EFFECT OF HERMAL PLANT ON CELLULAR VIABILTY AND SKIN REGENERATION ABILITY OF ACID BURN RATS

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EFFECT OF HERMAL PLANT ON CELLULAR VIABILTY AND SKIN REGENERATION ABILITY OF ACID BURN RATS

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RESEARCH COMPLETION CERTIFICATE

It is certified that **Ms. Faria Zainab** of B.Sc (Hons) (Session 2018-2022), Department of Zoology has carried out this research work entitled **"Effect of hermal plant on cellular viability and skin regeneration ability of acid burn rats"** under my supervision. It is assured that this research work is original and has not been published anywhere.

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ABSTRACT

Peganum Harmala has been used in traditional medicine as a disinfectant and pain reliever for a long time. It is extensively dispersed and utilized as a medicinal plant and also has antibacterial, antifungal, antiviral, antioxidant, anti-diabetic, anti-cancer, and anti-leishmanial effects. The aim of this study is to confirm the cytotoxicity and wound healing potential of the aqueous extract of *Peganum harmala*. The wound healing capacity of *Peganum harmala* extracts at different doses (50 and 100 mg/kg body weight) was assessed using the wound index, sandwich enzyme-linked immunosorbent assay (ELISA), antioxidant estimation, and histological examination. The maximum potential for wound healing was suggested at extract doses of 50 mg per kg by raising levels of vascular endothelial growth factor (VEGF). Additionally, a dose of 100 mg of *Peganum harmala* plant extract per kilogram demonstrated remarkable wound healing capacity with elevated VEGF levels. The cytotoxic potential of *Peganum harmala* extracts on HEK cell line at 570 nm was assessed using the MTT test. The *Peganum harmala* plant extract was shown to have decreased cytotoxicity or increased cytoprotectivity in the aqueous extracts, indicating that it is safe to use with an IC50 against the HEK cell line. Based on the aforementioned findings, it is strongly advised as a possible continuation of the current effort to extract, identify, and purify phytochemicals from *Peganum harmala* extracts.

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No.	Abbreviations	Full Forms
1.	ELISA	Enzyme-linked immunosorbent assay
2.	VEGF	Vascular endothelial growth factor
3.	TGF-a	Transforming growth factor alpha and
4.	PDGF	Platelet-derived growth factor
5.	HGF	Hepatocyte growth factor
6.	IL-8	Interleukin 8
7.	DNA	Deoxyribonucleic Acid
8.	RNA	Ribonucleic Acid
9.	HSV	Herpes Simplex Virus
10.	ALT	Alanine aminotransferase (ALT)
11.	AST	Aspartate aminotransferase
12.	DMEM	Dulbecco's Modified Eagle Medium
13.	FBS	Fetal bovine serum
14.	PBS	Phosphate Buffer solution
15.	SDS	Sodium dodecyl sulphate
16.	HPR	Horseradish Peroxidase
17.	PMS	Phenazine methosulphate
18.	NBT	Nitrobluetetrazolium

CHAPTER I

INTRODUCTION

Natural products generated from plants contain a considerable mixture of compounds that have been shown to aid wound healing (2). Plants and their extracts have a lot of potentials when it comes to wound management and therapy. The herbal treatments for injury healing are not only inexpensive and accessible but they are also said to be harmless, as hypersensitive reactions are uncommon when these agents are used (3). Decreased angiogenesis has come a long way in terms of understanding a non-healing wound. There are few effective herbal medicines available in the clinical context to speed wound healing and closure by promoting blood clotting, fighting infection, and hastening wound healing (4). These herbal medicines are made from plants that have pro-angiogenic properties like Aloe vera, Panax ginseng, Blechnaceae, Peganum harmala Azardicaindica and Cinnamomum cassia and many more (5).

In semiarid temperatures and sandy locations, Hermal *(Peganum harmala)* grows wild. The plant is well-known in the Middle East, North Africa, and the Central Asia where it is extensively dispersed and utilized as a medicinal plant for centuries (6). *Peganum Harmala* (hermal) had been widely used in traditional medicine as a disinfectant and discomfort reliever for a long time (7). Alkaloids are abundant in Hermal, including several quinazoline and carboline alkaloids, collectively recognized as alkaloids of harmala. The alkaloids peganine, harmalol, harmaline, harmol, harmane and harmine are the most common harmala alkaloids. These alkaloids have a large varieties of biological activities inclusive of antioxidants, antiviral, antifungal, antibacterial, anti-diabetic, anticancer, antileishmanial, insecticidal, hypothermic, hallucinogenic hepatoprotective and antinociceptive effects are also found in P. harmala (8).

Cytotoxic properties are also observed and hermal extract has also been shown to hasten the healing of skin wounds. In convential medicine, this plant has long been employed as an emmenagogue and an abortifacient (9). Intoxication signals would

appear a few minutes after the seeds were consumed. Hallucinations, neuro-sensory syndromes, bradycardia, and gastrointestinal (GI) problems and bradycardia, are all symptoms of a P. harmala overdose (10). Harmaline and harmine are two poisonous alkaloids found in P. harmala seeds, causing tremors and clonic convulsions at low doses but there is no significant increase in spinal reflex excitability (11). Due to the significant depressing influence on the central nervous system, lethal doses cause convulsions, which are quickly followed by motor paralysis followed by drop in body temperature (12). With the exception of the uterus, smooth muscle contraction are decreased as the perfused heart is driven into diastole, and there is a drop in blood pressure over a large dosing range due to a significant weakness of the heart muscle (13).

A wound is merely the rupture of a tissue's cellular and anatomic continuity. A chemical, thermal, physical, immunological or microbiological insult to the tissue might cause a wound (14). Wound healing is a collective events of interconnected cellular and biological activities that causes the restoration of structural and functional integrity as well as the strength of wounded tissue (15). Non-healing, under-healing, and over-healing are all common in clinical practise. As a result, the goal of injury curation is to either reduce the duration it takes for a wound to heal or to limit the negative repercussions (16). The focus should be on finding an agent that can hasten the recovery of wounds (17).

In order for wound healing and regeneration to occur, angiogenesis is required, that is the origination of new blood vessels (18). This plays a crucial role in production of new vessels from already present capillaries because it penetrates the injury clot and organizes into a network of microvasculars all over the granulation tissue (19). The rate and extent of the damage may limit the repair process, thus indicating the potential involvement of angiogenesis in wound healing (20).

New stroma, also known as granulation tissue, starts to build roughly four days post injury during the early stages of cutaneous wound recovery (21). When newly developing tissue is sliced and visually examined, it has a granular appearance,

hence the name. The granular appearance of the neo stroma is due to numerous new capillaries (22). Fibroblasts, macrophages, and vessels all travel in the injury space together, which is consistent with their suggested biological interdependence throughout tissue repair (23). Macrophages continuously release cytokines that stimulate fibroblasts, fibroplasia, and angiogenesis to produce fresh extracellular matrix to sustain internal growth of cells, and vessels take nutrients and oxygen to maintain cell viability (24).

The growth of new vessels from already existing ones is known as angiogenesis. It lasts the entirety of a person's life, starting in the womb and ending with death, in both health and disease. (25). It is a symbol of embryonic development in tumor growth and proliferation, psoriasis, age-relevant macular degeneration, arthritis and diabetes (26). The balance of numerous stimulating and inhibitory elements appears to be important (27). Angiogenesis is a multi-stage process that is similar to blood coagulation in some ways; and the induction of neovascularization is linked to a range of biological and pathologic processes involving injury healing, corpus luteum maturation, severe inflammation, and delayed hypersensitivity (28). Angiogenesis is excessively protracted yet self-limiting in certain pathologic conditions that are not malignant. Pyogenic granuloma, keloid development, and retrolental fibroplasia are some examples (29). Angiogenesis in cancer, on the other hand, is not self-limiting. When tumor-induced angiogenesis is activated, it continues endlessly until the tumour is completely eliminated or the host dies (30).

The regulation of separate phases of angiogenesis is referred to as molecular regulation (31). An angiogenic factor causes tube formation, migration and endothelial cell proliferation, whereas an inhibitor prevents all these processes from taking place (32). Various angiogenic growth factors have been identified thus far (33). The very well-known fibroblast growth factors are transforming growth factor alpha and (TGF-a), platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF, scatter factor), vascular endothelial growth factor (VEGF), and interleukin 8 (IL-8). (34).

In healthful individuals, angiogenesis is promoted by VEGF throughout development of embryo and aids in wound healing. (35). VEGF, the most vital regulator of angiogenesis in cancer, is upregulated by the expression of oncogenes, hypoxia and a number of growth factors. (36). VEGF is a glycoprotein that functions as an angiogenesis regulator during embryogenesis, skeletal growth, and reproductive processes (37).It is a heparin-binding angiogenic growth factor also used in pathological angiogenesis involving tumours and neurovascular disorders (38).In embryogenesis and in early post-natal development, VEGF plays a vital role in both (39).VEGF plays a chief character in wound healing and the menstrual cycle by promoting angiogenesis, giving rise to new vessels (40).Because of its major role in tumour formation, VEGF is an important target for anticancer therapy (41). Angiogenesis is induced by VEGF by a direct influence on endothelial cells. In vitro experiments revealed that VEGF induced capillary-like tubule formation by causing microvascular endothelial cells grown on the surface of three-dimensional collagen gels to penetrate the underlying matrix (42).

