

Wound Healing Activity of *Alari* Flower (*Nerium oleander linn*) Powder on Excised Wound in Wistar Albino Rats– A Five Arm Randomized Controlled Study

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Abstract: Wound healing is a dynamic and intricate biological process that plays a pivotal role in tissue repair and regeneration. There are several topical medications used for wound healing. Anyhow their efficacy is challenged because of development of bacterial resistant. This study aimed to evaluate the wound healing efficacy on excised wound with *Nerium oleander* flower powder in Wistar albino rats. Study was conducted by using, healthy albino rats. They were grouped into negative Control, positive control I, positive control II, Test Drug I and Test Drug II. Groups with each comprising of six rats. A wound size of 120mm² was inflicted on the dorsolateral aspect of the thorax of the rats. Negative Control group left untreated, positive control I group received Neosporin powder, Test Drug 1 received *Nerium oleander* flower powder, positive control II received 5 % Neosporin ointment, Test Drug II received *Nerium oleander* flower powder mixed with ointment base. Until the 14th day, the wound's size was measured once every two days. The proportion of the wound contraction was calculated as a percentage change in the original size of the wound across different days. Based on the results of the Bates Jensen Wound Assessment tool, the parameters of the wound edges, exudate type, exudate amount, and epithelialization are used for the overall evaluation of the wound. One-way ANOVA in SPSS was used for the statistical analysis.

Results shows value < 0.05 with 95% of confidence interval was noted in all Test groups. Test Drug 2 shows 83% of wound contraction, and Test Drug 1 shows 74% contraction. Positive control I shows 80% and positive control II shows 85%. Negative Control group shows 55% of wound contraction. Further, Test groups establishes a good grip on the wound edges, early reduction of exudates amounts and causing shorter epithelialization time in the wounds compare than Standard group. It is concluded that flower powder of *Nerium oleander* is moderately significant, and ointment form has highly significant wound healing effect in excised wounds.

Keywords: Anti-Inflammatory, Anti-Microbial, Epithelialization, Exudate, Wound Healing.

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I. INTRODUCTION

Siddha Medicine is an ancient traditional healthcare system that has its roots in the Indian subcontinent. With a history spanning thousands of years, Siddha medicine is considered one of the world's oldest healing systems. It is deeply rooted in the ancient Indian philosophy and draws inspiration from various ancient texts, including the Siddha manuscripts and the teachings of Tamil sages. Siddha medicine utilizes a wide range of natural remedies, including

herbs, minerals, and animal products, to treat various ailments and promote overall wellness. (Yogeshwari, *et al.*,2022)

Plants or chemical entities derived from plants need to be identified and formulated for treatment and management of wounds. In this direction, a number of herbal products are being investigated at present. Various herbal products have been used in management and treatment of wounds over the years. *Nerium oleander* is an evergreen shrub or small tree in the dogbane family Apocynaceae. It is commonly known as

oleander but has many other names like *Nerium indicum* mill. And *Nerium odorum* soland. It bears flowers in clusters with white, pink, yellow and red colours. It contains plumericin, alpha-amyrin, beta-sitosterol, kaempferol, cardioactive glycosides named Odorosides A-H obtained from the root bark. Leaves contain the cardiac glycosides kaneroside, Neriumoside, digitoxigenin, alpha -L-olendroside -5 α -adynerin and other glycosides. (Chaudri, *et al.*, 2014)

Oleander is a plant which is used to make medicine for over 2000 years. Historically, oleander flowers have been used for skin infections, rashes, and wounds. Herbal products have the immense potential for the management and treatment of wounds without adverse effects. These natural agents induce healing and regeneration of lost tissue by multiple mechanisms. However, there is a need for scientific validation, standardization and safety evaluation of plants of the traditional medicine before these could be recommended for proper usage. (Chaudry, *et al.*, 2014). Its ethnomedicinal uses include treatment of diverse ailments such as heart failure, asthma, corns, cancer, diabetes, and epilepsy. Less well appreciated are the skin care benefits of extracts of *Nerium oleander* that include antibacterial, antiviral, immune, and even antitumor properties associated with topical use. (Kathleen, *et al.*, 2022)

A wound is a break in the epithelial integrity of the skin or may also be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissue. According to the Wound Healing Society, wounds are physical injuries that result in an opening or break of the skin that cause disturbance in the normal skin anatomy and function. They result in the loss of continuity of epithelium with or without the loss of underlying connective tissue (Strodtbeck, 2001). Wounds are a common medical problem affecting millions of individuals worldwide. They can result from injuries, surgical procedures, or chronic conditions like diabetes. Delayed or ineffective wound healing can lead to infections, complications, and significant suffering. Advancements in wound healing research can directly improve the quality of life for countless people. (Timothy, *et al.*, 2015)

Healing of wounds starts from the moment of injury and can continue for varying periods of time depending on the extent of wounding. The process can be broadly categorized into three stages; inflammatory phase, proliferative phase, final the remodeling phase which ultimately determines the strength and appearance of the healed tissue (Sridhar, 2014). Collagen is a crucial protein involved in wound healing, as it provides structural support to the newly formed tissue. Some studies suggest that oleander extracts may stimulate collagen production, which could accelerate the healing of wounds and the closure of tissue gaps. Oleander has been reported to exhibit antimicrobial properties, which can be beneficial in preventing wound infections. By reducing the risk of microbial contamination, oleander extracts may support the natural healing process. (Heather, *et al.*, 2023)

Current estimates indicate the worldwide nearly 6 million people suffer from chronic wounds. Unhealed wounds constantly produce inflammatory mediators that

produce pain and swelling at the wound site. Wounds are a substrate for infection and prolong the recovery of injured patients. Chronic wounds may even lead to multiple organ failure or death of the patients. Wounds are the physical injuries that result in an opening or breaking of the skin and appropriate method for healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin (Roberts, 1998). Plant based drugs have been a part of the evolution of human, healthcare for thousands of years.

