral, also referred to as trans-citral and cis-citral, are two stereoisomeric mono-terpene aldehydes that make up citral (125). On average, citral oil makes up more than 45% of lemon grass oil; however, the proportion varies greatly between animal species. Citral content ranges from 30-94 percent in East Indian lemon grass (108, 126). According to research, terpenes, alcohol, ketones, and esters are among the hydrocarbons discovered in the production of EO (127). Phytochemicals such as anthraquinones, phenols, flavonoids, tannins, saponins, and alkaloids have been found in lemon grass. There are also trace amounts of citronellol, terpineol, borneol, kaempferol, nerol, luteolin, caffeic acid, chlorogenic acid, glycosides, catechol, geranyl acetate, valerichyde, and methylheptenone fumesol (108, 128, 129). Lemon grass has been shown to contain phytochemicals, orientin, swertiajaponin, and isoscoparin (130, 131). The figure depicts high geranial and neral concentrations as well as low levels of myrcene, limonene, nerol, citronellol, and geraniol (132, 133). Some of the minerals that have been discovered are silica (9.02%), calcium (25.6%), potassium (54.02%), and phosphorus (1.57%) (117). Along with protein, carbs, and fat, there are also niacin, vitamins A, C, and E, folate, riboflavin, and pyridoxine (134).

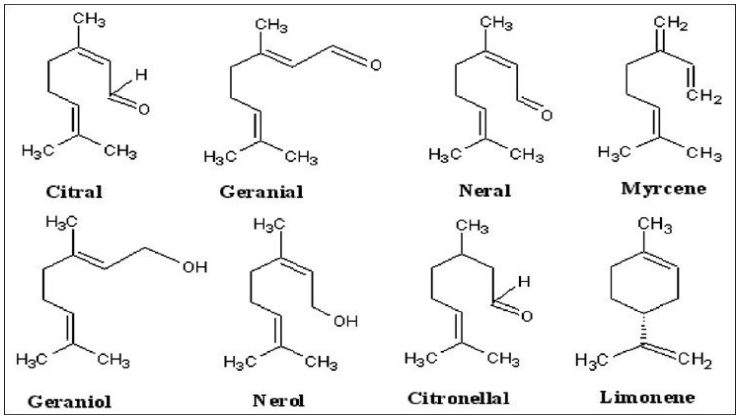


Figure 2.4: Chemical structures of lemon grass essential oil's principal ingredients (135)

Table 2.1: Composition of oil in lemon grass

Oil Composition	Components (%)
Alpha Pinene	0.07
Borneol	0.1-0.4
Beta Pinene	0.04
Beta caryophyllene	Traces
Citral alpha	40.8
Citral beta	32
Citronellal	2.10
Geraniol	3.04
Geranyl acetate	0.83
Limonene	Traces
Linalyl acetate	0.1
Myrecene	0.72
Nerol	4.18
Terpinolene	1.23
Terpinol	0.45

2.5.4 Lemongrass Properties

According to scientific research, lemon grass is an antifungal, antibacterial, and antiprotozoal agent (114, 136-143). Several investigations have also verified lemon grass anti-nociceptive and anti-inflammatory properties (144-146).

The biological activity of various components in lemon grass essential oil has been investigated in a number of studies. Citral and geraniol have antifungal properties, and the presence of myrcene boosts that activity even more (147). Lemon grass antibacterial activity was attributed to cinnamic aldehydes, linalool, alkaloids, and phenols (148). Lemon grass antibiotic activity is more likely due to the combination activities of its constituents than a single element (111).
Lemon grass has been proven to have anti-tumor, anti-carcinogenic (149), anti-mutagenic (150), anti-amoebic (151), anti-diarrhoeal (152), anti-filarial (153), larvicidal and ascaricidal activities (154).

The entire plant's lemon essence can be efficiently added to traditional cuisines, and its aqueous extract is frequently used to make fragrant drinks. It was widely used in traditional medicines. Lemon grass tea was widely used for its antibacterial, antifever, antidyspeptic, carminative, and anti-inflammatory properties, making it popular in South American, Asian, and West African nations. Others include stomachic agents, diuretics, analgesics, spasmolytics, antipyretics, and febrifuges (21).

In recent articles, it is stated that lemon grass has high vitamin C content, and its oil has antifungal properties against phytopathogenic fungi (155, 156). The extract of this plant proved effective in lowering blood cholesterol levels since it contained an active ingredient and raw fiber (112). (157) Lemon grass has been discovered to contain antioxidants, and dried lemon grass has more phenols and flavonoids than fresh lemons (158). In this research, both in vitro and in vivo evaluations of lemon oil's remarkable anti-fermentation properties were conducted. This essential oil is deemed toxicologically acceptable for use in food preservation because of its vital role in avoiding lemon grass oil fermentation in mixed juices (159). Cristiane de Bona da Silva gave a paper on this

topic. The report states that numerous essential oils have been investigated for their potential to inhibit mycotic growth both in vivo and in vitro, with some demonstrating potential antifungal properties (160). Lemon grass oil has been studied for its antifungal properties against dermatophyte species and Candida albicans, two forms of human illnesses (161, 162).

2.6 Pharmacology

2.6.1 Antibacterial Effect

Lemon grass also possesses antibacterial properties and is used as an herbicides and pesticides. Salmonella paratyphi, Shigella flexneri, Staphylococcus aureus, Shigella flexneri, Bacillus subtilis, and Escherichia coli were all successfully eradicated by the chromatographic extract of the oil on an agar plate (163, 164). Myrcene, the third component, has no antibacterial activity by itself, whereas both the geranial and neral citral components possess antibacterial activity against both gram-positive and gram-negative bacteria (163). The volatile oil extract was still functional after being oxidized with active oxygen (145, 165-167).

2.6.2 Antifungal Activity

Lemon grass is effective against Epidermophyton floccosum, Trichophyton mentagrophytes, Trichophyton rubrum, and Microsporum gypseum (168). Lemon grass oil has also been shown to be effective against keratinophilic fungus, food storage fungi, (169, 170) and 32 ringworm species (171). Lemon grass is frequently used in traditional medicine as an antibacterial agent, and its essential oils, which are combinations of naturally occurring volatile molecules that are obtained through distillation, have long been known to have antifungal properties (146).

2.6.3 An anti-inflammatory effect

Lemon grass tea contains several compounds and essential oil extracts that possess anti-inflammatory effects (31).

2.6.4 Antimutagenicity Effect

In a number of species, lemon grass ethanolic extract has an anti-carcinogenic action (150, 172, 173)and it prevents lung metastasis in mice by reducing the development of fibrosarcoma cells implanted in the lungs (174). It has been shown that the plant extract prevents the growth of DNA adducts and abnormal crypt foci brought on by azoxymethane in the rat colon (149). In 344 male Fischer rats, the plant extract had a suppressive effect on the development of the earliest stages of hepatocarcinogenesis after diethylnitrosamine exposure (175). Lemon grass was reported to have a promising anticancer activity (176).

2.6.5 Antioxidant Effects

Citrus juices and fruits are good sources of antioxidants, which include ascorbic acid, flavonoids, and phenolic compounds (130). Citrus fruits, such as lemon, have the highest antioxidant activity. Lemon grass extract and essential oil have been reported to have strong antioxidant properties (177).

2.6.6 The Hypocholesterolemic Effect

Costa et al. (178) reported the safety of lemon grass intake at the doses used in folk medicine and indicated the beneficial effect of reducing the blood cholesterol level. In a 2015 research study, the mice that received the plant extract had much lower levels of elevated cholesterol. It was determined that this decline was dosage-dependent. This demonstrates the extract's potential to lower cholesterol (179).

CHAPTER 3

METHODOLOGY

3.1 Plant Powder Preparation

Lemon grass is a tropical plant with gently fragrant leaves that is native to South and Southeast Asia (102). A fresh lemon grass plant was taken and ground to a fine powder.

3.2 Aqueous Extract Preparation

Aqueous plant extract was prepared by dissolving 10 grammes of plant powder in 100 millilitres of water and filtering the solution after two days. The filtered extract containing water-soluble fractions was evaporated using petri dishes placed in the refrigerator (180).

3.3 Antifungal Activity Evaluation

3.3.1 Preparation of culture media

According to the manufacturer's instruction, Ampoule containing freeze dried form of the microorganism was opened and the contents were added to the Yeast Extract Peptone Dextrose broth which was incubated at 25 ± 2°C for 72 h (181). The 30 ml of molten sterile agar was poured aseptically in each four sterile petri plates and were allowed to solidify at room temperature. Hundred microliter of inoculum was spread with a sterile steel spreader to prepare a lawn of microorganism.

