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WOUND HEALING EFFICACY OF A POLYHERBAL TOPICAL GELIN RAT MODELS OF EXCISION WOUND, INCISION WOUND, AND THERMAL BURN INJURY

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Keywords:

Aloe vera extract, turmeric, Honey, Karanj oil, and cow ghee; Excision wound; Incision wound; Burn injury

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ABSTRACT: Background: In Ayurvedic literature, Aloe vera, turmeric, honey, karanj oil, and cow ghee are documented for medicinal properties like anti-infective, anti-inflammatory, anti-oxidant, anti-coagulant and wound recovery. **Objective:** In this study, we aimed to evaluate the efficacy of a topical gel containing Aloe vera extract, turmeric, honey, karanj oil, and cow ghee for wound healing. **Methods:** Topical gel was prepared and evaluated for skin irritation and efficacy. Skin irritation studies were performed on rabbits according to OECD guidelines 402. Wound healing activity was performed on Wistar rats in the excision wound model, incision wound model, and burn injury model. **Results:** Treatment with formulation significantly ameliorated wounds compared to the vehicle control group in each wound injury model. Efficacy was comparable to the standard which was confirmed by amelioration of wound contraction, increase in tensile strength, attenuation of inflammatory cytokines TNF-α, IL-6, IL-10 and histological studies. Skin irritation studies on rabbits showed that formulation is non-irritant. **Conclusion:** In the topical gel used in this experiment showed enhanced wound healing properties in all three models of the experimental wound without skin irritation.

INTRODUCTION: Wound healing is a dynamic process in which the wound caused by physical, chemical, and microbial processes on the tissue is repaired or regenerated [1]. The process of wound healing starts with an injury where the loss of epithelium occurs [2, 3]. The skin can repair wounds after a series of processes like hemostasis, inflammation, proliferation, and remodeling, which ensures structural and functional integrity of the tissue [4].

A chronic wound can lead to organ failure in an individual, which may also cause death [3]. There are different types of wounds: open wounds, incised wounds, superficial wounds, laceration wounds, puncture wounds, gunshot wounds, penetration

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wounds, close wounds, bruises, hematomas, crush injury, acute wounds and chronic wounds [5]. Lack of proper diet, less oxygen supply, drugs, aging, wasting diseases like Diabetes mellitus, bacterial infections at the wound site, sterility, obesity, movement of wound edges and site of wound affects wound healing [5, 7]. The initial phase of wound healing involves the inflammatory phase, which is followed by collagen and other cellular matrix synthesis and may form a scar [8]. Wound healing is done by a network of blood cells, growth factors, and cytokines which work together to restore the normal condition of the damaged tissue [7]. Different methods are used for healing or management of wounds that include anti-inflammatory agents, dressing, pain killers and anti-bacterial, and agents which can promote wound healing in short duration with less pain & discomfort [9].

It is well known that herbal remedies are used to treat several diseases, including wounds, cuts and burns [10]. In comparison to the standard treatment available for wound healing, herbal treatments show fewer side effects [8]. Also, polyherbal preparations provide safe and cost effective option for wound management [11]. Aloe vera (Liliaceae) is an herbaceous succulent plant having an anti-inflammatory, anti-bacterial, anti-fungal and anti-oxidant activity that promotes wound closure and wound healing [12, 13]. Phytochemical present in herbs promotes fibroblasts' adhesion and proliferation that increases collagen production and secretion from fibroblasts which in turn promotes healing [14, 16].

Turmeric (*Curcuma longa* Fam. *Zingiberaceae*), a widespread herb in Asia, has various medicinal properties like anti-inflammatory, anti-infective, anti-coagulant, anti-oxidant, and radical scavenging activity [17, 18]. It has been used for various dermal infections and is a part of the South Asian diet. Curcumin, its major bioactive component, promotes wound healing by increasing fibroblast production, granulation tissue formation, collagen deposition, tissue remodeling, and increased vascular density and contraction of wound [19, 20]. Oral administration of turmeric has lesser bioavailability [21, 22]. However, topical administration is much effective as it is in direct contact with wound site [23, 26]. Honey is found to have various properties like anti-inflammatory, anti-oxidant, anti-bacterial. It increases the rate of re-epithelialization and helps in wound closure [27]. The application site absorbs all the water and dehydrates bacteria, restricts the pH of wound exudate, thus prevents bacterial colonization. Its high sugar content, phenolic content and non-peroxide factors contribute to wound healing [28].