Despite the fact that several authors have studied the effictiveness of P. harmala in the curation of various dermatoses, the impact of this extract on healing of the wound has not been the subject of published research in the literature. (6). P. harmala extract was employed to exaggerate experimental skin healing of wounds in rats and to examine the cytotoxic properties in this research.

RATIONALE

The purpose is to investigate the cytotoxic properties and the regeneration ability of Peganum Harmala (hermal). Along with other plants being used for therapeutic purposes hermal has also been used for its antibacterial, antifungal, antiviral, antioxidant, anti-diabetic, antitumor properties previously in various Ayurveda medicines. For the betterment of health issues of animals and human, present investigations are carried out to enlighten the importance of medicinal plants that have minimal side effects, less expensive with no age or gender limit. So it's very significant to study the cytotoxic properties and angiogenic potential of hermal.

OBJECTIVES

- To acquire the skill of preparing an aqueous extract from hermal plant powder.
- To analyze the skin regeneration in acid burn rats.
- To gain an understanding of the cytotoxic properties of hermal

CHAPTER II

LITERATURE REVIEW

2.1 Plant overview

Harmala (Peganum harmala) is a perennial grass with glabrous, ramified, densely leafy stems that measures 120 cm in width and 60 cm in height. Peganum harmala is indigenous to the Africa, Mediterranean and Near East and has also been reported by Uzbekistan, Tajikistan, Russian, Chinese, Turkey and Mongolian, Afghanistan, Iran, India, Iraq and Pakistan (43). Peganum harmala, often called Syrian Rue or Wild Rue, was also adopted in the America and Australia. In North Africa, the plant is known as "Harmal," while in the America, it is known as "African Rue," "Mexican Rue," or "Turkish Rue." There are no records of its cultivation in the past. It's been used as a psychoactive substance for a very long time and has long served as an entheogen in the Middle East (44). The plant had significant commercial importance in the past, primarily as a source of grain-based seed dyes, notably in Turkey, where grains were converted into a red color that was used to kill decorated teapots, despite their traditional use as an ethnomedicine. Seeds of hermal are used as incense in Africa since the beginning of time. For weaving, the Greeks used powdered harmala seeds. Since the beginning, harmful seeds have had hypothermic and hallucinogenic qualities. Different segments of the hermal have a long record of use in traditional medicine for treating lumbago, asthma, colic, and jaundice, among other ailments (45).

Figure 2.1: Hermal plant (46)

P. harmala is a bright green plant with thickly foliated leaves, herbicide, and a permanent timbered rootstock grower. While the plant's multi-branched, smooth stems can reach 4 feet or more, they rarely grow taller than 2 feet and are usually spherical and bushy. Due to its low maintenance and susceptibility to draught, the white blooming plant is an attractive ornamental plant (47). The leaves are 2 inches long, beautifully split, and separated in long, thin strips. Five long elliptical petals and five thin sepals that are much longer make up the relatively wide and conspicuous blooms. These are about an inch and a half wide. On the trunk, each blossom might develop into a three-valved leathery seed capsule (48).

In earlier times, the fruit is used as an analgesic and antiseptic. The seeds possessed hypothermic and hallucinogenic properties. However, it is unclear whether or not narcotics are used to induce vision (49). Further drug administration of P. harmala have shown anti-tumor effects based on existing pharmaceutical trials, anti-leishmanial, anti-spasmodic, anti-histamine effects healing wound, the activity of anti-oxidants, properties of immunomodulators, leukemia., hypoglycemic effects, antinociceptive effects and analgesic, antitumor activity, and anti-inflammatory properties, hepatoprotective effect, and cytotoxic activity (50).

The beautiful color of fruits of hermal are a source of scarlet color and oil. The alkaloid content of unmature seeds is lower than that of mature seeds. P. harmala's -carboline alkaloids, harmalins, Harmans, peganines, isopeganins, dipeganins, deoxypeganins, and quinazolines, such as vasicine, vasicinones, and deoxyvalins, may be responsible for some of the pharmacological effects seen (51).

Lamchouri et a. described that fever, diarrhea, miscarriage, subcutaneous tumours, and a variety of other illnesses are treated with *Peganum Harmala* seed powder, decoction, macerate, or infusion (52). According to Rashan et al. and Farouk et al. antimicrobial, antifungal, antiviral (53), central nervous system stimulant, analgesic, vasorelaxant, and wound-healing activities have been discovered in *P. harmala* seed (54).A seed extract has been utilised to treat dermatoses in humans as well as theileiosis in animals (55). Furthermore, Mirzaei studied that in streptozotocin-induced diabetic rats, the seed extract has been demonstrated to have

hypoglycemic action(56). According to Singhet al. a number of phytochemicals identified in *P. harmala* are thought to be in charge for its pharmacological and therapeutic properties (57).

Traditional medicine has also used *P. harmala* to treat cancer, according to Sobhani et al. In Iran, an ethno-botanical preparation is used to treat neoplastic (58). Chen, Chao et al. described that *P. harmala* powdered seeds are utilized in Chinese herbal recipes to treat digestive system cancers and sometimes used on skin and subcutaneous tumours in Morocco(59).

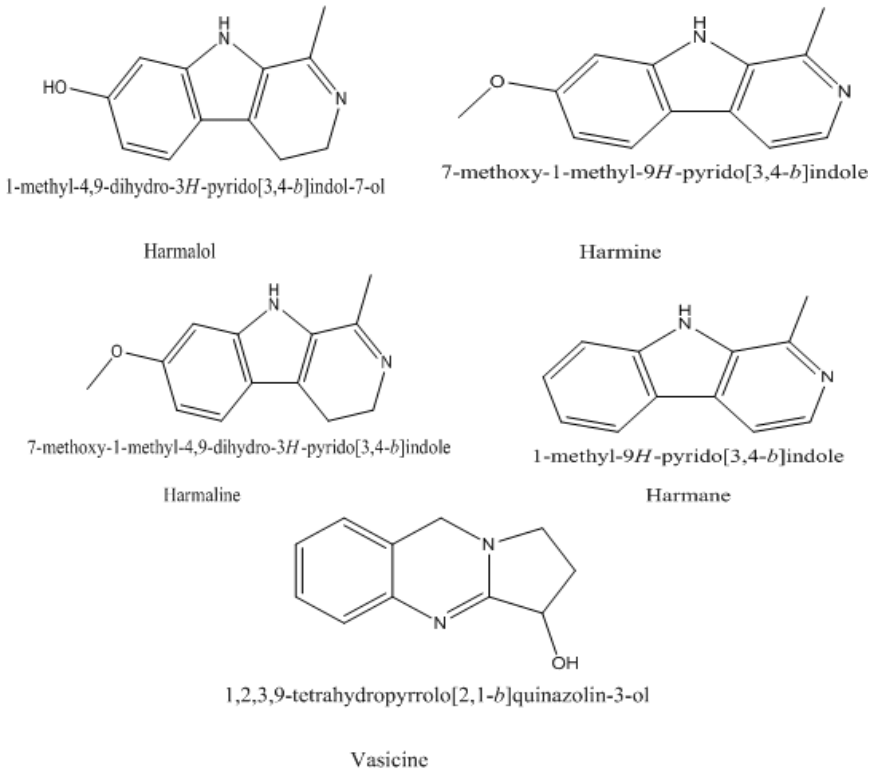

Figure 2.2: Structures of alkaloids of hermal (60)

2.2 Chemical Composition

Alkaloids, flavonoids, and anthraquinones all well-known phytochemicals are present in P. harmala. The total quantity of harmaline, harmalol, and harmine in P. harmala ranged from 2% to 5%. The two main beta-carbonic alkaloids present in P harmala formulations are harmol and tetrahydroharmine. Alkaloid concentrations were highest in the roots and seeds and lowest in the stems and leaves (60).

Harmaline is up to 4.3 percent in dry seeds, whereas harmaline is up to 5.6 percent w/w, harmalol is up to 0.5 percent, and tetra-hydroharmine is about 0.2 percent (61). Harmol and harmine are 2.1 and 1.5 percent in the roots. Peganine, also known as Harmaline, is the main alkaloid in the plant and was first obtained from the roots and seeds of P hermal. Harmine is contained in hermal and has pharmacological properties similar to harmaline, but is less damaging to its effects. Vasicinone and Vasicine are alkaloids of quinazoline that were initially discovered in the flora of hermal (62).

There is aslo a new alkaloid of carboline derivative obtained from the upper portions of hermal recently (63). Four new flavonoids, including, 7-O-6"-Oglucosyl-2"-O-(3"'-acetylrhamnosyl) glucoside, glycoflavone 2"'-O rhamnosyl-2"-O-glucosylcytiso, 7-0-(2"'-0- rhamnosyl-2"-O-glucosylglucoside), and the acacetin 7-O-rhamnoside (58).