3.3.2 Ditch plate method

Four wells on two plates were set up. The wells were assigned numbers between 1 and 8. Using a micropipette, a predetermined volume (250 ul) of the appropriate stock solution of lemon grass extract was added to each well. Then three wells were made similarly on the fourth plate. Fluconazole served as the positive control, while the same plate also included 50% DMSO and sterile distilled water to serve as the negative controls. The

plates were all then incubated for 24 hours at 25 °C while standing upright. The entire process was carried out twice. After 24 and 48 hours, the inhibition zones were measured on the underside of the plates using the Hi-Media zone scale.

Table 3.1: Samples and control groups incorporated in the 8 compartments of petri dish

Group 1	Normal
Group 2	Injection water 20 µl
Group 3	Treated-20 µl
Group 4	Treated-40 µl
Group 5	Amphotericin B
Group 6	Fluconazole
Group 7	Placebo-20 µl
Group 8	Placebo-40 µl

3.4 Animal Model

Albino rats (100-120g) were received 14 days before the experiment from the animal house of the University of Lahore. They were provided with proper food and water in their cages.

3.5 Ethical Clearence

For using animal (albino rats) Ethical Clearence was taken from Ethical Committee of Kinnaird College for Women, Lahore.

Figure 3.1: Albino rats

3.6 Grouping of rats

Albino rats were separated into 6 groups, each with n = 3 rats in each group. One group of rats was not given any injury while the other 5 groups were given acid burn injury.

Table 3.2: Categorization of rats

No.	Group Name	Group detail
1	Normal rats	Rats given no injury
2	Untreated rats	Injured rats left untreated
3	Placebo (Plac) 1	Injured rats treated with 50 mg/ml placebo
4	Treated 1	Injured rats treated with 50 mg/ml aqueous extract
5	Placebo (Plac) 2	Injured rats treated with 100 mg/ml placebo
6	Treated 2	Injured rats treated with 100 mg/ml aqueous extract

3.7 Acid Burn Injury on Skin of Rats

First the rats were anesthetized with combined injected ketamine/xylazine.

Table 3.3: Dose, Route and Duration of Anesthesia given to Rats before Acid Burn Injury

Drug	Dose	Route	Duration of Anesthesia
Ketamine + xylazine (Rompun®)	40-90 mg/kg ket + 5-10mg/kg xyl.	IP, SQ	45-90 minutes

Acid burn injury was conducted to check influence of the lemon grass extract on healing of the wound or if the lemon grass extract can be used for the treatment and cure of burn injury. Total number of rats (n=6) out of which 1 was normal (with no injury) and five (n=5) were anaesthetized with xylazene and ketamine solution (anaesthesia) to whom acid burn injury were given (Duan et al., 2009).

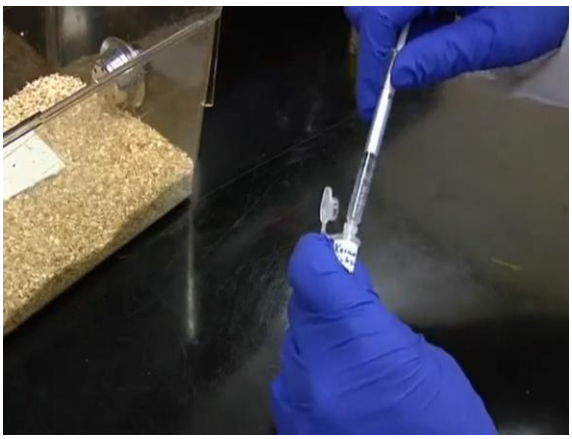


Figure 3.2: Anaesthesia Injected to Rats before Acid Burn Injury.

The dorsal side hairs of rats were removed.

Figure 3.3: Rat's Hair Removed by Veet Cream

Small cube shaped filter papers (2x2cm) were soaked in acid for few seconds.

Figure 3.4: Filter Paper Soaked in Acid

Acid burned area of rats then highlighted by marker.

Figure 3.5: Acid-burned Area of Rat, Highlighted by Marker

Acid-socked filter papers were then applied to the shaved region for 20 seconds as directed. Two injuries were given to each rat.

Figure 3.6: Acid Soaked Filter Paper was placed to the shaved region of Rats

3.8 Treatment of rats

One of these five rat groups was not given any treatment. Two groups were treated with the aqueous extract of lemon grass in different concentrations (50mg/ml and 100mg/ml), and normal saline in different concentrations (50mg/ml and 100mg/ml) was given to the remaining two groups, with the help of gavage tube (181).

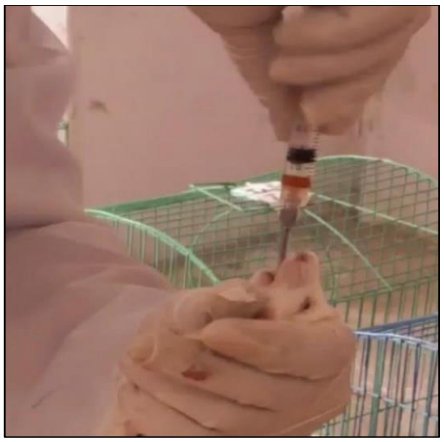


Figure 3.7: Gavage feeding of rat with extract

3.9 Wound Index Measurement

The reduction in the wound was measured by wound index measurement assay on days 7. Transparency clear sheet of plastic was placed on sedated rats wounds with the ventral side down. A pointer was used to draw a line through the wounded region on the sheet (182). The initial and current wound sizes were used to compute the percentage reduction in wound size.

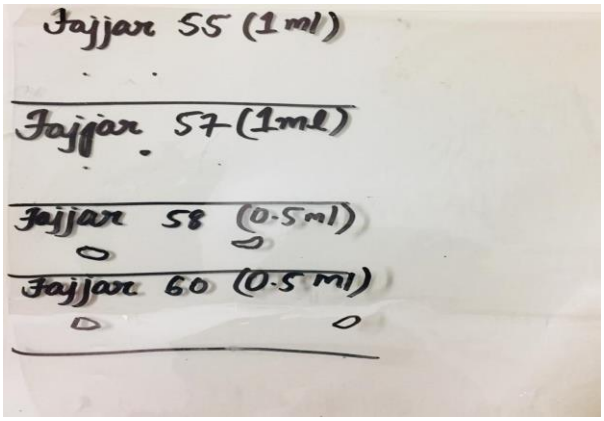


Figure 3.8: Wound Index Measurement through Transparency Sheet

3.10 Sandwich ELISA

ELISA was performed on the blood sample, taken from the body of rat after sacrifice. Rat's blood was calculated and the serum was isolated from that blood and then this serum was subjected to ELISA for skin regeneration and apoptosis.

In a 96-well plate, ELISA was used to test VEGF and annexin V. The VEGF and annexin V antibodies were applied to the plate and incubated for 100 minutes. Following 3 times TBS-T washes, 1% BSA was applied for a 35-minute blocking period. After blocking, each well was loaded with 210 µl of sample (serum taken from the treated rat's blood) and incubated for seventy minutes. The sample was taken out & thoroughly washed 3 times before being incubated for 115 minutes at 37°C with a HRP (Horse raddish peroxidase) conjugated donkey anti-rabbit secondary antibody. After washing for 10

minutes, 110 µl of chromogenic solution 3, 3, 5, 5-tetramethylbenzidine (TMB) and H2SO4 was added to terminate the reaction and at 450nm, absorbance was taken (183).