In present era, number of drugs are developed from plant which are active against a number of diseases. A survey conducted by the WHO reports that more than 80% of the world's population still depends upon the traditional medicines for various diseases (Gulzar, *et al.*, 2011). The siddha stanza mentioned in the textbook of Kuna Padam states the wound healing property of *Nerium oleander* flower which has not been proven by any scientific studies. Therefore, the scientific experimental studies of wound healing property of *Nerium oleander* in Wistar albino rats will be help to discover the new cost effective and sustainable topical wound healing agent in medical practices which is more congruent with the own values, beliefs, and philosophical orientations toward health and life.

II. METHODOLOGY

➤ Study Design

A five-arm randomized controlled animal experimental study

➤ Selection of Plant Material

Nerium oleander Linn. (“Alari”) was selected based on its general characteristics described in the reputed Kunapadam text (Dr. K.S. Murugesamuthaliyar, 1990). Fresh red-colored flowers were collected from the Nilaveli area, Sri Lanka. The collected plant material was authenticated by the Head, Department of Gunapadam, Faculty of Siddha Medicine, Trincomalee Campus, Eastern University, Sri Lanka.

➤ Preparation of Test Drug

Freshly collected flowers were cleaned to remove debris and washed thoroughly with tap water. The flowers were chopped and boiled in cow's milk for one hour, followed by shade drying for seven days. The dried flowers were powdered using a mechanical grinder. Fine powder was obtained through the Vasthirakayam process and stored in an airtight labeled container. For ointment preparation, 10 g of flower powder was uniformly mixed with 100 g of ointment base.

➤ Study Population

Thirty healthy Wistar albino rats of either sex weighing 150–200 g were procured from the Medical Research Institute (MRI) animal house. Well-being animals with normal behavior and body weight within the specified range were included in the study. Diseased animals, rats outside the weight range, or those exhibiting abnormal behavior were excluded.

Animals were housed in standard polypropylene cages with sterilized wood chip bedding under controlled laboratory conditions (temperature $22 \pm 2^\circ\text{C}$, relative humidity 50–60%, and 12-hour light/dark cycle). Rats were provided standard pellet feed and water ad libitum. Animals were acclimatized for one week prior to the commencement of the experiment.

➤ Randomization

Animals were randomly allocated into five experimental groups using a computer-generated randomization sequence. Each rat was assigned a unique identification number to minimize allocation bias and ensure equal probability of group assignment.

➤ Grouping of Animals

Animals were divided into five groups:

- Group I (Negative Control): Left untreated
- Group II (Positive Control I): Treated with 10% Neosporin powder (Ikobi et al., 2012)
- Group III (Positive Control II): Treated with 5% Neosporin ointment
- Group IV (Test Group I): Treated with *Nerium oleander* flower powder
- Group V (Test Group II): Treated with *Nerium oleander* flower ointment

$$\frac{\text{wound area on 1st day} - \text{wound area on day (n)}}{\text{a}^{\text{n}}\text{wound area on 1st day}} \times 100$$

The epithelialization period was defined as the number of days required for complete shedding of the scab without residual raw wound (Demilew et al., 2018). Wound characteristics including wound edges, exudate type, surrounding skin coloration, and necrotic tissue were evaluated and converted into Bates-Jensen Wound Assessment Tool scores (Ehab et al., 2018).

➤ Welfare Diary & Monitoring

Animals were observed daily for food and water intake, behavioral changes, physical appearance, and signs of distress. All observations were recorded in a welfare diary maintained throughout the study.

➤ Humane Endpoints

Animals were humanely euthanized prior to study completion if they exhibited severe distress, $\geq 20\%$ body weight loss, inability to access food or water, or significant wound complications.

➤ Euthanasia

At the conclusion of the experimental procedures, animals were euthanized using Ether overdose ($>5\%$) followed by cervical dislocation as a secondary confirmatory

➤ Experimental Procedure

All surgical procedures were performed under sterile conditions using ketamine anesthesia (120 mg/kg, intraperitoneal) as described by Nayak et al. (2009). The dorsal thoracic region of each rat was shaved, and full-thickness circular excision wounds measuring approximately 120 mm² were created using toothed forceps, scalpel, and pointed scissors following the standard excision wound model (Demilew et al., 2018).

Hemostasis was achieved by blotting the wound with sterile cotton swabs soaked in normal saline. The wounds were left open without suturing. Test drugs and standard drugs were applied topically once every two days starting from the day of wound creation and continued until complete epithelialization, up to 14 days.

➤ Outcome Measures

Wound contraction was assessed by tracing the wound margins on transparent paper on days 2, 4, 6, 8, 10, 12, and 14. The wound area was measured using 1 mm² graph paper as described by Demilew et al. (2018).