Table 2.1: Chemical Profile of Peganum Harmala (1)

Alkaloids	Root	Stem	Leaves	Flowers	Seeds	%age
Harmine	+	+	-	-	+	2.5-3%
Harmaline dehydrharmine	+	+	-	-	+	2.5-3%
Peganine	.	+	.	+	+	2.5-3%
Quinazolone	.	.	.	.	.	2.5-3%
Harmalol	.	+	.	.	+	2.5-3%
Harmalidine	.	.	.	.	+	2.5-3%
Harmaline	.	.	.	.	+	2.5-3%
Pegamine	+	+	+	.	+	.
Vascinones	+	+	+	.	+	.

2.3 Peganum harmala properties

2.3.1 Antibacterial and Antifungal properties

Studies and comparisons have been done on the bectericidal properties of the leaf, root, flower, seed, and stem of hermal. Gram +ve bacteria like Bacillus pumilus, Bacillus cereus, Bacillus anthracis, Streptococcus pyogenes, Staphylococcus epidermidis, Staphylococcus aureus and Listeria monocytogenes, as well as Gram -ve bacteria like Pseudomonas Brucella and aeruginosa, were most effectively inhibited by the root and seed extracts of hermal (48).

It is extremely possible that secondary metabolites with antibacterial action are kept in the root and seed of the hermaphrodite because these parts of the plant are known as reservoir sections. When compared to seed extract, the root extract shows greater antibacterial action against Gram positive bacteria, according to the findings. DNA intercalation has been revealed to be a mediator of the antibacterial activity of harmane, a highly aromatic planar alkaloid; as a result, this mechanism needs to be taken into account for the extract of hermal root and seed (64). The high concentration of polyphenols in P. harmala, which are known to have strong antibacterial characteristics, may be responsible for the plant's antibacterial activity (65). In vitro testing revealed that there is significant antibacterial activity of various concentrations of hermal seed aqueous extract against S. mutans. Alcoholic and aqueous extracts of hermal reduced the growth of Candida albicans and Lactobacilli, two common oral bacteria. A 50% concentration of alcoholic and aqueous extracts outperformed 0.2 percent chlorohexidine for both microorganisms under investigation. This might be because certain alkaloids, like harmaline, harmine, harmalol, and peganine, have the ability to interpolate with the DNA of microbes (66).

P. harmala's in vitro antifungal effectiveness was examined against six and three different species of Aspergillus and Candida, respectively. In an ethanolic extract of P. harmala seeds, Candida glabrata and Candida albicans demonstrated the lowest and highest inhibitory impacts. The extract's lowest fungicidal concentration on Candida obtains was also found to range from 0.625 to 2.5 mg/ml (67).

C. glabrata had the strongest fungicidal activity (0.625 mg/ml), while C. albicans had the weakest (2.5 mg/ml). The results of the Aspergilli assay were dependant on the species. Aspergillus fumigatus and Aspergillus niger were unable to grow because of the extract (68). Harmaline is primarily responsible for the antifungal and antibacterial properties of P. harmala seeds extract (67).

2.3.2 Anti-tumor activities

Spinal-Z is an Iranian ethnomedical medicine made from Dracocephalum kotschyii leaves and hermal seeds. For many years, it has been used to treat several types of cancer. The (MTT) assay was used to determine Spinal-Z's in vitro effects. Spinal-Z inhibited cell proliferation in a selected dose, and its components reduced the viability of cells (69). They both together were able to stop tumor proliferation (70).

2.3.3 Antidiabetic Properties

The results of recent studies clearly proved that the alcohlic extract of hermal seed remarkably (P0.001) lowered glucose levels in normal and diabetic rats at different dose levels (150 and 250 mg/kg) (71). The application of the ethanolic extract increased their ability to use the external sugar load. There is strong evidence that the sample works just as well as the well-reputed oral low glycemic medicine at lowering glucose levels after a sucrose challenge in both healthy and diabetes induced rats (72).

2.3.4 Antioxidant Properties

A substance that significantly slows or inhibits the oxidation of the substrate at low concentrations in comparison to the oxidizable substrate is referred to as an antioxidant (73). They are of high importance to biologists and clinicians because they continue to shield the body from the damage wrought by free radicals produced by Alzheimer' disease, Parkinson's disease, stroke, heart disease, atherosclerosis and normal ageing. The importance of antioxidant properties in natural components and products, as well as their connections to cancer prevention, anti-aging, and inflammation, are well supported by data. New studies have showed that the ethanol extract of hermal seed has significantly reduced anti-aging, cancer, and anti-inflammatory and hypolipidemic effect (74).

The ammonium thiocyanate technique was used to evaluate the anti-oxidant capacity of hermal leaves and determine how well it inhibited lipid peroxidation. After five days of incubation, the alcoholic extract from hermal leaves (75.9 ± 0.3) successfully halted the oxidation of linoleic acid. Tocopherol (80.12 ± 0,4) in a methanol extract was found to be extremely antioxidant when matched to control. The presence of phenols in the methanolic extract, such as flavonoids and tannins, is primarily responsible for this powerful antioxidant action. An intriguing characteristic that supports the idea that the former chemicals directly contribute to the function of antioxidants is a link between phenolic content and antioxidant activities of plants (75).

2.3.5 Antiviral Properties

All around the world, human herpes viruses are common sources of viral infections in both immune-compromised and immune-deficient people. One of these contagious illnesses that affects many body parts is the herpes simplex virus (HSV) infection (76). Up to a concentration of 667 g/ml, On Vero cells, hermal seed extract showed no cytotoxicity. According to the suppression of virus yield, giving the extract to the cells an hour after infection may drastically lower the virus titer in the first phase and entirely stop virus production in the third or last phase (77).

2.3.6 Protective Effects

Even though P. harmala has been shown to have somewhat cytotoxic action, it has also been shown to have protective properties against thiourea-induced diseases in adult male rats. This suggests that the samples can save the body from the impacts of thiourea on cancer. To evaluate the potential hepatoprotective effect, the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities as well as the blood bilirubin level were examined. The findings showed that hermal samples saved the animal from thiourea's cancer-causing effects since the levels of cancer-causing chemicals normalized. (78).

2.4 Angiogenesis and Wound Healing

In angiogenesis, existing blood vessels are used to create new blood vessels from scratch. John Hunter, a Scottish anatomist and surgeon, was the first to notice that

overall angiogenesis regulation follows Aristotle's primary law of nature, which is "form follows function" (79). The contemporary history of angiogenesis began with Judah's hypothesis (published in 1971) that tumour growth is dependant on angiogenesis (80). With only 40 papers published in 1980 and approximately 6000 in 2010, the field of angiogenesis has evolved tremendously in the last 30 years (81).

Contributing to the early methods to study angiogenesis, Sandison invented a transparent chamberin 1928 that could be placed in the rabbit ear, making it feasible to examine angiogenesis in living mammalian animals for the first time (82). Many workers extended the chamber approach to various tissues over the years, but two contributions stand out. Beginning in the 1940s, Algire et al. employed a chamber implanted in mouse skin to explore tumour angiogenesis (83). Greenblatt and Shubik studied the creation of new capillaries by tumours over a millipore filter in the hamster cheek pouch. They also gave two significant disadvantages of transparent chambers: a high likelihood of infection and difficult access for introducing tumour specimens (84). Then in 1967, following the full description provided by Leighton, the chick embryo chorioallantoic membrane became beneficial for studying and analysing tumour progression (85).

A systematic study by Bonanno and Iurlaro et al. confirmed the extracellular matrix (ECM) is required for angiogenesis at all stages (86). According to Stratman, Malotte, and colleagues, angiogenesis in adults begins with endothelial cell stimulation, vascular basement membrane disintegration, and vascular sprouting within the interstitial matrix (87). According to Senger and Claffey et al. as ECs multiply and migrate, ECM attachment to integrins offers important signalling assistance. The ECM also acts as a substrate for a number of cytokines that have critical signalling roles during angiogenesis (88).

Carmeliet, De Smet et al. describe that in both utero and adulthood, sprouting angiogenesis and intussusceptive angiogenesis occur (89). Angiogenesis can sprout in areas of the body that were previously lacking blood vessels (90). Interstitial tissue infiltrates existing capillaries to create new blood vessels, resulting in

transvascular tissue pillars that expand, is known as intussusceptive angiogenesis (91).

In the paper presented by Ingber et al. he stated that a variety of in vivo and in vitro angiogenesis models have been developed, all of which have considerably contributed to the field's outstanding progress (92). According to Stogard et al. this test is useful for gathering information about angiogenesis since it may be administered intravenously or topically, it is a relatively quick test, and it can be easily modified to study angiogenesis-dependent processes such as tumour growth (93). Importantly, Jain et al. proved that the CAM provides a physiological environment in which pro- and anti-angiogenic drug interactions can be studied (94).

Giordano and Johnson et al.described that angiogenesis is described by hypoxia, pH levels, interstitial pressure, and vascular permeability in recent breakthroughs in the subject and what is currently known about how angiogenesis is set off (95). Shweiki et al. proposed the finding that hypoxia increases expression of the strong angiogenesis stimulator (VEGF) and highlighted a key relationship between oxygen levels and angiogenesis (96).