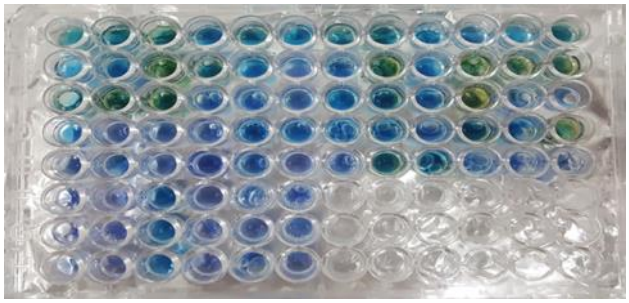


Figure 3.9: VEGF Elisa Plate after adding Substrate

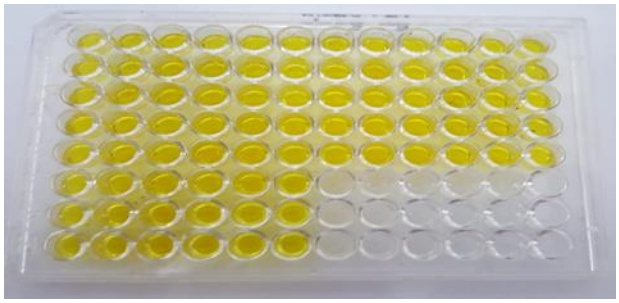


Figure 3.10: VEGF Elisa Plate after adding Stock Solution

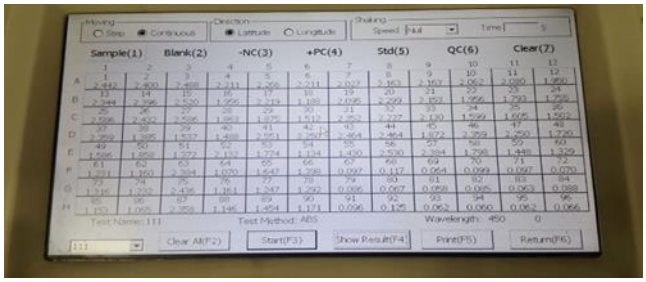


Figure 3.11: VEGF Elisa Readings

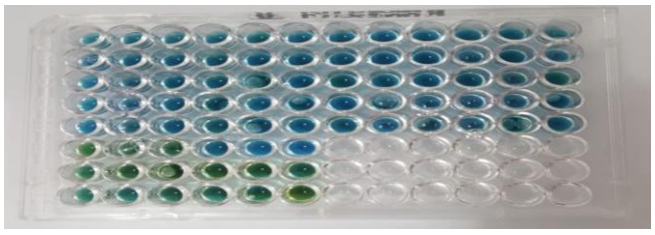


Figure 3.12: Annexin V Elisa Plate after adding Substrate

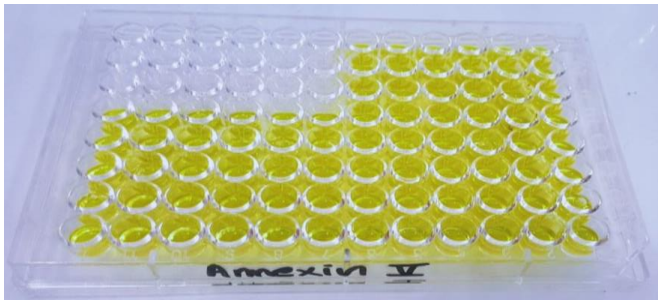


Figure 3.13: Annexin-V Elisa Readings after adding Stock Solution

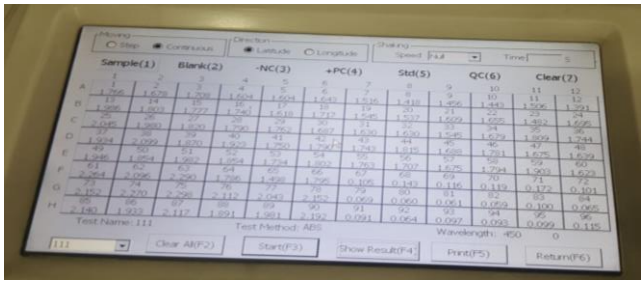


Figure 3.14: Annexin V Elisa Readings

3.11 Antioxidant analysis

3.11.1 Estimation of APOX

The techniques of Israr et al. and Nakano and Asada were utilised to examine APOX (184). One millilitre of the reaction mixture contained 0.3 mM H2O2, 0.5 mM ascorbate, 100 mM KH2PO4 buffer (pH 7.0), and post-treatment medium. After 3 minutes, OD290 was taken (as oxidation of ascorbic acid was estimated as abatement in absorption at 290 nm for 3 minutes). Enzyme activity was measured in units of the enzyme per gram of new weight (U g-1 FW). At 25 ° C, one unit of the enzyme is required to reduce the concentration of 1 mol H2O2 per minute.

3.11.2 Assessment of SOD

Using the technique of Kakkar et al., the activity of superoxide dismutase (SOD) was calculated (185). 0.1 ml of cell culture media was mixed with 0.1 ml of phenazine methosulphate (PMS) (186 lM), 1.2 ml of sodium pyrophosphate cushion (52 mM, pH

8.3), and 0.3 ml of nitrobluetetrazolium (300 lM), and with the incorporation of 0.2ml of nicotinamide adenine dinucleotide (NADH), the reaction was initiated. After 90 seconds of incubation at 30 C, the reaction was stopped by adding 0.1 mL glacial acetic acid. The reaction mixture was rapidly agitated with 4.0 mL of butanol. After a 10-minute incubation period, the mixture was centrifuged for 5 minutes at 2000 rpm for 5 minutes. The upper butanol layer's absorbance was estimated at 560 nm.

3.11.3 Glutathione measurement

Using the Beutler et al. approach, the diminished amount of glutathione (GSH) in cell culture medium is assessed (186). 0.5 ml of cell culture media was loaded up in a test tube from both gatherings, 0.25 ml (5, 50-dithiobis-(2-nitrobenzoic acid) or DTNB and 2.0 ml of disodium hydrogen phosphate cradle (0.3 M). After 15 minutes of incubation, the absorbance was measured using a spectrophotometer at 412 nm.

3.11.4 Assessment of catalase

The activity of catalase is estimated using the Sinha method (187). An amalgam of 0.1 ml of cell culture medium, 0.4 ml H2O2, and 1.0 ml phosphate cushion was prepared. The reaction was halted with the addition of 2.0 mL of dichromate acetic acid reagent. After 10 minutes in a bubbling water shower, the samples cooled and at 530nm, the absorbance was estimated.

3.12 Histopathology of Skin Sections from Wounds

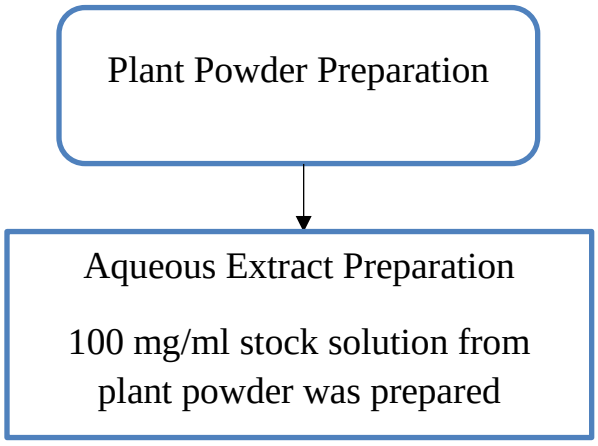
Skin samples of 0.5 cm2 were collected from the injured area 7 and fixed in a 10% neutral buffer formalin solution after slaughtering an animal. The specimens were then sliced into 46 microns thicker portions and stained with hematoxylin and cosine. Then they were dehydrated and washed into paraffin bedding (188).

3.13 Statistical Analysis

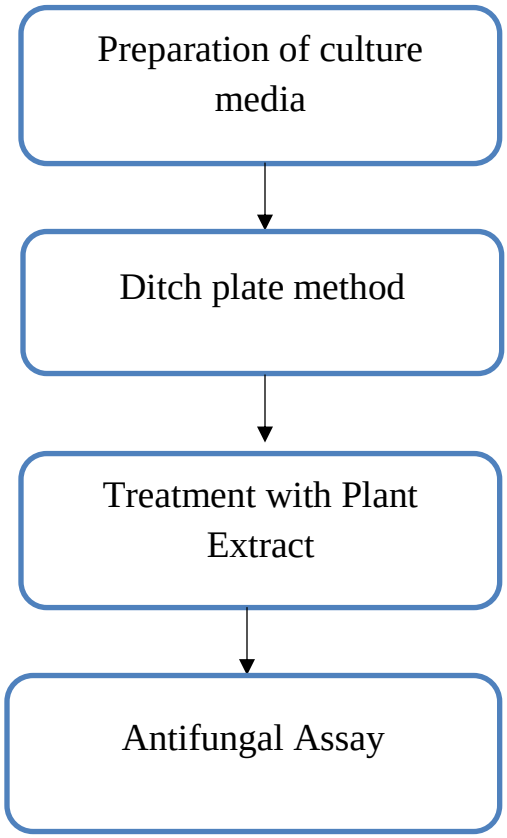
The whole set of experiments was used in a triplicate manner and expressed as mean ± EMS. One-way ANOVA and Bonferroni's test were applied for statistical analysis of different groups. To draw graphs for the quantitative data achieved from molecular analysis, Prism software has been used. For statistical analyses, P valve ≤ 0.001 was considered significant. Software Endnote X7 for adding reference has been used.