Karanj oil obtained from the plant *Pongamia pinnata* (*Fabaceae*) is a traditional remedy for treating wounds, piles, tumours, skin itches, rheumatic joints, ulcers, and inflammation. It acts on the proliferative and remodeling stage for wound healing. It increases Superoxide dismutase, an anti-oxidant enzyme that prevents the further generation of free radicals. It also increases the IL-10 level in T-lymphocytes and macrophages, leading to a decrease in pro-inflammatory mediator expression, decreasing inflammatory cells' recruitment to the wound. This oil is also used as a lubricant in industries [29, 30]. Cow ghee, also called clarified butter, is used as a base in ayurvedic medicines. It can transport drugs to deeper tissues in the body hence be used as a vehicle. Cow ghee has its use in wound recovery and is used for skin rashes and broken bones [31]. It contributes to the wound healing process by regulating prostaglandin synthesis, and if applied topically, it can manage pain and swelling. It has anti-microbial, anti-inflammatory properties. It maintains hydration and gives a moist environment for wound healing [32].

Based on the reports mentioned above, we aimed to formulate gel containing these ingredients for the treatment of excision, incision, and burn wounds. The objective of this study was to evaluate wound-healing efficacy of gel containing Aloe vera, Turmeric, Honey, Karanj oil and Cow ghee in animal models of incision wound, excision wound and burn wound.

MATERIALS AND METHODS

Chemicals and Reagents: Major chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Merck Specialities Pvt. Ltd. (Mumbai, India). The solvents used were of high purity and HPLC grade. All other chemicals and reagents used in the whole study were of analytical grade. Aloe vera extract, Turmeric, Honey, Karanj

oil, and Cow ghee were provided by Gufic Biosciences Ltd., Navsari, Gujrat, India.

Experimental Animals: White New Zealand rabbits (2.5-3 kg) were procured from IPCA Lab. Mumbai India. Male Wistar rats (230-250 g) were procured from the National Institute of Biosciences, Pune, India. They were kept in the departmental animal house facility at 25 ± 2ºC and relative humidity 45-55%, light and dark cycles of 12h each, throughout the experiments.Animals were provided with a standard rodent pellet diet and water Ad libitum. The study protocol was reviewed and approved by the Institutional Animal Ethical Committee(Protocol No.: CPCSEA\IAEC\P-36\18)and were conducted in accordance with the guidelines of the 'Committee for the Purpose of Control and Supervision of Experiments on Animals' (CPCSEA), Ministry of Environment and Forests, Government of India.

Preparation of Topical Gel: Topical gel formulation containing Aloe vera, turmeric, honey, karanj oil, and cow ghee was prepared to investigate its wound healing efficacy. To the gel base Aloe vera, turmeric, honey, karanj oil, and cow ghee were added with the following formula to get the final gel formulation.

Aloe Vera: 18.0 % w/w.
Turmeric Extract equivalent to Turmeri: 1.25% w/w.
Cow Ghee: 3.0 % w/w.
Honey: 3.0 % w/w.
Karanj Oil: 3.0 % w/w.
Rose Water: 3.0 % w/w.
Gel base: up to 100%.

Evaluation of the Polyherbal Test Gel: Test gel formulation was evaluated for different pharmaceutical parameters like appearance, homogeneity, pH, spreadability and viscosity.

Skin Irritation Study in Rabbits: The acute dermal irritation study was performed in accordance with the OECD Guidelines 404 for "Acute dermal irritation/corrosion" on White New Zealand rabbits (2.5-3 kg). Briefly, approximately 24 h before the test, around 5 cm × 5 cm of rabbit's trunk was unclipped for experimental use. 0.5 g gel was then applied under a 2.5 cm × 2.5 cm gauze patch and wrapped with an occlusive dressing. The test sites were observed and scored for erythema and oedema at 1 h, 24 h, 48 and 72 h post-exposure with gel. Dermal responses were determined in accordance with OECD guidelines. Erythema and oedema were scored on a scale of 0-4, with 0 showing no effect and 4 representing severe symptoms.