According to Clark and Madri et al. wound healing relies on the formation of new blood vessels. Angiogenesis was first described by Gross et al. in translucent, two-dimensional wounds, such as frog's webs and rabbit's ear chamber, which led to the sprouting model of angiogenesis (97). Schreiber et al. added histopathology and electron microscopy of muscle and corneal restoration to these findings (98). According to Juliano and colleagues, endothelial cells are the key players; they consume the basement membrane of the vascular system, penetrate the stroma of ECM, and form cylindrical structures that emerge, expands, and form networks as a consequence of endothelial cell proliferation and chemotaxis in the form of developing capillary sprouts (99). Arnold et al. proved that endothelial cells, angiogenesis factors, and surrounding ECM proteins must interact in a dynamic, time- and space-controlled manner for these events to occur and the wound to heal (100).

A "wound" is defined as harm or disruption to the typical anatomical structure and function. A skin epithelial integrity rupture might be the extent of it, or it might affect subcutaneous tissue and destroy bones, organs, nerves, arteries, muscles and tendons (101). Injury may form as a consequence of pathological processes that begin outside or inside the affected organ. They could be the result of a illness, or they could be an unintended or deliberate source. The tissue is harmed, and the environment is disrupted, regardless of the cause of the wound or its appearance (102).

A physiological response to the toxic substance results in bleeding, vascular constriction with coagulation, complement activation, and an inflammatory response (103). Normal wound healing includes blood clotting, the beginning of an acute inflammatory response to the initial injury, connective tissue and parenchyma cell regeneration, migration, and proliferation, as well as the synthesis of extracellular matrix proteins, remodelling of new parenchyma and connective tissue, and collagen deposition. The orderly healing of damaged tissues completes the process of improving wound strength (104).

The processes underlying mechanisms the above include:

i. Inflammatory growth factors and mediators;
ii. Cell-cell connections between cells and extracellular matrix that control cell migration, cell proliferation, and differentiation
iii. Activities related to fibroplasia, epithelialization, and
iv. Wound contraction
v. Angiogenesis remodeling

These systems are began as soon as a physical injury occurs and continue during the entire repair process (105).

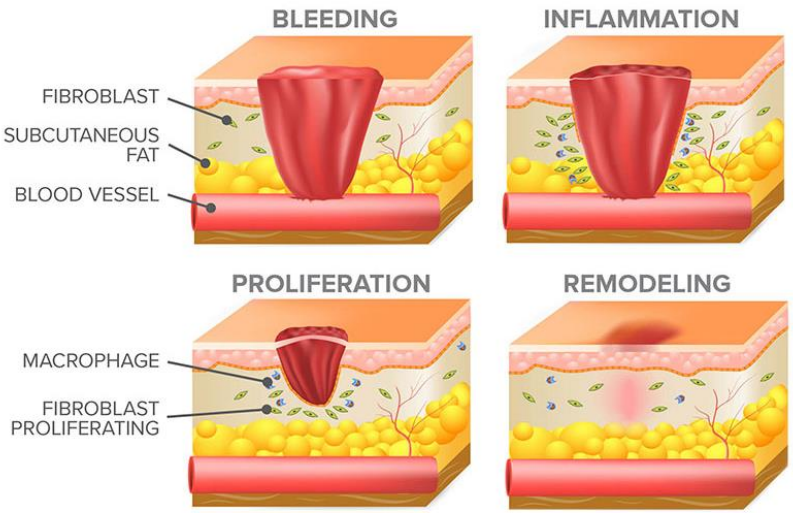

Figure 2.3: Stages of Wound Healing (105)

Burn injuries are underappreciated and have a high rate of morbidity and mortality. Burn injuries, especially severe burns, cause metabolic anomalies, distributive shock, and inflammatory and immunological reactions that can be difficult to control and culminate in the failure of numerous organs. Burn injuries are brought on by warmth from warm liquids, solids, or flames, not by friction, cold, heat, radiation, chemical, or electric driven resources. Energy transfer produces tissue damage in all burn injuries, although diverse sources can have a variety of physiological and pathophysiological repercussions (105). There is a considerable variety of chemicals included in natural products made from plants that have been found to promote wound healing. Understanding of decreased angiogenesis has advanced significantly, and few clinically useful herbal remedies are now available to improve wound healing and closure by stimulating blood clotting, thwarting infection, and accelerating wound healing. These herbal medications are created from plants that promote angiogenesis (106).

CHAPTER III

METHODOLOGY

3.1 Plant Powder Preparation

The region from where Peganum harmala was obtained is the Soon Valley which is one of Pakistan's most beautiful valleys. Located in the Khushab district of Pakistan's Central Punjab province. With an area of 780 km^2, its climate is hot in summer (with temperatures reaching 36°C in June) and dry in the winter (with a low of 1°C in January).The valley has a diverse range of plant species and is a potential source of medicinal herbs due to the different altitude gradients and weather conditions (45). After obtaining the plant, it was dried and ground to make a fine powder using an 80 mesh sieve, and it was fed orally to the rats through gavage feeding (45).

3.2 Aqueous Extract Preparation

An aqueous extract was made by dissolving 10 g of Peganum harmala powder in 100 ml of water for 2 days and was filtered through Whatman filter paper. All the water soluble fractions were present in that water, which was evaporated by placing it in petri dish. After evaporation, the leftover content was weighed and stored for further experiments.

3.3 Sampling of Cell Lines

The cell culture facility at The University of Lahore provided normal cell lines (HEK). To keep these cell lines viable, liquid nitrogen-filled cryovials were employed. Cryovials were processed after being resurrected.

3.4 Culturing of Cell Lines

A liquid nitrogen cylinder was used to defrost the cryovials. The normal cell lines were then cultured in DMEM-HG supplemented with 10% fetal bovine serum (FBS), 100 mg/mL penicillin G (Sigma), and 100 U/mL streptomycin (Sigma) in a culturing flask. At 37°C, the cultures were kept humidified in a humidified incubator with 5% CO2. Experiments were carried out thrice (107). Sub-culturing

of cells were done after they reach 70-80% confluence in culture. In order to remove cells off the flask's surface, they were rinsed in 1X phosphate buffer saline (PBS) and then incubated with 0.05 percent trypsin-EDTA. The flask was observed under an inverted microscope, which indicated that the cells had been separated. The flask was then filled with a few drops of FBS and carefully swirled. The mixture was centrifuged for 5 minutes at 2000 rpm in a 15 ml tube. The supernatant was removed and pellet was re-suspended after centrifugation (108).

3.5 Treatment of Cell Lines with Hermal Plant Powder

The cells that had formed a contact with the culturing flask's walls were rinsed in phosphate buffer saline (PBS), then triedpsin-EDTA was applied to them until they separated from the flask's exterior. Four sets of cells were formed from the cell line, first group was kept untreated. The second group was treated with hermal powder, third with ethanol extract and fourth group with water extract. Plant extract was used to treat each cell line at following concentrations of (0µg/ml, 100µg/ml, 200µg/ml, 500µg/ml, 1000µg/ml, and 2000µg/ml) in DMEM (Dulbecco's Modified Eagle Medium). Treatment was provided to the cultured cells for 24 hours. It was estimated using 96-well plates of post-treated cells after 24 hours (109).

3.6 Calculation via MTT assay

To calculate cell viability, 3-(4, 5-dimethylthiazol-2- yl) -2, 5-diphenyltetrazolium bromide (MTT) assay was done utilizing a 96-well plate (110). Cells were washed out with phosphate buffer saline (PBS) after 24 hours of treatment with different doses of hermal extracts powder, and then were incubated for 3-4 hours with 100µl of DMEM and 25µl MTT solution. After 4 hours, the formazan crystals were dissolved in 10% sodium dodecyl sulphate (SDS) and the absorbance at 570 nm was measured. The percentage viability was estimated using the following formula:

$$\% \text{ Cell viability} = \frac{\text{Experimental } (OD_{570})}{\text{Control } (OD_{570}} \times 100$$

Experiments were repeated three times for the IC50 calculations (111).

3.7 Animal Model

Albino rats (100-120g) were obtained from the University of Lahore's animal house 14 days before to the experiment, and they were kept at a constant temperature of 25°C. They received nutritious meals and had regular, unrestricted access to food and water in their cages.

3.8 Ethical Clearance

The Ethical committees of University of Lahore and of Kinnaird College for Women in Lahore provided approval for the use of animals (albino rats).

3.9 Grouping of Rats

Table 3.1. Categorization of Rats

No.	Group Name	Group Description
1	**Group 1 Normal (N)**	Normal rats with no injury
2	**Group 2 Injured (INJ)**	Acid-burned injured rats with no treatment
3	**Group 3 0.5 ml Normal saline (Placebo 1)**	Acid-burned injured rats given 50 mg/ml Normal saline
4	**Group 4 Treatment 1 (T1)**	Acid-burned injured rats given 50 mg/ml *Peganum harmala* aqueous extract
5	**Group 5 1 ml Normal saline (Placebo 2)**	Acid-burned injured rats given 100 mg/ml Normal saline
6	**Group 6 Treatment 2 (T2)**	Acid-burned injured rats given 100 mg/ml *Peganum harmala* aqueous extract

3.10 Acid Burn Injury on Rats

First, injections of ketamine and xylazine were used to anaesthetize the rats.