PLAN OF WORK

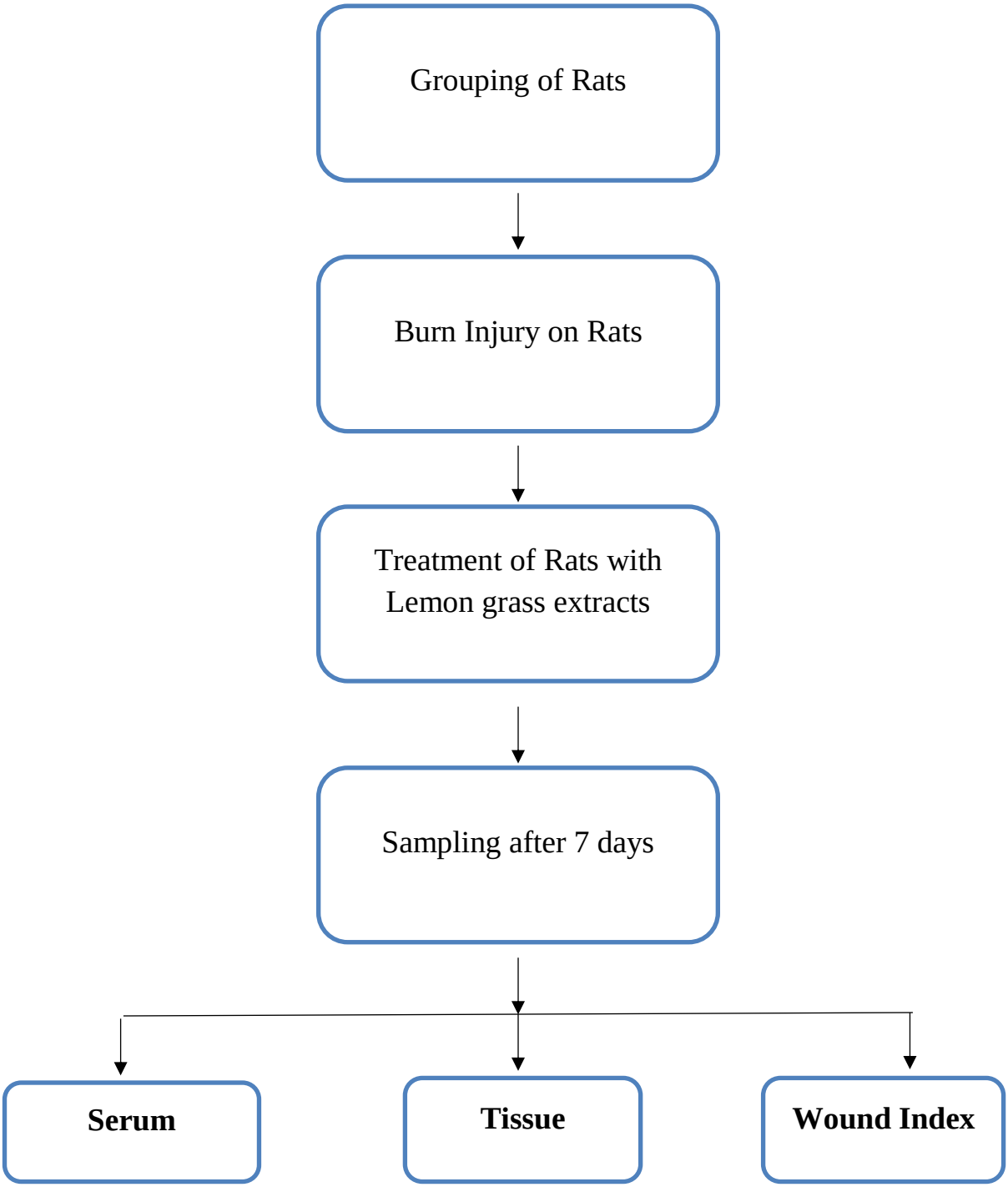
IN-VITRO ASSAYS

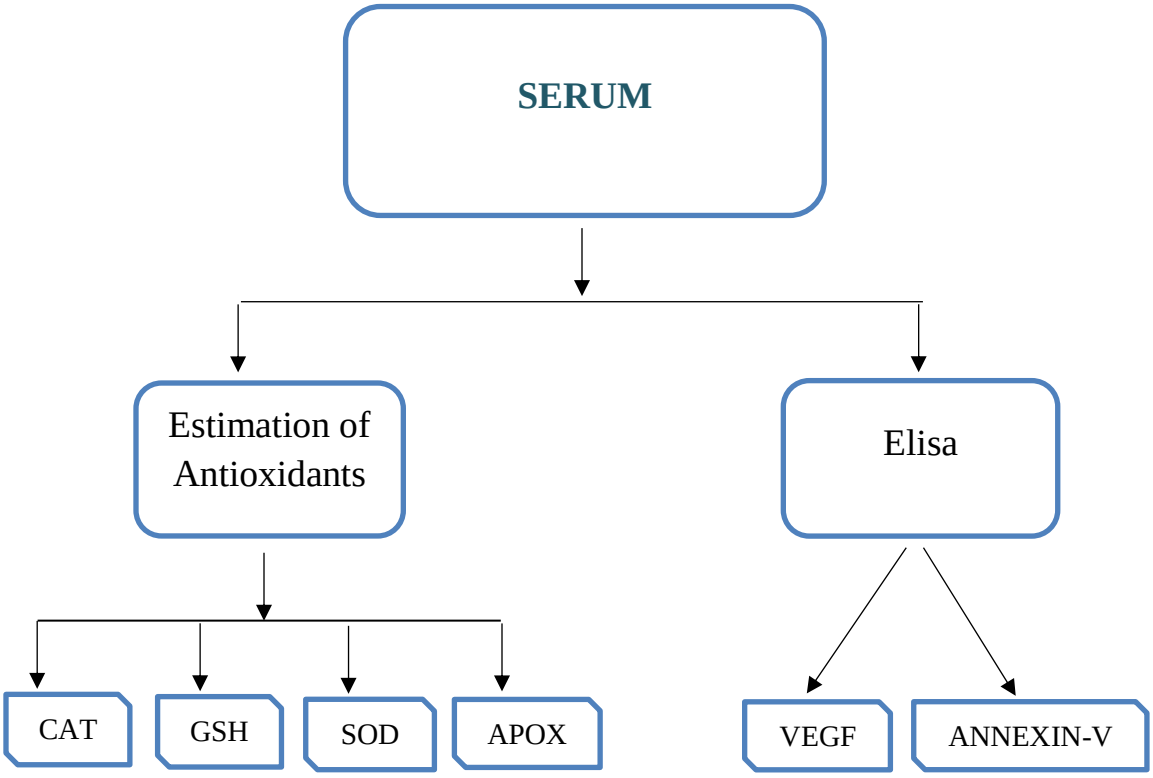


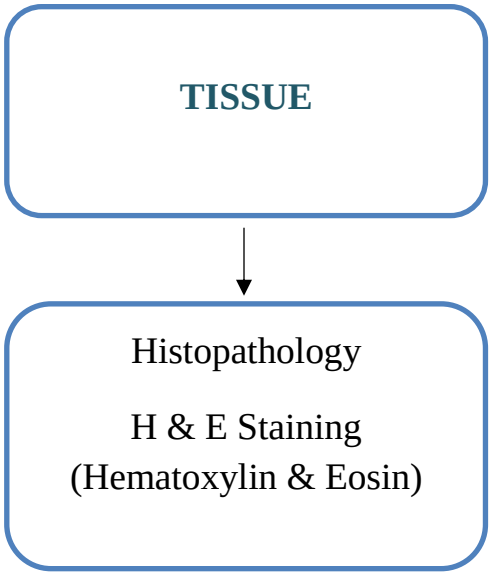
Antifungal Assay



IN-VIVO ASSAYS




SERUM
Estimation of Antioxidants
Elisa
CAT
GSH
SOD
APOX
VEGF
ANNEXIN-V


TISSUE
Histopathology
H & E Staining
(Hematoxylin & Eosin)

CHAPTER 4

RESULTS

4.1 Plant powder

Lemon grass was grounded and sieved to form a yellow-colored plant powder.

Figure 4.1: Lemon grass Powder

4.2 Aqueous extract

The mixture of weighted lemon powder and water was filtered after 2 days and then evaporated to obtain the extract of lemon grass.

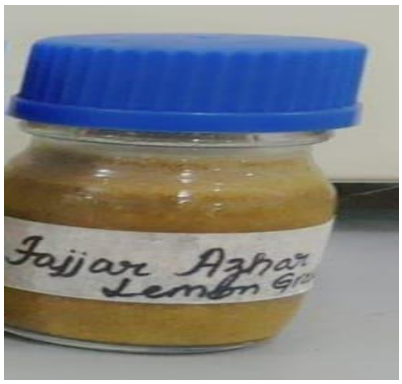


Figure 4.2: Filtered lemon grass powder

4.3 Antifungal property of lemon grass

The purpose of this assay was to determine the antifungal property of lemon grass extracts. Lemon grass extracts was tested against Candida albicans and the result obtained was highly significant.