Wound Healing Studies in Rats:
Experimental Protocol: The rats were acclimatized for one week before the commencement of the experiments. Three separate studies were performed for three types of wound models: excision, incision, and thermal burn. For each wound model, animals were divided into three groups of six animals each (n = 6 per group).The grouping of animals was done as follows: Group I (Placebo): treated with gel base (without any drug), Group II (Standard): treated with a reference standard Povidone-iodine 10% (for excision wound and incision wound) and silver sulfadiazine 1% (for burn injury), Group III (Test gel): treated with 0.5 g gel with test drugs.

Excision Wound Model: The excision wound model was used to study the wound contraction rate and epithelization. Animals were anesthetized with ketamine hydrochloride (100 mg/kg, i.m.), and the back hair of the animals was cleared. An impression was made on the dorsal thoracic region 1 cm away from the vertebral column and 5 cm away from the ear on the anesthetized rat. An excision wound of size 3 cm^2 and 2 mm depth was made by cutting out a layer of skin from the shaved area [33]. The entire wound was left open. All the groups received respective treatment once daily for 21 days. The wound closure rate was evaluated by tracing the wound on 0th, 3rd, 6th, 9th, 12th, 15th and 18th post-wounding days using tracing paper and a marker. The traced papers were then scanned and the wound areas were measured using Image J software. Rate of wound contraction was indicated by calculating the changes in wound area. The percentage of wound closure was calculated. The period of epithelialization was also calculated as the number of days required for falling of the dead tissue remnants without any residual raw wound.

Incision Wound Model: All the animals were anesthetized with ketamine hydrochloride (100 mg/kg, i.m.) and hair on the abdomen of the

animals was shaved. A 4 cm long liner incision was made with a sterile surgical scissor through the midline of the abdomen. The wound was then closed with three interrupted surgical sutures of 1 cm apart [34]. The test article and standard were applied to the wound once a day for 21 days. The Control group was left untreated. On the 21st day, all the animals were euthanized. One portion of linear incised skin was measured using a tensiometer for its tensile strength. Wound-breaking strength was evaluated on the 21st day of the experiment.

Burn Injury Model: A second-degree burn was induced on the rat's skin. Initially, the hair on the back of each rat was shaved well, and then the animal was anesthetized using ketamine hydrochloride (100 mg/kg, i.m.). A metal coin with an approximate diameter of 2.5 cm was heated up to 80 °C and placed on the back of rats for 20 seconds [35]. The percentage of healing thermal burn wounds was calculated. The period of epithelialization was also calculated as the number of days required for falling of the dead tissue remnants without any residual raw wound.

Evaluation of Pro-Inflammatory (IL-6, TNF-Alpha) and Anti-Inflammatory Cytokine (IL-10) Levels: Blood samples were collected from all the animals during the study on 1st, 7th, 14th and 21st day. The blood was centrifuged at 5000 rpm for 10 min to separate the plasma. The plasma was used to determine the levels of Pro-inflammatory cytokine (TNF-alpha and IL-6) and Anti-inflammatory cytokine (IL-10) production during healing. The cytokine levels were determined by using ELISA Kits (Kinesis Dx, USA).

Histopathological Analysis: At the end, animals were sacrificed and a 0.5 cm × 0.5 cm skin sample was taken from the wound surface for histopathological analysis. The tissues were preserved in 10% phosphate-buffered formalin. Tissue samples were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. All the sections were observed under a light microscope for evaluation.

Statistical Analysis: The results were calculated and expressed as Mean ± Standard deviation. The data obtained in the studies were subjected to one way of analysis of variance (ANOVA) followed by Dunnet's t-test. A p-value <0.05 was considered to be significant. All the statistical analysis were performed using Graph Pad In Stat Version 3.06 (Graph Pad Software, Inc. La Jolla, CA, USA).