Percentage of wound contraction was calculated using:

method. Death was confirmed by the absence of respiration, heartbeat, and pedal withdrawal reflex.

➤ Risk-Benefit Assessment

The study was ethically justified based on its potential scientific benefits, including validation of traditional Siddha medicinal claims and development of plant-based therapeutic agents. Potential risks to animal welfare were minimized through adherence to the 3Rs principles, use of anesthesia, continuous monitoring, and predefined humane endpoints.

➤ Statistical Analysis

Data were entered and analyzed using IBM SPSS Version 20. Statistical analysis was performed using descriptive statistics, one-way Analysis of Variance (ANOVA), and Tukey-Kramer multiple comparison test as recommended in similar experimental studies (Masuram et al., 2014). A p-value < 0.05 was considered statistically significant.

➤ Confidentiality & Data Management

All experimental data were recorded using standardized formats, securely stored, and maintained in accordance with institutional research policies to ensure data integrity and traceability.

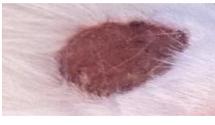
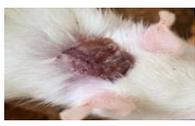
III. RESULTS

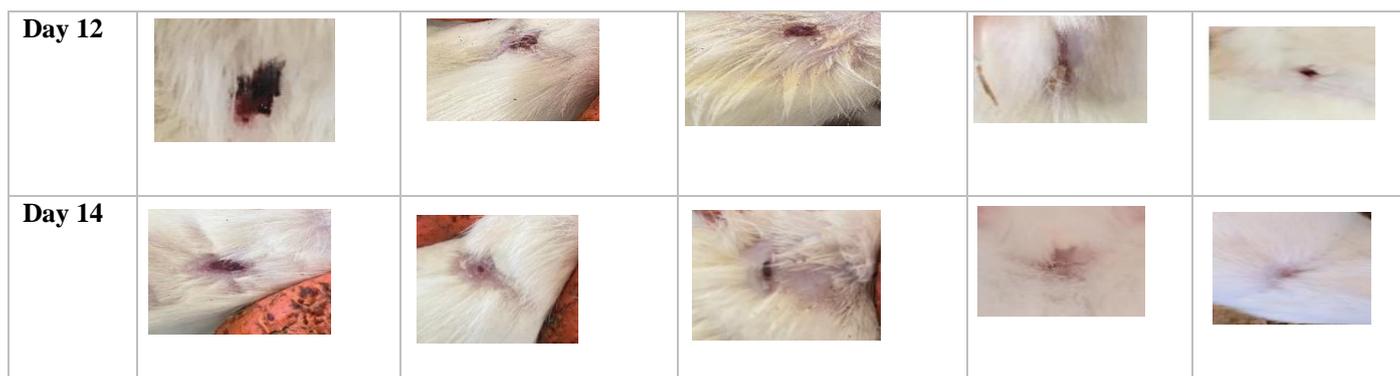
Table 1 Mean Wound Circumference in Day 00 and Day 14 Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Negative_Control_Day_00_Wound_Circumference	119.0000	6	1.26491	.51640
	Negative_Control_Day_14_Wound_Circumference	36.3333	6	4.88535	1.99444
Pair 2	Positive_control_I_Day_00_Wound_Circumference	119.5000	6	1.37840	.56273
	Positive_control_I_Day_14_Wound_Circumference	23.5000	6	2.16795	.88506
Pair 3	Positive_control_II_Day_00_Wound_Circumference	119.8333	6	1.60208	.65405
	Positive_control_II_Day_14_Wound_Circumference	11.5000	6	1.87083	.76376
Pair 4	Test_Drug_I_Day_00_Wound_Circumference	119.0000	6	1.09545	.44721
	Test_Drug_I_Day_14_Wound_Circumference	19.6667	6	2.16025	.88192
Pair 5	Test_Drug_II_Day_00_Wound_Circumference	119.2000	6	1.09545	.48990
	Test_Drug_II_Day_14_Wound_Circumference	13.4000	6	3.28634	1.46969

Analyzed by: SPSS IBM 20 version Paired Sample t-test

Table 2 Gross Assessment of Wounds

Day	Negative Control	Positive Control I	Positive Control II	Test I	Test II
Day 00					
Day 02					
Day 04					
Day 06					
Day 08					
Day 10					



IV. DISCUSSION

The wound healing undergoes tissue repair and regeneration proceeds immediately following an injury. The wound healing process consist of mainly four phases such as hemostasis, inflammation, proliferation and remodeling. The natural chemical components expose anti-microbial, anti-inflammatory, antioxidant, analgesic and vasodilator activities actions mainly which are facilitating the wound healing in natural ways. When a wound occurs, whether it's a

small cut or a surgical incision, the body's first response is to defend against infection. Inflammation helps to activate the immune system and recruit immune cells like neutrophils and macrophages to the site of the wound. These cells help to clear away any potential pathogens, preventing infection. The balance between proinflammatory and anti-inflammatory processes is critical for efficient wound healing (Michel, 2010). This study poses the *Nerium oleander* flower powder in the topical application for wound healing.