Table 4.1: Observed Antifungal Activity

No.	Groups	Antifungal activity
1	Normal	-ve
2	Injection water 20 µl	-ve
3	Treated-20 µl	+ve
4	Treated-40 µl	++ve
5	Amphotericin B	++ve
6	Fluconazole	+ve
7	Placebo-20 µl	-ve
8	Placebo-40 µl	-ve

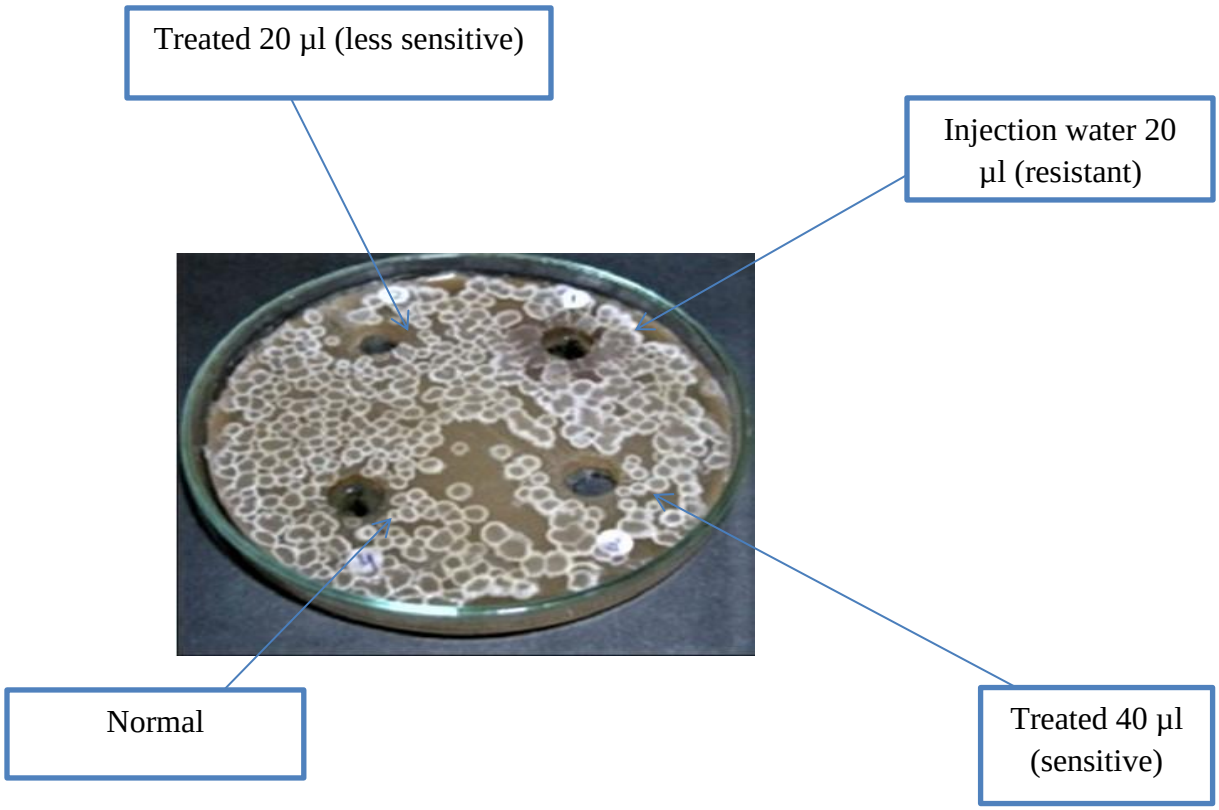


Figure 4.3: *C. albicans* petri dish showing inhibition zone in treated group (40 µl)

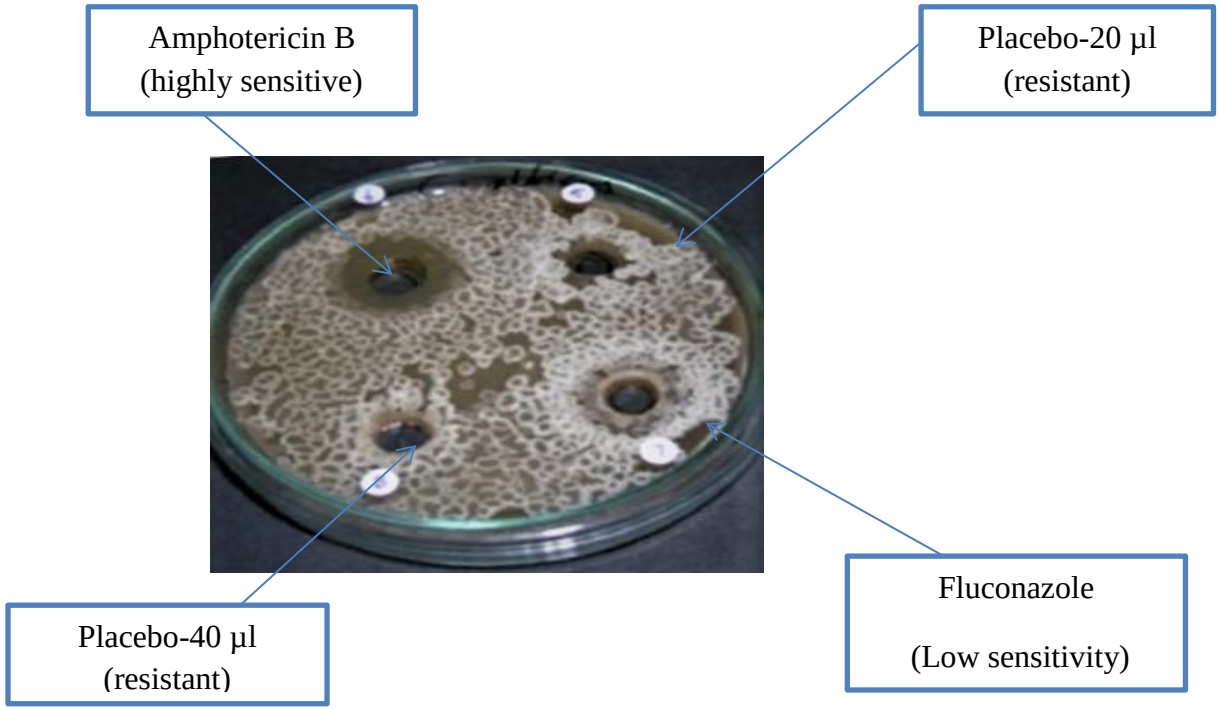


Figure 4.4: *C. albicans* petri dish showing inhibition zones in amphotericin B, and Fluconazole

4.4 Wound Index measurement

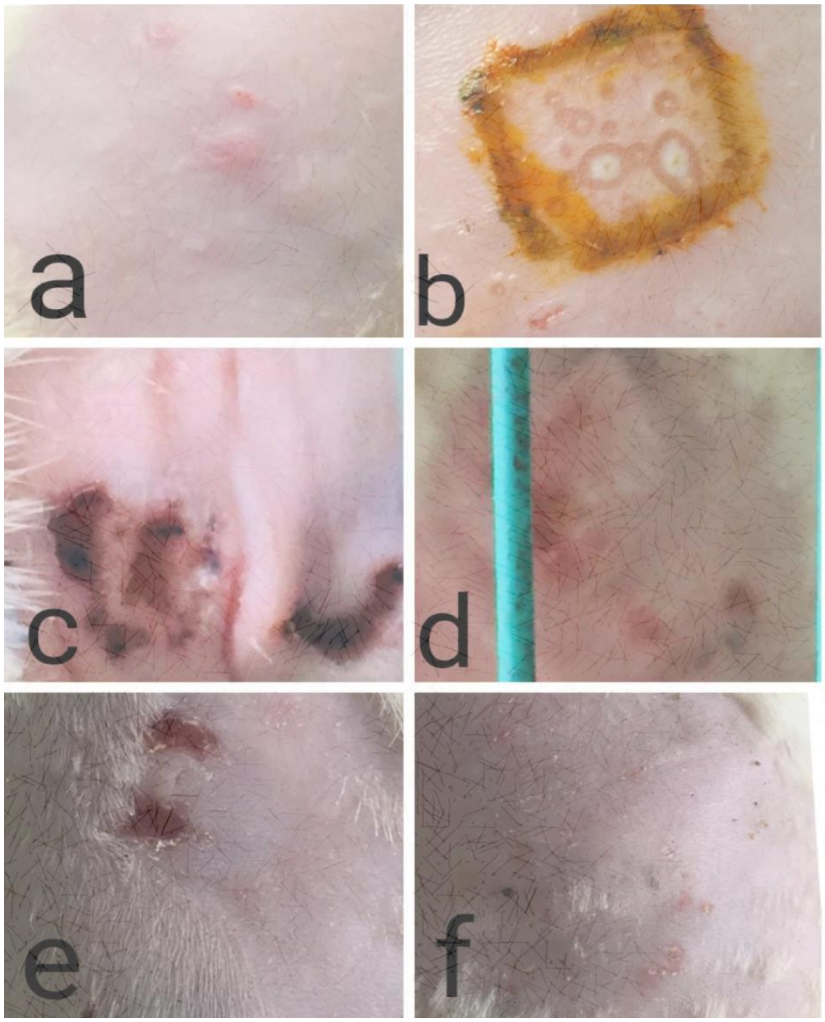


Figure 4.5: A shows normal non-injured rat skin, B shows injured non-treated, C shows placebo 1, D shows treated rats 1, E shows placebo 2 and F shows treated 2

This graphical data shows that the group of rats treated with lemon grass plant extract in comparison to injured groups shows significant values of wound index and an increase in wound contraction. The results were estimated by applying one-way ANOVA using graph pad.