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

Figure 3.1: Dose, Route and Duration of Anesthesia given to Rats before Acid Burn Injury.

Acid burns were utilised to test whether plant extracts had any effect on the healing of the wound or whether they might be used to treat and cure burn injuries. Six rats were used in total, one was normal (uninjured), and the remaining five (n=5) were given acid burn injuries while under the influence of xylazene and ketamine solution (anaesthesia). One was kept injured, two were placebo at 50 mg/ml aand 100 mg/ml and two others were given hermal at 50 mg/ml aand 100 mg/ml cocentrations (112).

Figure 3.2: Removing rat's body hair by applying hair removal cream

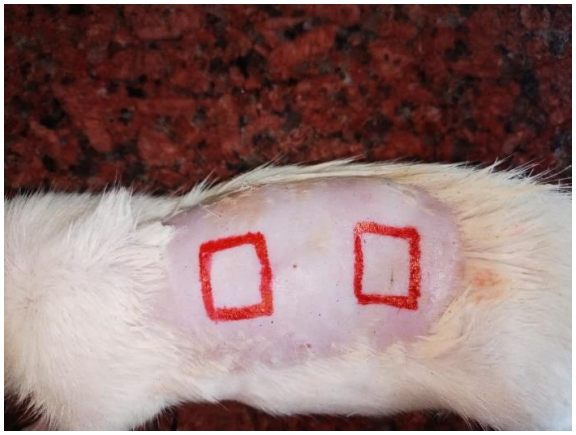

Figure 3.3: Points for acid burn injury are marked by a marker

Figure 3.4: Filter paper is soaked in acid

Figure 3.5: Acid soaked filter paper is placed on rats

3.11 Treatment with Peganum Harmala

The acid burned injured rats were treated with giving different doses of Hermal plant to them. 2 groups were made, 1 group was given 50mg/ml of hermal and the other group was given 100mg/ml hermal. The results were obtained after 7 days by measuring wound index.

3.12 Wound Index Measurement Protocol

The measuring of wounds is an important aspect of the wound assessment process. It was noted at the time of the original presentation, as well as at frequent intervals during the review process. A wound healing assay was performed in alternate days to measure how much the size of wounded area is reduced (113). The wounds of anaesthetized rats were covered with a transparent piece of plastic sheet with the ventral surface down. Any pen/pointer was used to draw a line through the wound on the sheet. To preserve the record, their sketches were drawn and photographs were taken. The initial and final wound sizes were used to compute the percentage reduction in wound (114).

3.13 Enzyme-linked immunosorbent assay (ELISA)

In a 96-well plate, solid phase sandwich ELISA was carried out for VEGF and annexin V. The plate was coated with VEGF and annexin V antibodies and was incubated for 120 minutes. After washing three times with TBS-T, 1 percent BSA were added for 30 minutes to block. After blocking, incubate each well for 60 minutes with 200 µl of serum collected from the blood of the treated rat (115). After removing the sample and rinsing it three times with horse reddish peroxidase (HRP) conjugated donkey anti-rabbit secondary antibody, it was incubated for 120 minutes at 37°C. As soon as the washing is complete, 100 µl of chromogenic solution 3,3,5, 5-tetramethylbenzidine was added and 0.18 M (H_2SO_4) was also added after 15 minutes to halt the reaction before taking the 450 nm absorbance (116).

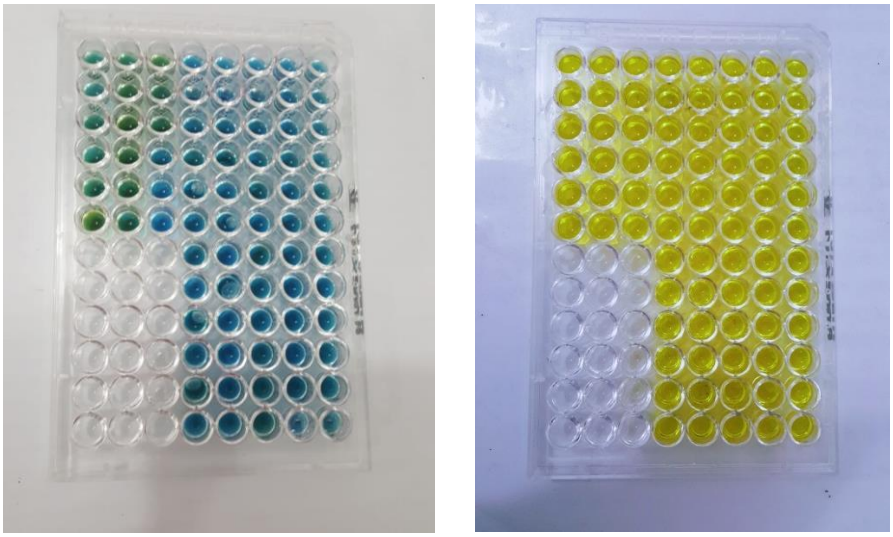

Figure 3.6: At the addition of substrate solution then addition of stop solution

3.14 Estimation of Antioxidants

3.14.1 Ascorbate peroxidase (APOX) assay

The APOX assay will be done on a 96-well plate. The reaction will include 100m M KH_2PO_4 buffer (pH 7.0), 0.5mM ascorbate, and 0.3mM H_2O_2, with the remaining will be post treatment media from a separate cell line. The optical density will be measured at 290 nm after 3 minutes (117).

3.14.2 Estimation of SOD

The activity of superoxide dismutase (SOD) was measured using the method suggested by. 0.1 ml cell culture media was combined with 1.2 ml sodium pyrophosphate buffer (52 mM, pH 8.3), 0.1 ml of phenazine methosulphate (PMS), and 0.3 ml of nitrobluetetrazolium (NBT), and the reaction was initiated with the addition of 0.2 ml nicotinamide adenine dinucleotide (NADH).The reaction was halted by adding 0.1 ml glacial acetic acid after 90 seconds of incubation at 30 C. With 4.0 mL n-Butanol, the reaction mixture was rapidly agitated. After a 10-minute incubation period, the mixture will be centrifuged at 2,000 rpm for 5 minutes. The upper butanol layer's absorbance will be measured at 560 nm (118).

3.14.3 Estimation of glutathione

A method proposed by was used to calculate the amount of reduced glutathione (GSH) in cell culture media. 0.5 ml cell culture medium from both groups, 2.0 ml

disodium hydrogen phosphate buffer (0.3 M), and 0.25 ml (5, 50-dithiobis-(2-nitrobenzoic acid) or DTNB were added to a test tube (0.001 M). After 15 minutes of incubation, the absorbance was measured utilizing a spectrophotometer at 412 nm.

3.14.4 Estimation of Catalase

Catalase activity was determined by combining 0.1 ml of cell culture media with 1.0 ml of phosphate buffer (10 mM, pH 7.0) and 0.4 ml hydrogen peroxide in a mixture (0.2 M). By adding 2.0 ml of dichromate acetic acid reagent, the reaction was stopped. As soon as the samples were cooled after 10 minutes in the water bath, they were tested for absorbance at 530 nm (119).

3.15 Histopathology Assay

Seven days after the rats were burned, skin samples from the burned area were collected that were formalin-treated. Skin samples that had been dehydrated were combined, cut, and waxed using a microtome. H&E was used to stain the skin segment and examine the structure of the skin.

3.16 Statistical Analysis

All readings and the data collected will be statistically analyzed by using graph pad prism software.

CHAPTER IV

RESULTS

4.1 Reduced Cytotoxicity of HEK Cells following Peganum Harmala Treatment

The MTT assay at 570 nm does not demonstrate cytotoxicity of HEK cell lines after treatment with Peganum harmala, demonstrating the safety of the Hermal plant extract.

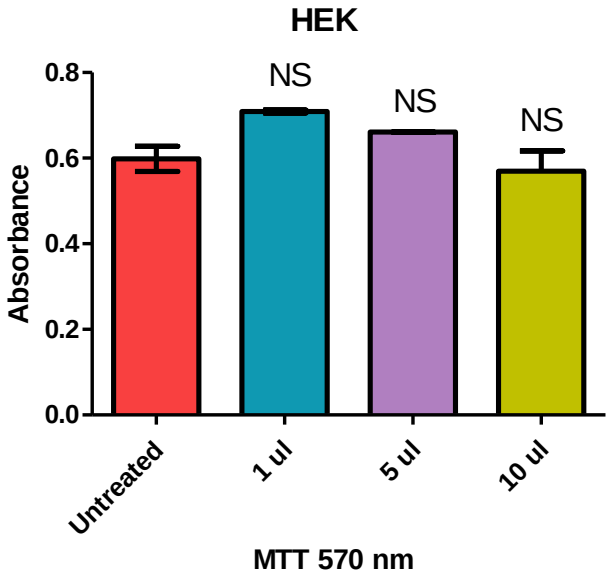

Figure 4.1: The graph shows decreased cytotoxicity of HEK cells after treatment with Peganum harmala. NS shows that there is statistical insignificance in the results (P > 0.05).

The mean ± SEM is used to express the values.

Table 4.1: The cell viability values mean ±SEM of HEK cell line obtained after trypan blue assay.