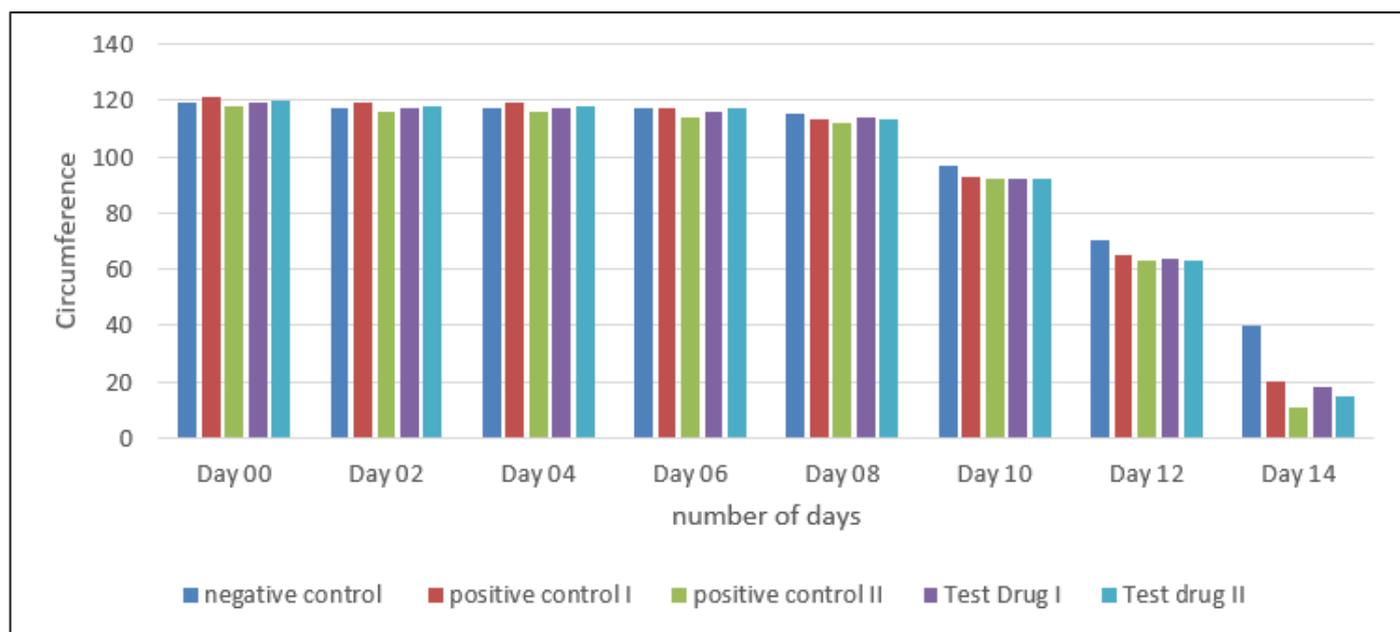


Fig 1 Mean Wound Circumference Among with the Number of Days

Figure 1 indicates, mean wound circumference in day 00, day 02, day 04, day 06, day 08, day 10, day 12 and day 14 among the groups. on the day 00 the mean wound circumference was nearly 120mm² in all groups. on the day 02, all the groups show reduction in wound circumference. In Negative control group until day 06 wound circumference did not change. From day 08, it was gradually reduced and reached 40mm² at the end of the study. In Positive control I group wound circumference was gradually reduced and reached 20mm² at the end of the study. In Positive control II wound circumference from day 02 and reached 11mm² at the day 14. In Test Drug I, Test Drug II groups shows fast reduction of wound circumference at shows 18mm², 15mm² at the end of the study respectively.

The test compounds like crude methanolic extract (both concentrations) and different fractions like chloroform, methanol and ethyl acetate of nerium oleander flower shows significantly increase in tensile strength and the rate of wound contraction compared to the control and nitrofurazone. Antimicrobial activities were evaluated against seven microorganisms namely. *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *A. Baumannii*, *Candida albicans*, *aspergilus Niger*. The different extracts and fractions of nerium oleander flower showed remarkable antioxidant activity which facilitates early wound healing among the groups (Rout *et al.*, 2014).

Antibacterial substances, such as antibiotics or antiseptics, are used to kill or inhibit the growth of bacteria that can enter the wound. Bacterial infections often lead to increased inflammation at the wound site. This can delay the healing process and cause more pain and discomfort for the patient. By controlling bacterial growth, antibacterial agents

help reduce inflammation, promoting a more favorable environment for wound healing. Thus, the flower powder of nerium oleander shows a marked anti-bacterial activity against gram-negative and gram-positive bacteria which supports the wound healing (Yousra Shafiq *et al.*,2021).