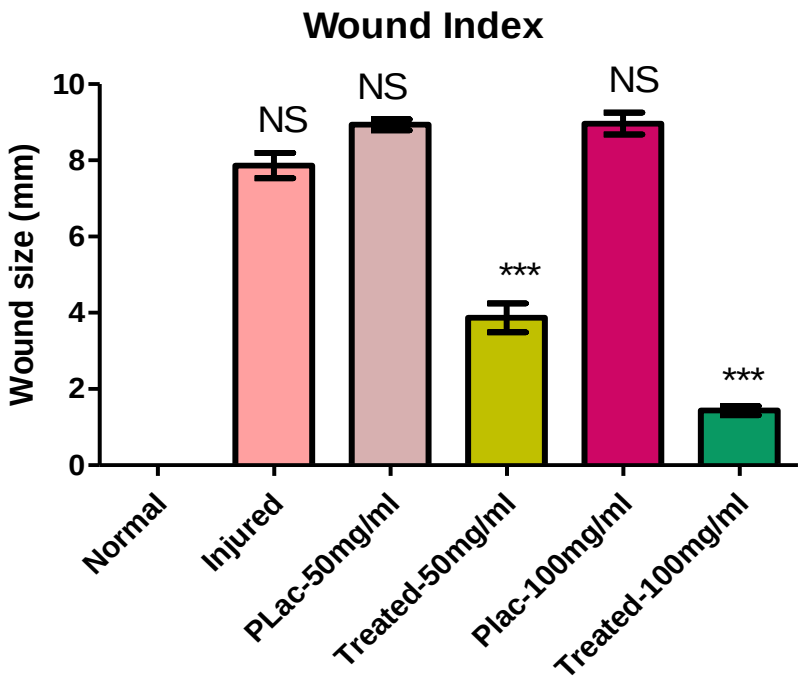


Figure 4.6: Wound index levels in treated groups of rats with selected doses of Lemon grass extract decreases as compared to injured groups of rats. Asterisk symbol *** indicates high statistical significance in results ($P < 0.001$). The mean ± SEM is used to express the values.

Table 4.2: Graphical results values of Wound index expressed in mean ± SEM

	Normal	Injured	Placebo 50 mg/ml	Treated 50 mg/ml	Placebo 100mg/ml	Treated 100mg/ml
WOUND INDEX	0.0±0.0	7.86±0.33	8.93±0.14	3.86±0.37	8.96±0.29	1.43±0.12

4.5 ELISA VEGF

The graph data shows that the group of rats treated with lemon grass plant extract in comparison to injured groups shows a significant value of VEGF markers and results were estimated by applying one-way ANOVA using graph pad.

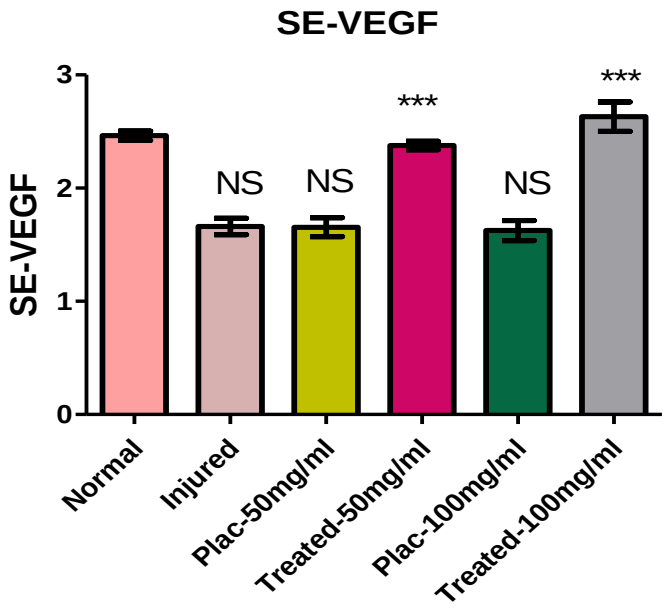


Figure 4.7: VEGF levels in treated groups of rats with selected doses of Lemon grass extracts increases as compared to injured groups of rats. Asterisk symbol *** indicates high significant in results ($P < 0.001$). The mean ± SEM is used to express the values.

Table 4.3: Graphical results values of ELISA VEGF expressed in mean ± SEM

	Normal	Injured	Placebo 50mg/ml	Treated 50mg/ml	Placebo 100mg/ml	Treated 100mg/ml
VEGF	2.46±0.04	1.66±0.07	1.65±0.08	2.37±0.03	1.62±0.08	2.63±0.12

4.6 ELISA Annexin V

The graph data shows that the group of rats treated with lemon grass plant extract in comparison to injured groups shows a significant increase of annexin marker and decreased apoptosis after treatment with lemon grass. The results were estimated by applying one-way ANOVA using graph pad.

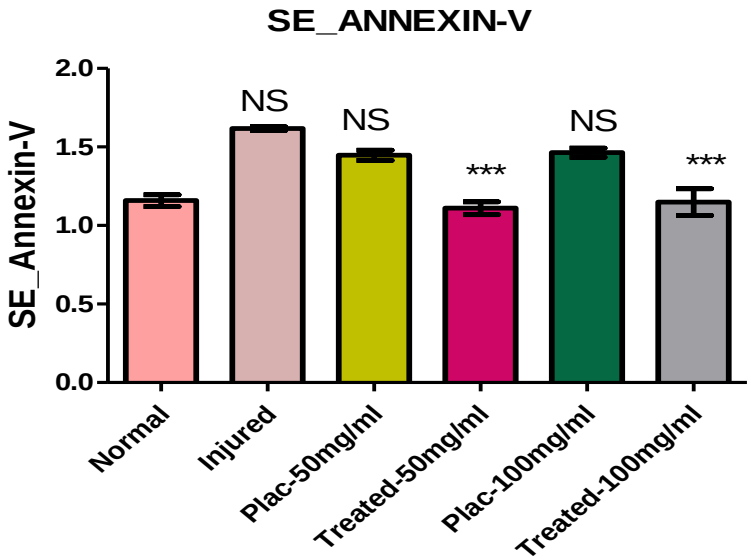


Figure 4.8: Annexin V levels in treated group of rats with selected doses of Lemon grass extract decreased as compared to injured group of rats. *** indicates high significant in results ($P < 0.001$). The mean ± SEM is used to express the values.

Table 4.4: Graphical results values of Annexin V expressed in mean ± SEM

	Normal	Injured	Placebo 50mg/ml	Treated 50mg/ml	Placebo 100mg/ml	Treated 100mg/ml
ANNEXIN V	1.15±0.03	1.61±0.01	1.44±0.03	1.11±0.04	1.46±0.02	1.14±0.08

4.7 Antioxidant Analysis

4.7.1 Estimation of APOX

A biochemical test which is responsible for finding out the estimation of APOX level is Lemon grass.

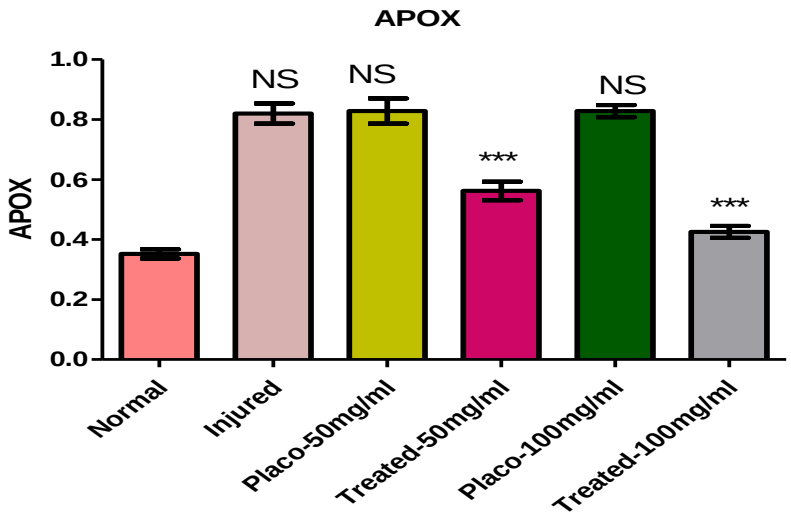


Figure 4.9: APOX levels in treated group of rats with selected doses of Lemon grass powder in comparison to injured group of rats. *** indicates that there is high statistical significance in the results ($P < 0.001$). The mean ± SEM is used to express the values.