	Untreated	**1 ul**	**5 ul**	**10 ul**
ABSORBANCE	0.60 ± 0.030	0.71±0.00 33	0.66±0.0 058	0.57±0.047

4.2 Peganum harmala's capacity for wound healing

The pictures below shows the result of wound index in 6 different groups. A shows non-injured rat, then B shows injured rat with no treatment, C shows placebo 50 mg/ml of saline water (Placebo 1) , D shows treated rats with 50 mg/ml of hermal extract (treatment 1), E shows placebo 100 mg/ml (Placebo 1) and F shows 100 mg/ml of hermal extract (treatment 2). It demonstrates how the recovery was speedy when aqeous plant extract was given as compare to placebo and non-treated.

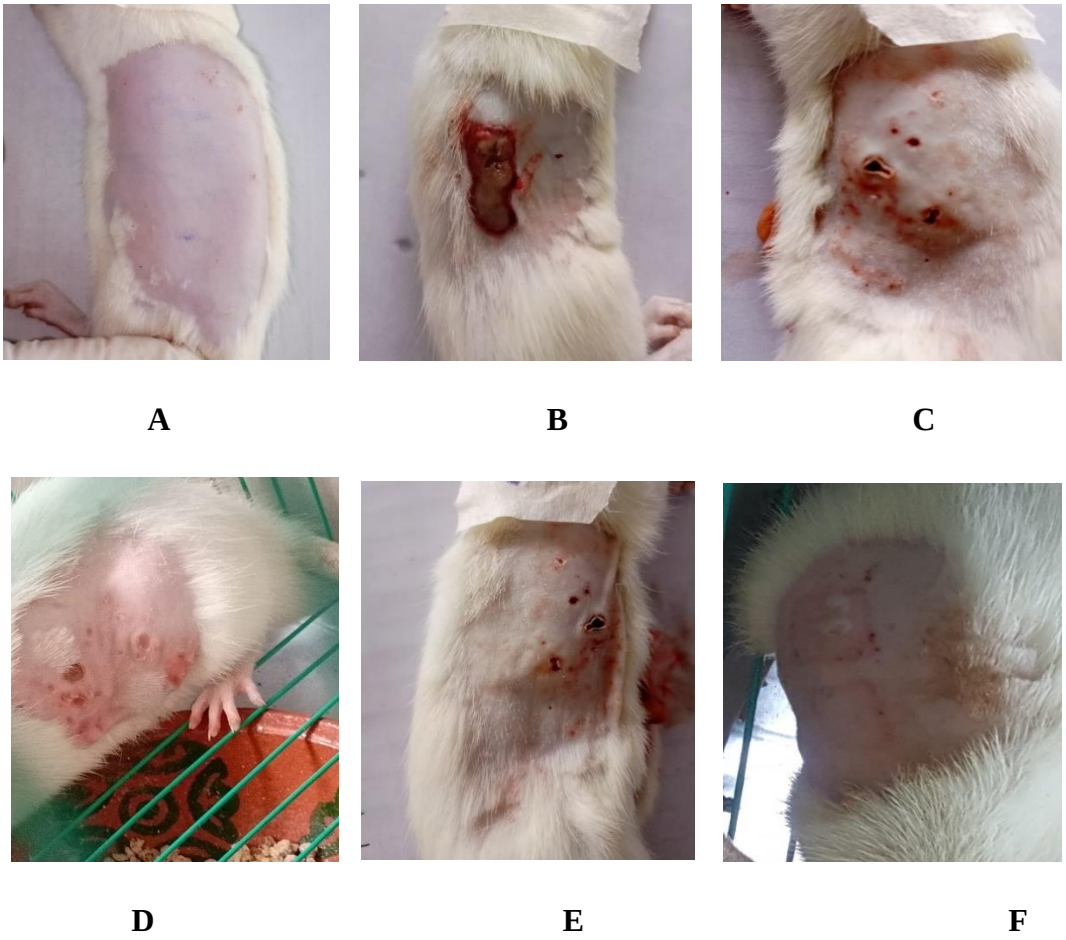

Figure 4.2: A shows non-injured rat, B shows injured non-treated, C shows placebo 1, D shows treated rats 1, E shows placebo 2 and F shows treated 2

The values for wound index were calculated by tracing transperancy sheets and rsults were concluded. graph data demonstrates that the groups of rats given the Peganum harmala plant extract exhibit significant wound index values as compared to uninjured groups, and the outcomes were calculated using one-way ANOVA in graph pad.

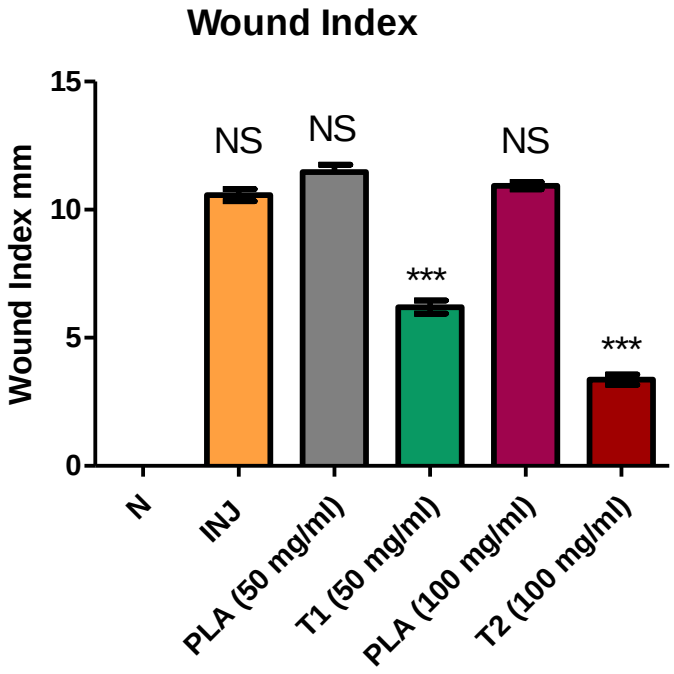

Figure 4.3: Compared to untreated groups of rats, those given specific doses of Peganum Harmala experience lower wound index levels. Results that are highly statistically significant (P 0.01) are shown by the asterisk symbol. The values are expressed using the mean ± SEM.

	Normal	**Injured**	**Placebo 50mg/ml**	**Treated 50mg/ml**	**Placebo 100mg/ml**	**Treated 100mg/ml**
WOUND INDEX	0.00±0.00	11±0.23	11±0.29	6.2±0.25	11±0.15	3.4±0.20

Table 4.2 Wound Index Levels mean ±SEM values.

4.3 Enhanced Angiogenesis following Peganum Harmal Treatment

The graph data demonstrates that the groups of rats given the Peganum plant extract exhibit significant levels of VEGF when compared to the wounded groups, and the findings were calculated using one-way ANOVA in graph pad.

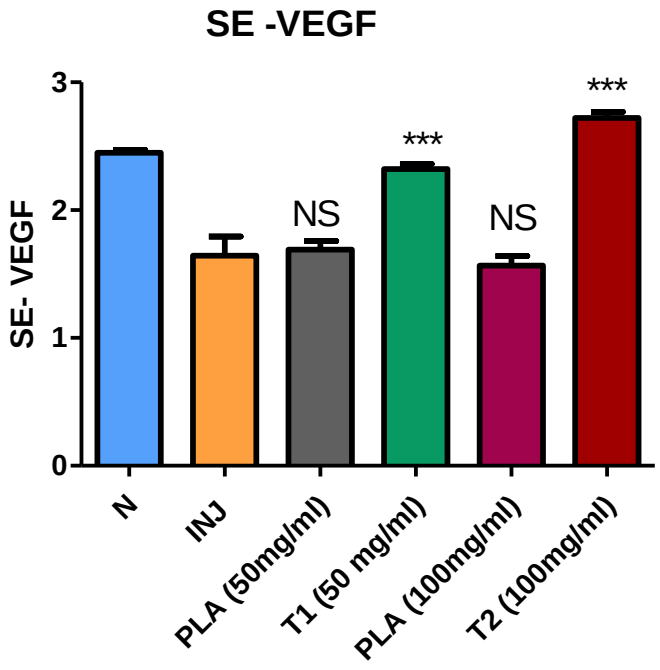

Figure 4.4: VEGF levels in treated groups of rats with selected groups of Peganum harmala extracts increases as compared to injured groups of rats. Asterisk symbol * indicates that the results are highly significant (P < 0.001). The mean ± SEM is used to express the values.**

Table 4.3 VEGF level measured via ELISA mean ±SEM values.

Normal	Injured	Placebo 50mg/ml	Treated 50mg/ml	Placebo 100mg/ml	Treated 100mg/ml
2.4±0.023	2.2±0.019	2.0±0.040	1.8±0.12	2.1±0.047	1.7±0.015

4.4 Decreased Apoptosis following Treatment with Peganum harmala

The graph data demonstrates that the rat groups given the Peganum harmala plant extract have lower annexin-V values than the wounded groups, and the outcomes were calculated using one-way ANOVA in graph pad.