RESULTS:

Characterisation of the Polyherbal Topical Gel: The developed topical gel was a light yellow in colour with good homogenicity and smooth texture. The pH of the formulation was found to be 6.6 ± 0.02. The spreadability was found to be 25 g.cm/sec, calculated using formula (S = M.L/T). Where S = spreadability, M = Weight tied to upper slide, L = Length of glass slides and T = Time taken to separate the slides completely from each other. The viscosity of the formulation was found to be 14001 cps at 30rpm at room temperature, which indicates that the prepared gel was easily spreadable. Based on these pharmaceutical evaluation results, the poly herbal gel was found to be best and satisfactory. The formulations was kept for the physical stability for 6 months and it was found to be stable with respect to all the above mentioned parameters.

Skin Irritation Studies: Skin irritation studies were performed as per OECD guideline 404. The gel formulation did not produce any skin irritation. No signs of erythema, oedema or any unusual changes were observed in rabbits when observed over a week **Fig. 1.** During the 14 days of observation, no irritation was observed. Thus, the formulations can be considered safe for skin application.

Effect of Test Gel on Wound Contraction and Epithelisation Time in Excision Wound: It was found that the treatment with the gel or the reference standard (povidone-iodine, 10%) on the wounds resulted in considerably faster healing compared to the negative control group. A significant attenuation was observed in the wound contraction rate in polyherbal gel treated, and standard treated animals compared to negative control animals ($p<0.05$, **Fig. 2.** The average number of days that took for the shedding of eschar without leaving any residual raw wound (epithelization period) was significantly less for test gel and povidone-iodine compared to the negative control group $P < 0.05$, **Fig. 3.**