Table 4.5: Graphical results values of APOX expressed in mean ± SEM

	Normal	Injured	Placebo 50mg/ml	Treated 50mg/ml	Placebo 100mg/ml	Treated 100mg/ml
APOX	0.35±0.01	0.82±0.03	0.82±0.04	0.56±0.03	0.82±0.02	0.42±0.01

4.7.2 Estimation of SOD

A biochemical test which is responsible for finding out the estimation of SOD level is Lemon grass.

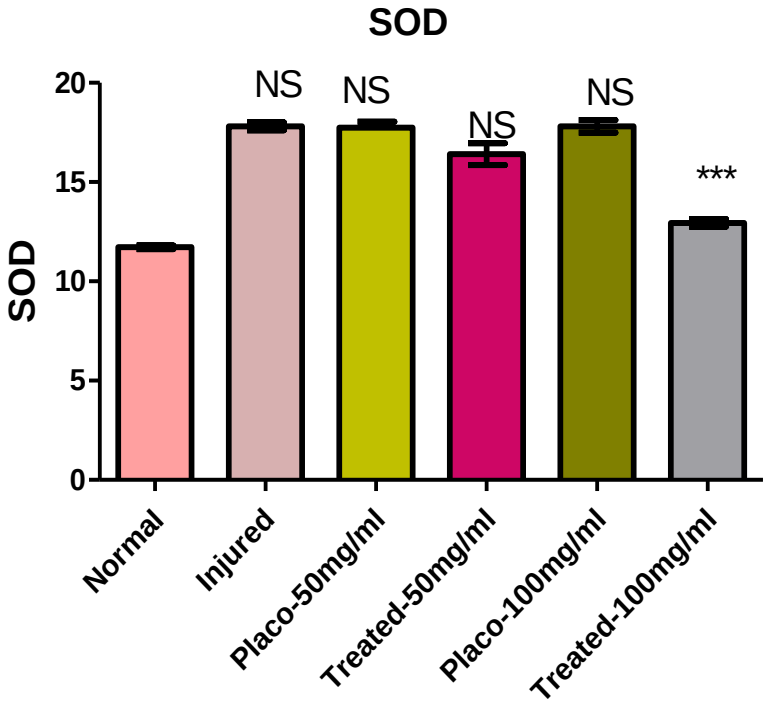


Figure 4.10: SOD levels in treated groups of rats with selected doses of Lemon grass extract in comparison to injured group of rats. *** Symbols indicate high significant in results ($P < 0.001$). The mean ± SEM is used to express the values.

Table 4.6: Graphical results values of SOD expressed in mean ± SEM

	Normal	Injured	Placebo 50mg/ml	Treated 50mg/ml	Placebo 100mg/ml	Treated 100mg/ml
SOD	11.72±0.09	17.81±0.20	17.74±0.30	16.41±0.55	17.81±0.31	12.94±0.19

4.7.3 Estimation of GSH

A biochemical test which is responsible for finding out the estimation of GSH level is Lemon grass.

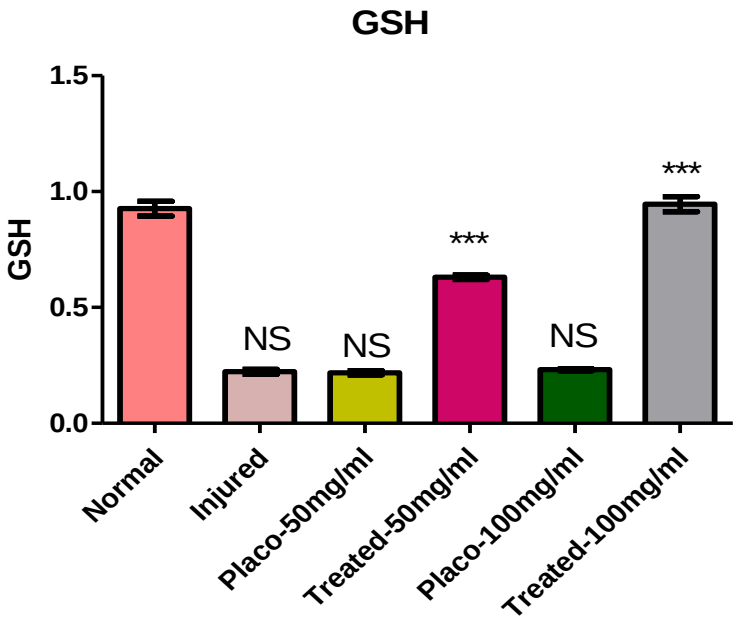


Figure 4.11: GSH levels in treated groups of rats with selected doses of Lemon grass extract in comparison to injured group of rats. *** Symbols indicate high significant in results ($P < 0.001$). The mean ± SEM is used to express the values.

Table 4.7: Graphical results values of GSH expressed in mean ± SEM

	Normal	Injured	Placebo 50mg/ml	Treated 50mg/ml	Placebo 100mg/ml	Treated 100mg/ml
GSH	0.92±0.03	0.22±0.01	0.21±0.008	0.63±0.009	0.23±0.004	0.94±0.03

4.7.4 Estimation of CAT

A biochemical test which is responsible for finding out the estimation of CAT level is Lemon grass.

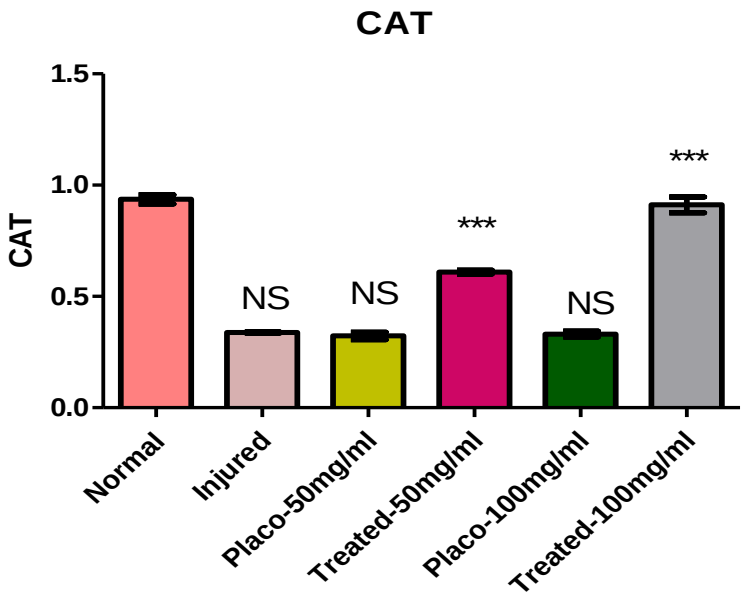


Figure 4.12: CAT levels in treated groups of rats with selected doses of Lemon grass extract in comparison to injured group of rats. *** Symbols indicate high significant in results ($P < 0.001$). The mean ± SEM is used to express the values.

Table 4.8: Graphical results values of CAT expressed in mean ± SEM

	Normal	Injured	Placebo 50mg/ml	Treated 50mg/ml	Placebo 100mg/ml	Treated 100mg/ml
CAT	0.93±0.02	0.33±0.002	0.32±0.01	0.60±0.008	0.33±0.01	0.91±0.03

4.8 Histopathology

4.8.1 Group 1: Normal group

Three layers of tissue make up the skin: Epidermis, Dermis, the middle layer and hypodermis, the fatty layer. The epidermis is composed of keratinized, stratified squamous epithelium. It is made of four or five layers of epithelial cells. The dermis, beneath the epidermis, contains tough connective tissue, hair follicles, and sweat glands. The above figure of normal group result shows the thickness of skin which means that histologically skin's glands are normal.