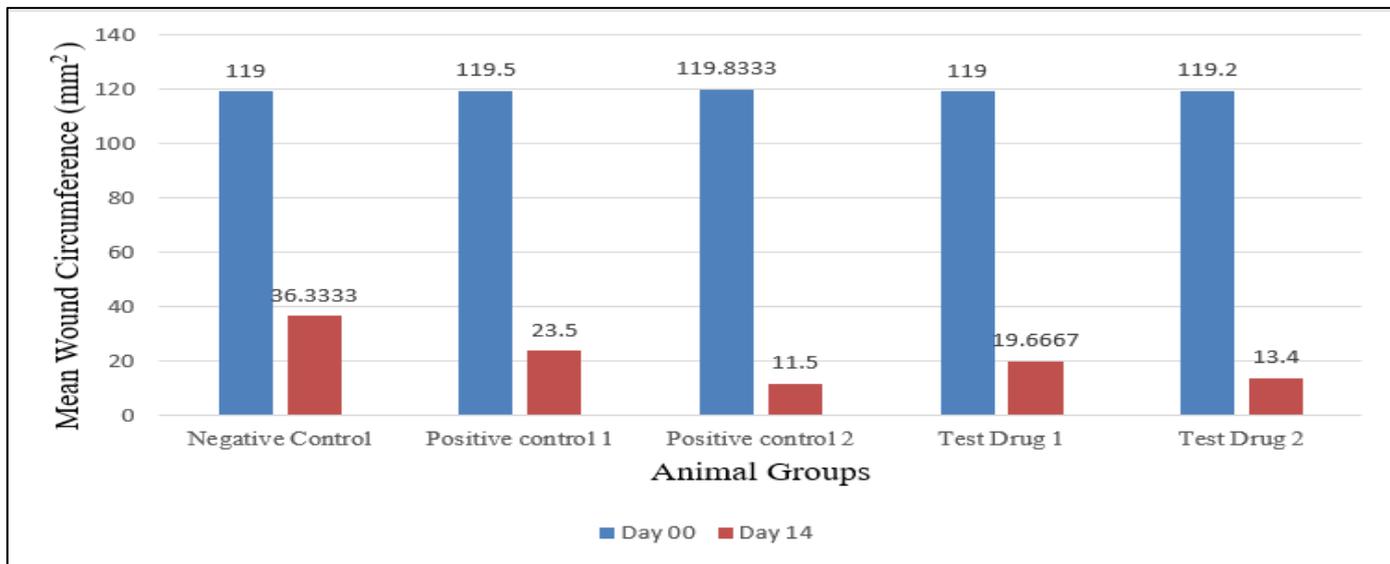


Fig 2 Mean Wound Circumference in Day 00 and Day 14 Among Animal Groups

Figure 2 indicates, mean wound circumference in day 00 and day 14 between the animal groups. At the day 00 the mean wound circumference was nearly 120mm² in all groups. At the end of the study, it was reduced to 36mm², 23.5mm², 11.55mm², 19.7 mm², 13.4mm² in the groups of Negative control, Positive control I, Positive control II, Test Drug I and Test Drug II respectively.

Anti-mycotic activity of the ethanol extracts from Nerium Oleander floral parts were screened in vitro against four important plant pathogenic fungi *Alternaria alternate*, *Fusarium oxysporum*, *Fusarium solani* and *Rizoctonia solani*

using agar dilution bioassay. Fungal infections can weaken the local immune response at the wound site. Fungal infections can inhibit the formation of granulation tissue, a crucial step in wound healing. Granulation tissue provides a foundation for new blood vessels and collagen formation. Nerium oleander flower can support collagen production by eliminating fungal pathogens, aiding in wound closure and scar formation. Further facilitate the development of healthy granulation tissue and promote epithelialization by removing fungal obstacles, leading to faster wound closure (Hadizadeh I, Peivastegan and Kolahi). Therefore, the Test Drug groups show highly fast wound healing than other groups.