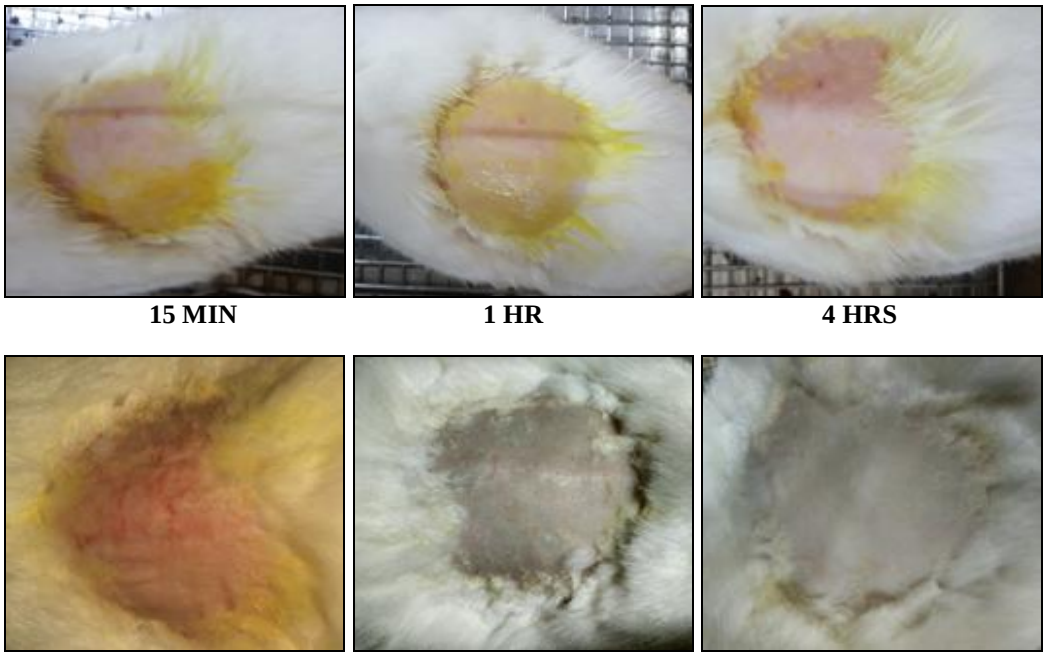


FIG. 1: APPLICATION OF TEST GEL ON RABBIT SKIN SHOWED NO IRRITATION TILL 14 DAYS

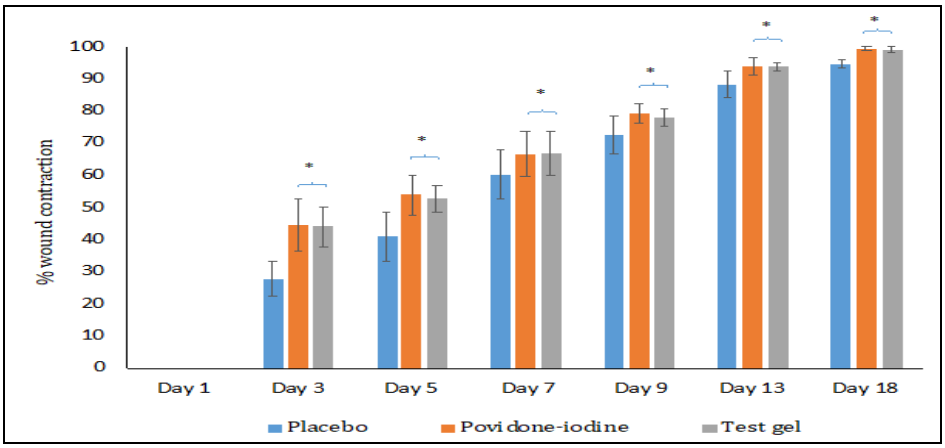


FIG. 2: EFFECT OF TESTGEL ON PERCENTAGE WOUND CONTRACTION IN EXCISION WOUND MODEL. VALUES ARE EXPRESSED AS MEAN ± S.D. (N = 6). STATISTICAL ANALYSIS WAS DONE BY ONE WAY ANOVA FOLLOWED BY DUNNET'S T-TEST. *P < 0.05 COMPARED TO THE NEGATIVE CONTROL GROUP

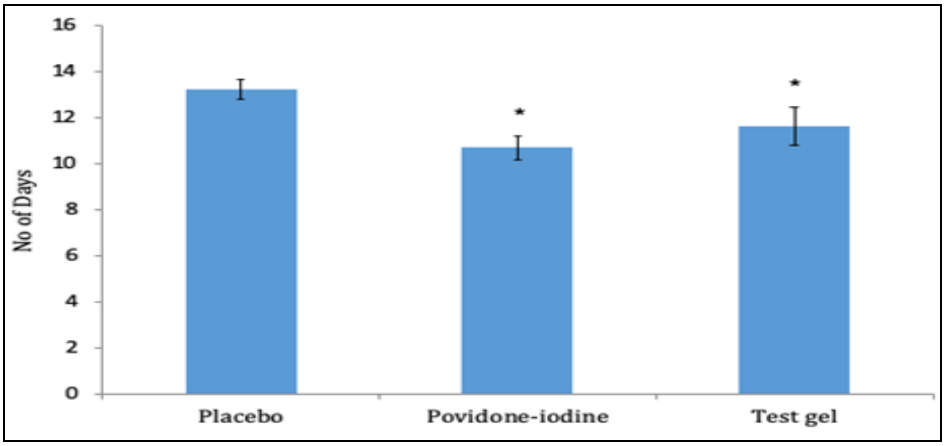
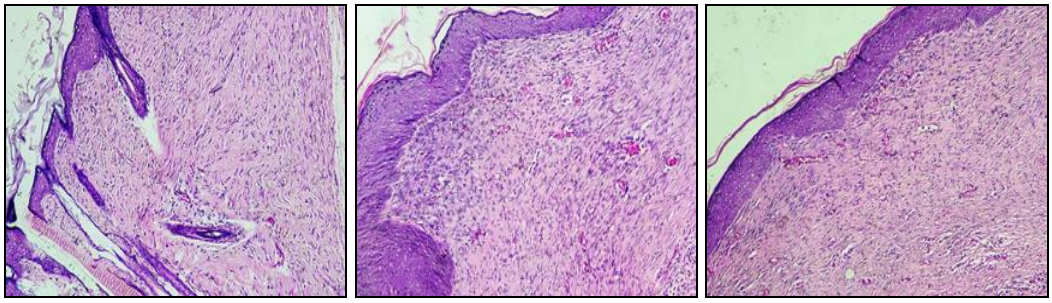