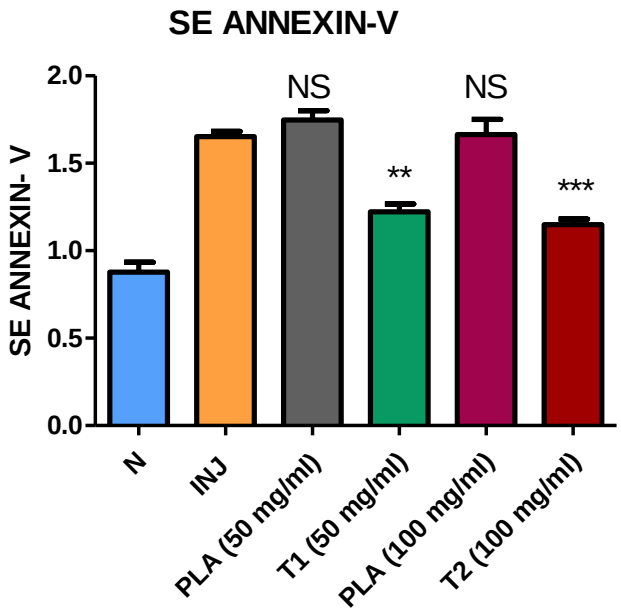

Figure 4.5: Annexin levels in treated group of rats with selected doses of Peganum harmala extract decreased as compared to injured group of rats. NS shows that there is statistical insignificance in the results (P > 0.05). The symbol * denotes statistical significant in results (P < 0.05). The mean ± SEM is used to express the values.

Table 4.4 Annexin level measured via ELISA mean ±SEM values.

Normal	Injured	Placebo 50mg/ml	Treated 50mg/ml	Placebo 100mg/ml	Treated 100mg/ml
1.2±0.043	1.7±0.0029	1.7±0.52	1.2±0.046	1.7±0.087	1.1±0.031

4.5 Antioxidant analysis

4.5.1 Estimation of APOX

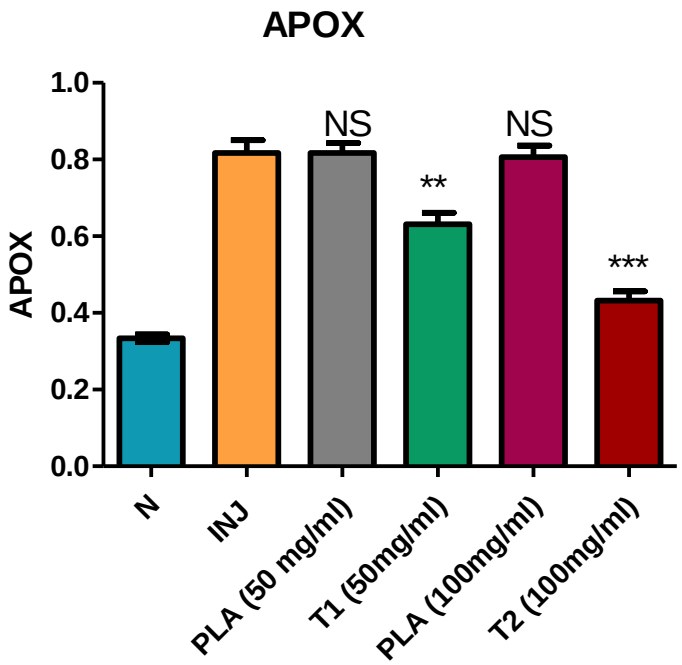

Figure 4.6 Apox levels in injury vs treated groups of rats with selected doses of Peganum harmala plant extracts. Asterick symbols * indicates that the results are highly significant (P < 0.001). The mean ± SEM is used to express the values.**

Table 4.5 Apox levels mean ±SEM values.

	Normal	**Injured**	**Placebo 50mg/ml**	**Treated 50mg/ml**	**Placebo 100mg/ml**	**Treated 100mg/ml**
APOX	0.33±0.010	0.82±0.073	0.82±0.026	0.63±0.030	0.81±0.030	0.43±0.024

4.5.2 Estimation of SOD

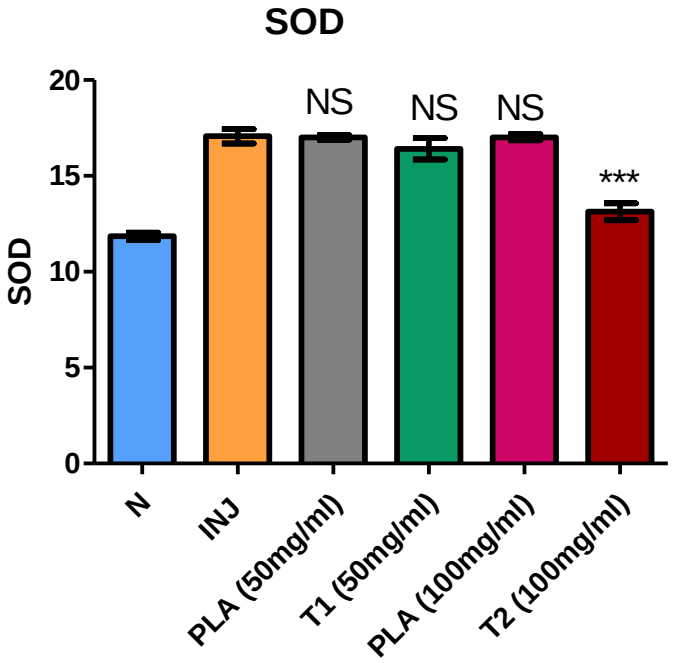

Figure 4.7: Apox levels in injury vs treated groups of rats with selected doses of Peganum harmala plant extracts. Asterick symbols * indicates that the results are highly significant (P < 0.001). The mean ± SEM is used to express the values.**

Table 4.6. SOD levels mean ±SEM values.

	Normal	**Injured**	**Placebo 50mg/ml**	**Treated 50mg/ml**	**Placebo 100mg/ml**	**Treated 100mg/ml**
SOD	12±0.20	17±0.038	17±0.12	16±0.56	17±0.16	13±0.44

4.5.3 GSH

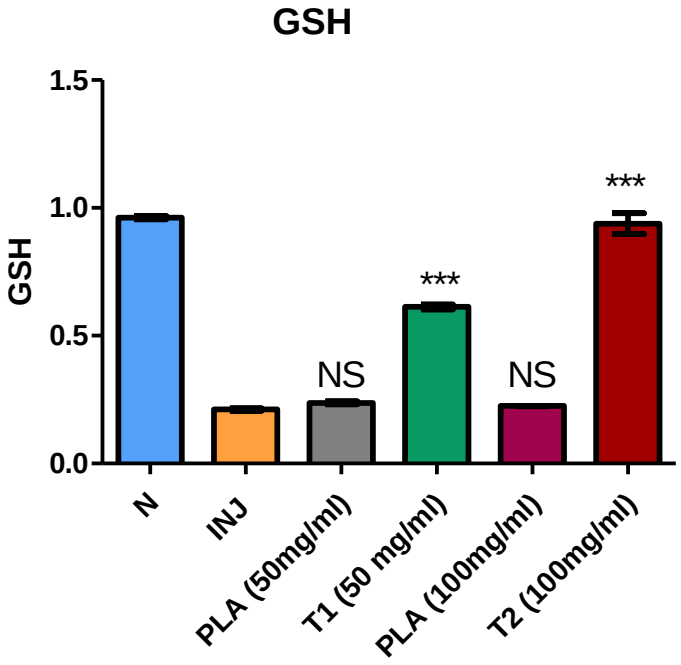

Figure 4.8: GSH levels in injury vs treated groups of rats with selected doses of Peganum harmala. Asterick symbols indicates that the results are highly significant (P < 0.001). The mean ± SEM is used to express the values.

Table4.7. GSH levels mean ±SEM values.

	Normal	Injured	Placebo 500mg/ml	Treated 50mg/ml	Placebo 100mg/ml	Treated 100mg/ml
GSH	0.96±0.0060	0.21±0.0043	0.24±0.0052	0.61±0.0097	0.23±0.008	0.94±0.041

4.5.4 CAT

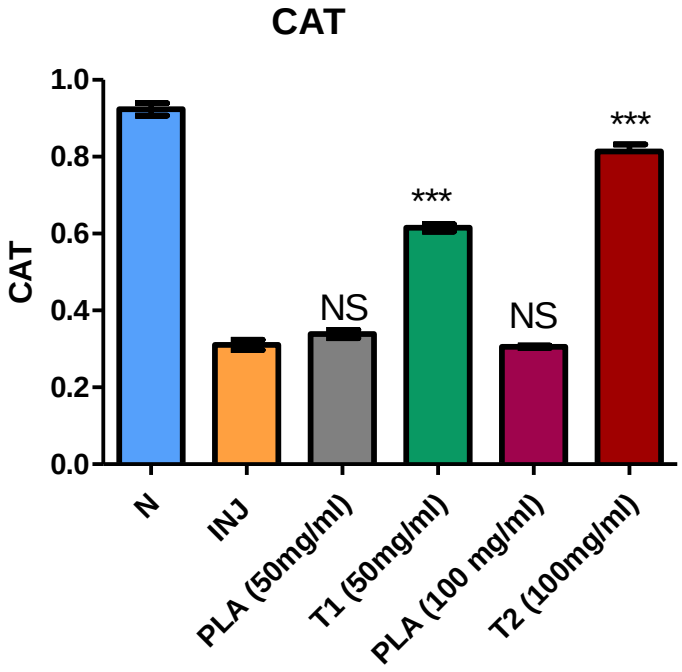

Figure 4.9: CAT levels in treated groups of rats with selected doses of Peganum harmala. Asterick symbols indicates that the results are highly significant (P < 0.001). The mean ± SEM is used to express the values

Table 4.8. CAT levels mean ±SEM values.