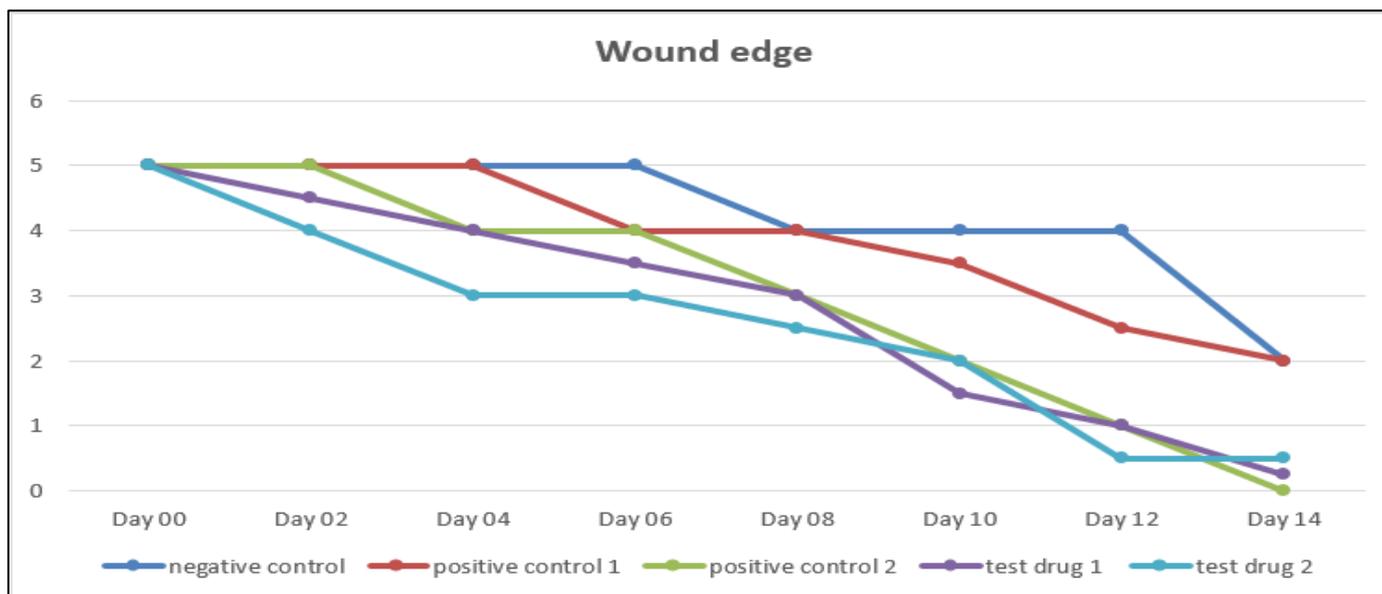


Fig 3 Relationship of Wound Edges with Number of Days Based on BJWAT

Before treatment all groups show well-defined fibrotic, scarred wound and it was scored as 5. Until 2nd day there was no changes in the wound edges in the groups except Test Drug II and Test Drug I. In the day 04 Positive control II and Test Drug II scored as 4, it was considered as well defined, not attached to base, rolled under. Test Drug II scored as 3 and showing well defined edges not attached to wound base. Negative control group shows until day 06 no changes in wound edges day 08 it turns to score 4 and day 14 showed score 2 which is distinct, outline clearly visible edges attached with wound base. Positive control I group also shows, score 2 at day 14. Positive control II group shows score 1 at day 12 and it considered as indistinct, diffuse none clearly visible wound edges. Test Drug I show at day 10 score 1, and Test Drug II shows at 12th day.

The most well-known effects of oleander are due neriin an alkaloid, oleandrin which shows, anti-inflammatory

action. Vitamin C is an essential, water-soluble vitamin which is present in nerium oleander flower it is known as a coenzyme and as an antioxidant. It plays a crucial role in wound healing by helping the body combat oxidative stress and inflammation, which are common components of the wound healing process. Which help promote collagen production. Collagen is a protein that is essential for the formation of new skin tissue and the repair of damaged skin. Adequate collagen synthesis is critical for wound closure and scar formation. It may help minimize scar formation by promoting healthy tissue regeneration and reducing the formation of hypertrophic scars or keloids. This enhanced oxygenation can facilitate the healing process by ensuring that cells have the necessary oxygen and nutrients to repair and regenerate. Flower extract of nerium oleander shows potent anti-oxidant activity in many researches. It could help to promote wound healing (İrem Atay Balkan, *et al.*, 2018).

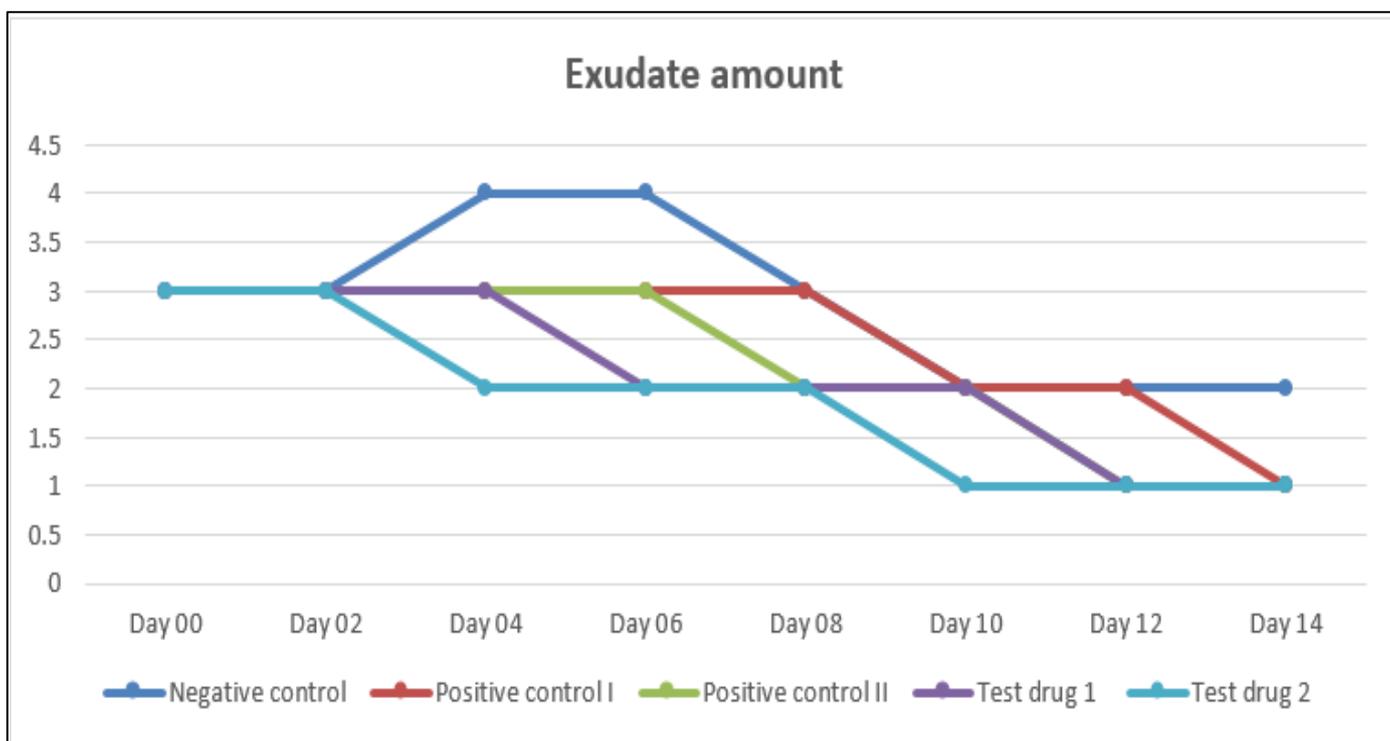


Fig 4 Relationship of Exudate Type with Number of Days Based on BJWAT

Before treatment all groups have been observed as 3 according to the score. It was considered as small amount of exudate excreted in the wounds. Until 2nd day no change in the exudate amount. Negative control group scored 4 on day 04 and it was considered as moderate amount of exudate was noted. In the 8th day it was scored 3 and from 10th day to until the end of the study it was scored 2 and it was considered as