FIG. 3: EFFECT OF TEST GEL ON THE PERIOD OF EPITHELIZATION EXCISION WOUND MODEL. VALUES ARE EXPRESSED AS MEAN ± S.D. (N = 6). STATISTICAL ANALYSIS WAS DONE BY ONE-WAY ANOVA FOLLOWED BY DUNNET'S T-TEST. *P < 0.05 COMPARED TO THE NEGATIVE CONTROL GROUP

Effect of Test Gel on Histology of Healed Skin in Excision Wound Model: Microscopic images of H & E stained excision biopsy of skin at day 21 at 100 × magnification is given in **Fig. 4.** Histological studies of the tissue sections revealed a maximum amount of scarring with signs of inflammatory cells infiltration, few blood vessels, fibroblast, and a sparing amount of collagen for the control group treated rats. Rat's treated with test gel formulation and povidone-iodine 10% showed almost healed skin structures with normal epithelization, restoration of the adnexa, reduction of scarring and inflammatory cells, an increase of fibroblast, blood vessels and collagen deposition. Improvement in histological grades and histological observation was better in the herbal formulation and povidone-iodine group compared to the negative control group.



PLACEBO **POVIDONE-IODINE 10%** **TEST GEL**

FIG. 4: HISTOLOGICAL EXAMINATION OF HEALED EXCISED WOUND TREATED WITH (A) PLACEBO (B) POVIDONE-IODINE 10% (C) TEST GEL

Effect of Test Gel on Incision Wound Injury:

Effect of Test Gel on Wound Breaking Strength: The incision wound healing was measured in terms of the wound breaking strength of the healed skin on the 21st-day post-wound. Results are shown in **Fig. 5**. The result revealed that the wound-breaking strength of the healed skin of rats treated with test gel and reference standard povidone-iodine increased significantly ($P < 0.01$) compared with the control group on the 21st day of post-wound. The wound breaking strength (WBS) for reference standard povidone-iodine was 965.33 ± 52.44 g, whereas for test gel, it was 917.40 ± 105.30. The wound breaking strength for the control group was 652.85 ± 48.17.

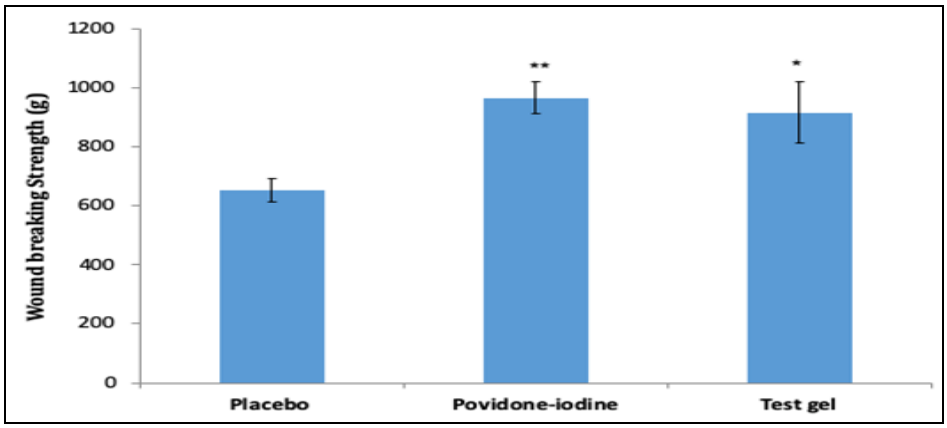
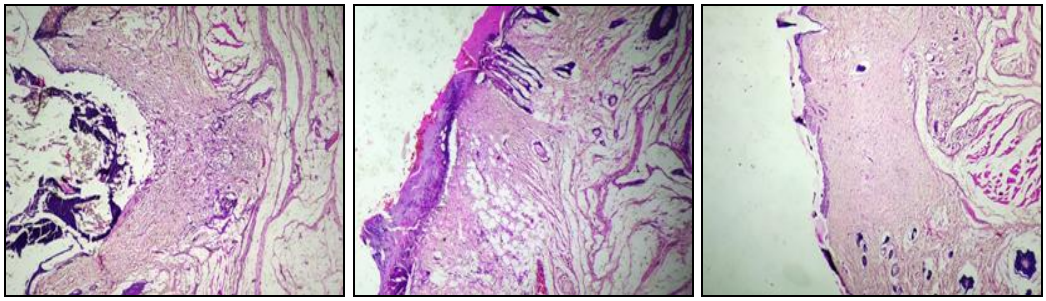