	Normal	**Injured**	**Placebo 50mg/ml**	**Treated 50mg/ml**	**Placebo 100mg/ml**	**Treated 100mg/ml**
CAT	0.92±0.016	0.31±0.013	0.34±0.0011	0.62±0.0092	0.31±0.0034	0.81±0.019

4.6 HISTOPATHOLOGY

4.6.1 GROUP 1: NORMAL GROUP

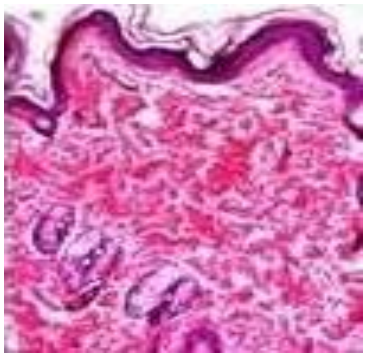

Figure. 4.10: Histopathological diagram of normal skin obtained from rats

The epidermis and dermis are the two primary layers of skin in the diagram. The hypodermis, a subcutaneous fascia deep in the dermis, is present. Thickness of the skin is normal. So, this shows that in normal group of skin the architecture of skin is normal.

4.6.2 GROUP 2: INJURY GROUP

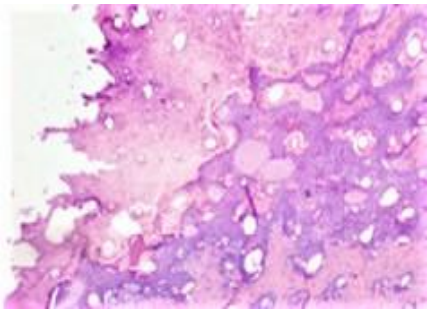

Figure. 4.11: Histopathological diagram of injured skin obtained from rats

A burn wound's physiology is characterised by an inflammatory response that triggers the rapid formation of oedema due to increased microvascular sensitivity, vessel dilatability, and extravascular osmotic activity. The little dots in the figure above depict the inflammatory cells that are integrated in burn skin. The figure also illustrates that the skin thickness has been weakened. The structure of the skin is completely damaged.

4.6.3 GROUP 3: PLACEBO 1

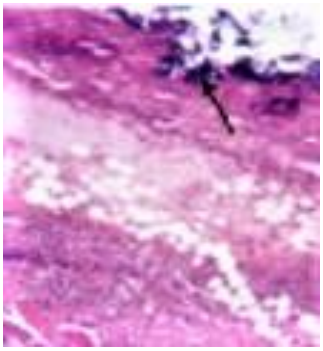

Figure 4.12: Histopathological diagram of skin obtained from placebo group 50 mg/ml of rats

The diagram shows a burn wound, which exhibits an inflammatory response and fast oedema production. Skin thickness is compromised. There is evidence of inflammatory cell infiltration.

4.6.4 GROUP 4: TREATED 1

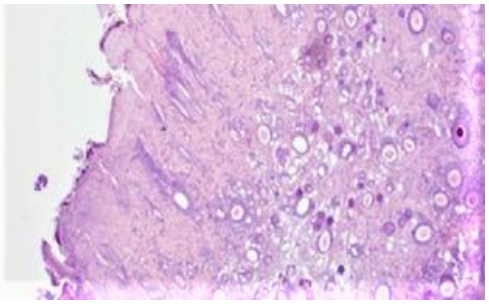

Figure 4.13: Histopathological diagram of skin obtained from treated group 50 mg/ml of rats

In the diagram above, using 50 mg of pomegranate peel powder had a limited impact on wound healing. There is a little infiltration of inflammatory cells. The healing process has begun.

4.6.5 GROUP 5: PLACEBO 2

Figure 4.14: Histopathological diagram of skin obtained from placebo group 100 mg/ml

The physiology of a burn wound is characterised by an inflammatory response that causes extravascular osmotic activity, enhanced microvascular sensitivity, and fast oedema production. 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 skin's entire structure is damaged.

4.6.6 GROUP 6: TREATED 2

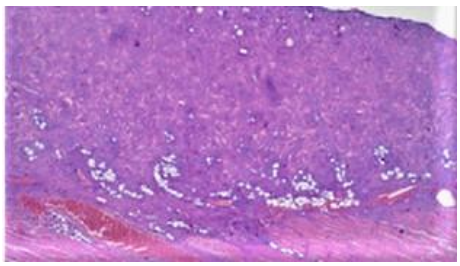

Figure 4.15: Histopathological diagram of skin obtained from treated group of 100 mg/ml of extract

Histopathology of rat’s skin treated with treatment 2 (1 ml plant aqueous extract) shows the morphology is really near to being normal. The skin's structure is supple. Rapid burn healing is ensured by the release of several substances by dendritic cells, which speed up early cell proliferation. Therefore, drugs that enhance dendritic cell function are regarded as drugs for burn wound treatment. The skin's whole framework is built.

CHAPTER V

DISCUSSION

P. harmala has pharmacological activities that include analgesic and anti-inflammatory properties, antinociceptive effects wound healing properties, anticancer, cytoprotective action, leukaemia healing, and a hypoglycemic effect. Additionally, this plant reportedly contains antiviral, antifungal, and antibacterial properties. The cytotoxicity and wound healing property of *Peganum harmala* were studied further by using MTT assay, wound index measurement, Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) Protocol, estimation of antioxidants and Histopathological testing at different doses of aqueous extracts of *Peganum harmala (60).* The assay for the recognition of cytotoxicity of *Peganum harmala* plant extract (MTT assay at 570 nm) showed the decreased cytotoxicity or enhanced protectivity of *Peganum harmala.*

Damage or disruption to the typical anatomical structure and function is referred to as a wound. Normal wound healing is a dynamic, intricate, and occasionally lengthy process, but there are various herbal formulations that can speed up wound healing and be helpful in its treatment (120).

A burn wound's physiology is characterised by an inflammatory response that triggers the rapid formation of oedema due to increased microvascular sensitivity, vessel dilatability, and extravascular osmotic activity (121).

ELISA assay was conducted and VEGF which is a wound healing agent is synthesised at the site of the injury. With higher VEGF levels in the treated group of rats given Hermal feed are compared to the untreated group, it is evident that the findings are highly significant ($P < 0.001$). The sandwich elisa results of ANNEXIN-V, which is a marker for apoptosis revealed that the level of apoptosis is decreased with treatment of plant extract of *Peganum harmala (122)*.

The *Peganum harmala* plant's capacity to heal wounds was demonstrated by the wound index measurement. One-way ANOVA was used to evaluate the results, which showed that the groups of rats given the *Peganum harmala* plant extract demonstrated significant wound index (reduction) values when compared to the wounded untreated groups. The (***) asterisk symbol denotes high statistical significant in results ($P < 0.01$).

Estimates were made for the antioxidant assays, including APOX, GSH, CAT, and SOD. By utilising one-way ANOVA in GraphPad, the APOX levels, GSH levels, CAT levels, and SOD levels in the injured vs. treated group of rats with specific doses of *Peganum harmala* reveal (***) that results are very significant (P 0.001).

The two major layers of skin are the epidermis and dermis, with the hypodermis acting as a subcutaneous fascia deep within the dermis. The epidermis is mostly made up of four to five layers of cells, most of which are keratinocytes, and three less abundant cells. The histopathology findings of the rats in the normal group revealed that the skin's glands are healthy. A burn wound's physiology is characterised by an inflammatory response that produces oedema quickly due to increased microvascular sensitivity, dilated vessels, and extravascular osmotic activity. The histopathological findings of the wounded group of rats demonstrated inflammation, impaired skin thickness, and inflammatory cells that were integrated into burn skin. The structure of the skin is completely damaged. Skins treated with placebo 1 and 2 demonstrated the same damaged skin structures. According to the histopathological results, the skin thickness of the treated group of rats is being reconstructed, and the entire structure of the skin is gradually returning to normal (123).

CONCLUSION

Peganum Harmala extracts demonstrated potential cytoprotective and wound-healing abilities in various experiments. According to preliminary cytotoxicity testing of cell lines by MTT assay it was concluded that P.harmala is not cytotoxic. The evaluation of the potential for wound healing at various doses of Peganum Harmala extracts used wound index assessment, sandwich enzyme-linked immunosorbent assay (ELISA) protocol, antioxidant estimation, and histopathological test. When extracts were administered in a certain amount, the level of vascular endothelial growth factor increased, indicating the possibility for optimum wound healing (VEGF). The results were good at low quantities but significantly better at high quantities. The demonstration extracts cytoprotective and wound healing abilities of Peganum Harmala extracts opens a new door for future research into the treatment of acid burn injuries and other wound treatments.

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