scant, wound moist but no observable exudate. Positive control I showed score 2 from 10th day and at the end of study scored as 1 it was indicating dry wound. Positive control II was on 8th day scored to 2 on 12th day scored to 1. Test Drug I showed score 2 on 6th day and 1 on 12th day. Test Drug II showed score 2 on 4th day and on 10th day of the study.

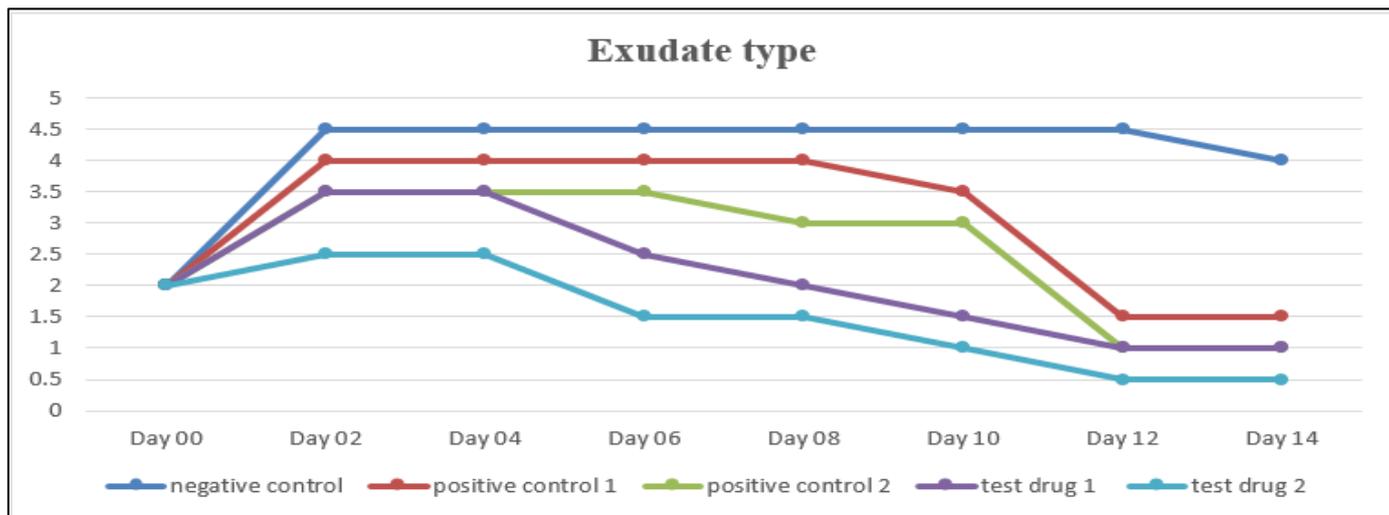


Fig 5 Relationship of Exudate Type with Number of Days Based on BJWAT

Before treatment all groups have been observed as 2 according to the score. Initially all wounds showed bloody discharge. At day 02 in groups of Negative control and Positive control I group scored 4, it was considered as serous, thin, watery discharge. In the groups, Positive control II and Test Drug I considered as score 3 which was serosanguineous, thin and watery discharge and Test Drug II scored 2 and considered as, bloody discharge. At 14th day, Negative control group shows Score 4, Positive control I group shows 1. Positive control II shows score 1 at 12th day. Test Drug I show score 1 at day 10 and Test Drug 2 shows score 1 in 6th day. Test Drug II showing fast wound healing rather than other groups.

Tannins in nerium oleander flower promote the wound healing through several cellular mechanism; chelation of the free radicals and reactive species of oxygen, promoting contraction of the wound and increasing the formation of capillary vessels and fibroblasts and including keratinocyte proliferation. The collagen composed of amino acid (hydroxyproline) is the major component of extra cellular tissue, which gives strength and support. Breakdown of collagen liberates free hydroxyproline and its peptides. On

the basis of the results finding in the present study shows nerium oleander flower produces highest wound healing activity and the animals treated with nerium oleander flower ointment showed highest wound healing potency treatment was continued up to 14th days (Kumar and Anand., 2010). Further, Ursolic acid in nerium oleander flower shows anti-bacterial and anti-fungal properties. Quercetin facilitating immunomodulatory actions which supports for wound healing (Zibbu and Batra., 2010).

The phytochemical screening and qualitative estimation of the plant studies showed that the flowers were rich in alkaloids, flavonoids, phenols and triterpenoids in all the extracts. Flavonoids are polyphenolic compounds and consist of flavones, flavanols, flavanols, flavanone and flavanonols. These compounds represent the majority of plant secondary metabolites and have shown to possess remarkable health promotory effects such as anti-inflammatory, antioxidant, antimicrobial and anticancer properties. Interception of free radicals or other reactive species is mainly by radical scavenging and is caused by various antioxidants like vitamin C and E, glutathione, other thiol compounds, carotenoids, flavonoids (Saranya *et al.*, 2017).