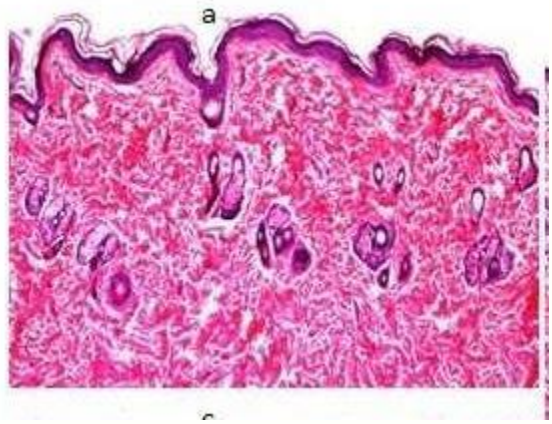


Figure 4.13: Histological Diagram of Normal Skin of Albino Rats

4.8.2 Group 2: Injury group

A burn wound's physiology is characterized by an inflammatory response that triggers the rapid formation of oedema due to increased microvascular sensitivity, vessel dilatability, and extravascular osmotic activity. The above figure shows the inflammation, skin thickness is compromised and the small dots in figure shows the inflammatory cells that are integrate in burn skin. The whole skin architecture is destroyed.

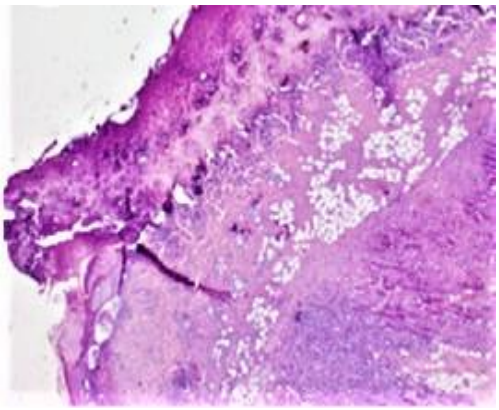


Figure 4.14: Histological Diagram of Injured Skin of Albino Rats

4.8.3 Group 3: Placebo 1

The physiology of a burn wound is distinct from other wound types because of the inflammatory response that causes extravascular osmotic activity, vessel dilatation, and enhanced microvascular sensitivity, which quickly generates oedema. In the image above, the damaged skin thickness can be seen together with the inflammatory cells that are incorporated into burn skin. The entire structure of the skin is damaged.

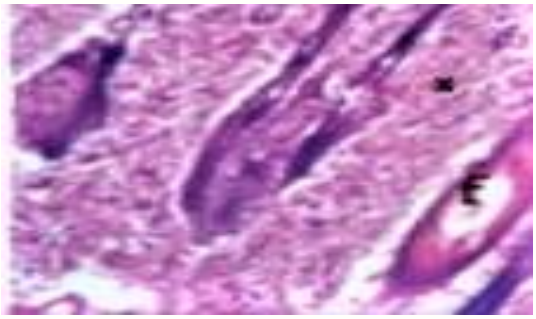


Figure 4.15: Histological Diagram (50mg/ml Normal Saline) Skin of Albino Rats

4.8.4 Group 4: Treated 1

The above result of histopathology of rat's skin treated with treatment 1 (50mg/kg plant aqueous extract) shows that the skin thickness is reconstructed. The entire structure of the skin is build.

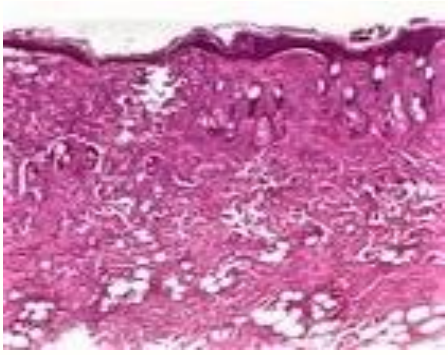


Figure 4.16: Histological Diagram of Treated 1 Skin of Albino Rats

4.8.5 Group 5: Placebo 2

A burn wound's physiology is characterized by an inflammatory response that results in rapid oedema generation as a result of increased microvascular sensitivity, vessel dilation, and extravascular osmotic activity. Inflammatory cells that are integrated into burn skin are visible in the above image as small dots, and the skin thickness is destroyed. The top pink colour indicates inflammation. The whole structure of the skin is disrupted.

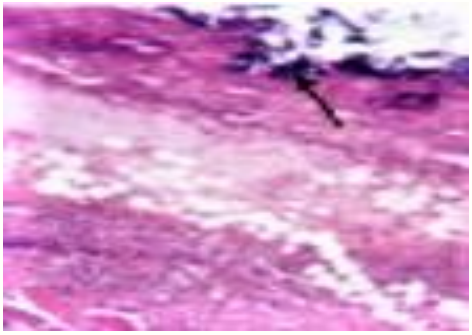


Figure 4.17: Histological Diagram (100 mg/ml Normal Saline) Skin of Albino Rats

4.8.6 Group 6: Treated 2

The above result of histopathology of rat's skin treated with treatment 2 (100mg/kg plant aqueous extract) shows that the skin thickness is reconstructed. The skin's overall structure gradually returns to normal.

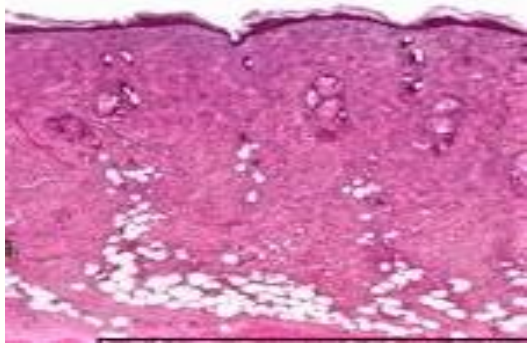


Figure 4.18: Histological Diagram of Treated 2 skin of Albino Rats

CHAPTER 5

DISCUSSION

The Wound healing and Antifungal Properties of lemon grass was investigated by using estimation of antioxidants, wound index measurement, sandwich enzyme-linked immunosorbent assay, and histopathological testing at different doses of aqueous extract of lemon grass (107, 189). A burn wound's physiology is characterized by an inflammatory response that triggers the rapid formation of oedema due to increased microvascular sensitivity, vessel dilatability, and extravascular osmotic activity. One of the most significant proangiogenic mediators in wound healing is VEGF (190). The increase in VEGF following damage is likely due to multiple cellular sources. Damaged skin produces more VEGF, which is typically expressed at low levels by epidermal keratinocytes (8). The Sandwich Elisa results of ANNEXIN-V demonstrated that the level of apoptosis is decreased with treatment of plant extract of lemon grass. ANNEXIN-V is a marker for apoptosis (191).

The antifungal, anti-inflammatory, antioxidant, antibacterial, antifever, antifilarial, antidyspeptic, anti-amoebic, analgesic, antimicrobial, antiviral, antihypertensive, antihyperglycemic, antinociceptive, and wound healing properties of the lemon grass plant have been demonstrated in vitro and in vivo using plant materials (31). The Lemon grass plant's potential to heal wounds was proven by the wound index measurement. Wound index level was measured in treated group of rats with selected doses of lemon grass extract in comparison to injured group of rats. The symbol *** denotes high statistical significance in results ($P < 0.001$). The rat groups treated with the lemon grass plant extract had remarkable wound index (reduction) values, and results were assessed using one-way ANOVA in graph pad (29).

Estimates were made for the antioxidant assays, including APOX, GSH, CAT and SOD. By utilising one-way ANOVA in Graph Pad, the APOX levels, GSH levels, CAT levels, and SOD levels in the injured vs. treated group of rats with specific doses of lemon grass reveal that the results obtained were highly significant with p value < 0.001. Antioxidants

are believed to facilitate the control of oxidative stress in the wound healing process (192).

The histopathology results of normal group of rats showed that the glands of the skin are normal. All the skin layers are present. The results of injured group of rats showed inflammation, skin thickness is compromised and inflammatory cells are formed that are integrated in burn skin. The whole skin structure is destroyed. Same damaged skin structures were showed in skins treated with placebo 1 (50mg/ml) and placebo 2 (100mg/ml). While the histopathology results of treated group of rats (50 and 100 mg/ml) showed that the skin thickness is reconstructed and the entire structure of the skin is gradually backed to normal.

All these results concluded that lemon grass has antifungal properties and wound healing potential and could be very effective in the treatment of skin wound injuries.

CONCLUSION

Lemon grass extracts showed potential antifungal and wound healing properties. The antifungal testing of lemon grass extracts on Candida albicans proved the antifungal potential of lemon grass extracts. At high and low doses of lemon grass extracts, it demonstrated remarkable antifungal ability by showing the inhibition zone. The wound healing ability of lemon grass extracts was confirmed using wound index measurement, antioxidant estimation, sandwich enzyme-linked immunosorbent assay (ELISA) protocol, and histopathological test. Lemon grass extract confirmed greatest wound healing potential by increases the vascular endothelial growth factor (VEGF) at various doses (50 and 100 mg/ml). The results obtained were highly significant. At high dose of lemon grass extracts, it demonstrated remarkable wound healing potential, whereas at low dose, it also demonstrated good results. The confirmation of antifungal and wound healing properties of lemon grass extracts open a new horizon for future research to treat acid burn injuries and wound treatments.

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