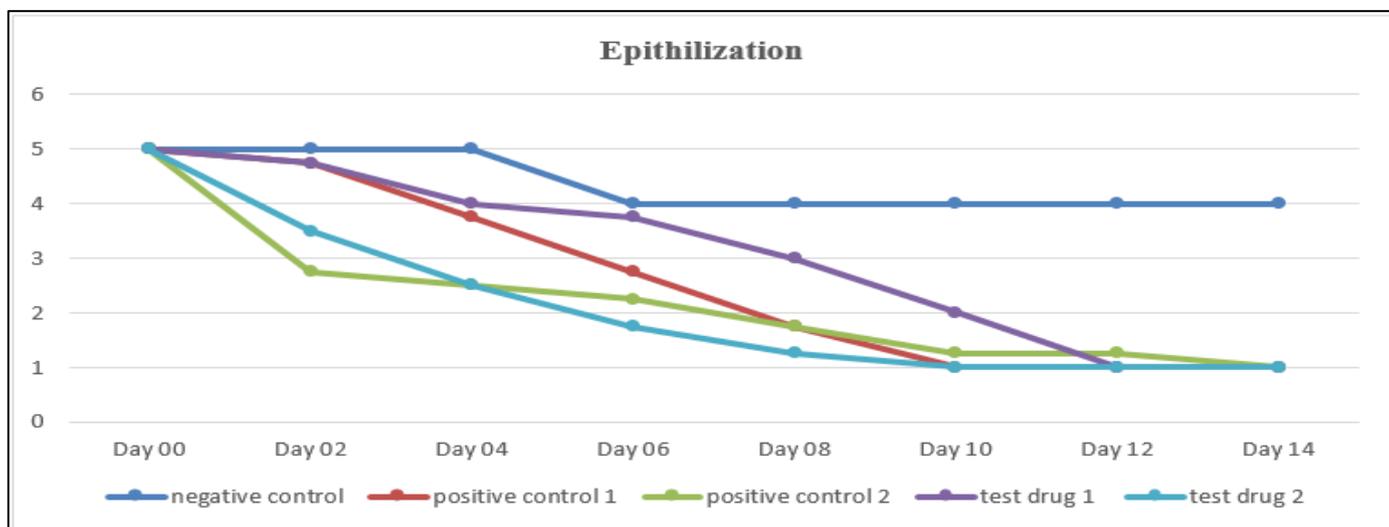


Fig 6 Relationship of Epithelialization with Number of Days Based on BJWAT

Before treatment in day 00 all groups showed, the wounds were raw and not formed epithelial tissue until day 02 and it was scored 5. In the Negative control until day 4 epithelization was not noted and at 6th day it was scored as 4 until 14th day. In this group at the end of the study only 25-50% of wounds were covered by epithelial tissue. In other groups, epithelial tissues were gradually formed. Positive control I scored 4, 3, 2,1 from day 02 to day 08 respectively, at the end of study all the wounds were epithelialized. Positive control II group showed score 2 at 2nd day at day 08 scored 1. Test Drug I scored 4 at day 2, scored 2 on 10th day and score 1 on 12th day. Test Drug II scored 3 at 2nd day and at 10th day all the wounds covered with epithelial tissue in this group.

Regeneration of the epidermis, reduction in infiltrated cell levels, the role of fibroblasts and endothelial cells in repairing the dermis, and reinforcement of capillary growth and collagen development was previously underlined. Collagen levels providing strength and integrity to the tissue matrix and playing an important role in hemostasis along with epithelialization in Test groups. The effects of antioxidant prevent the oxidation of biomolecules by eliminating overproduced free radicals, removing inflammation products, and facilitating a quicker wound healing process. Lipid peroxidation, which is considered to be one of the most deleterious events occurring in Test groups (Akgun *et al.*, 2017).

Antibacterial agents can facilitate the formation of granulation tissue by preventing bacterial interference in this process. Bacteria can form biofilms on the wound's surface, creating a protective shield that makes them resistant to the body's natural defenses. Infections can lead to more pronounced scarring. flower powder of nerium oleander controlling bacterial growth and promoting optimal wound healing conditions, can help minimize scarring and improve the cosmetic outcome of wound healing (Yousra Shafiq *et al.*, 2021).

The ethanolic extracts of Nerium oleander dried and fresh flowers exhibited potent anti-inflammatory activity against carrageenan-induced hind paw edema model in mice without inducing any gastric damage. It is significantly impeding the natural healing process and may lead to complications. Analgesic activity of nerium oleander flower which involves the relief of pain, can play a significant role in wound healing by comfort and overall well-being during the healing process. It could be enhancing the wound healing in Test groups (Vikas Gupta & Payal Mittal, 2010)

V. CONCLUSION

This pharmacological study consisted of five groups of wistar albino rats (n=6). Among the five groups, two groups were treated with Test drugs. Remaining three groups, one group was treated with Standard drug powder and one group treated with standard drug ointment, and another one was untreated as negative control. comparatively *Nerium oleander* flower ointment shows more significant effect than flower powder. Both test drugs show P<0.05 and it was

significantly effective, Therefore, this study is concluded *Nerium oleander* flower ointment showing high efficacy in wound healing on excised wounds.

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