FIG. 5: EFFECTS OF TOPICAL APPLICATION OF TEST GEL ON WOUND BREAKING STRENGTH IN AN INCISION WOUND MODEL. VALUES ARE EXPRESSED AS MEAN ± S.D. (N = 6). STATISTICAL ANALYSIS THE BY ONE WAY ANOVA FOLLOWED BY DUNNET'S T-TEST. **P < 0.01 COMPARED TO THE NEGATIVE CONTROL GROUP; *P < 0.05 COMPARED TO THE NEGATIVE CONTROL GROUP

Effect of Test Gel on Histology of Healed Skin In Incision Wound Model: Microscopic images of H & E stained excision biopsy of skin at day 21 at 100 × magnification is given in **Fig. 6.** In the negative control group, there was decreased epidermis thickness. Degeneration was determined in the hair roots. The degenerative findings in the sebaceous glands in the hair roots and the areas hosting the hair roots were observed to be smaller. Animals treated with test gel and povidone-iodine

showed almost healed skin structures with epithelization and restoration of skin. Improvement in histological grades and histological observation was better in the test gel and povidone-iodine group compared to the negative control group.



PLACEBO **POVIDONE-IODINE 10%** **TEST GEL**

FIG. 6: HISTOLOGICAL EXAMINATION OF THE HEALED INCISION WOUND TREATED WITH (A) PLACEBO (B) POVIDONE-IODINE 10% (C) TEST GEL

Effect of Test Gel on the Burn Injury Model:

Effect of Test Gel on Burn Wound Contraction: The percentage contraction of thermal wounds is given in **Fig. 7.** The results show that percent wound closure was significantly ($P < 0.01$) more in test gel and silver sulfadiazine 1% treated groups than in the negative control group. In the total study period of 21 days, maximum wound closure percent was observed in rats treated with silver sulfadiazine 1% followed by test gel treated group

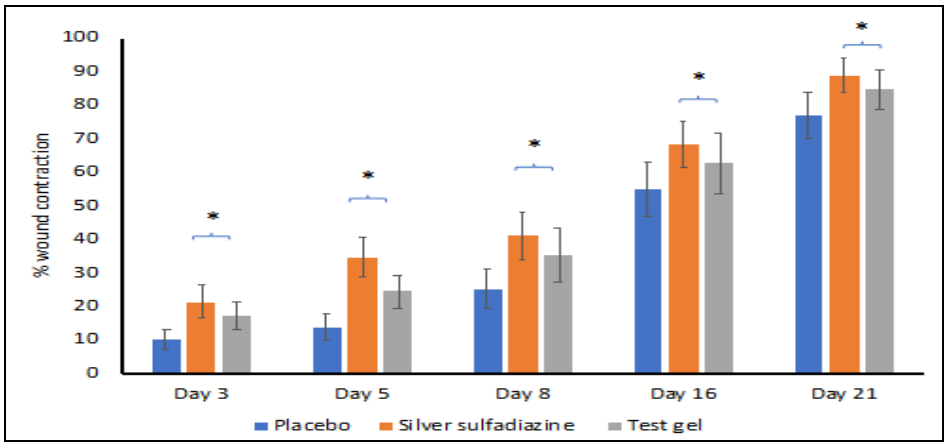


FIG. 7: EFFECT OF TEST GEL ON PERCENTAGE WOUND CONTRACTION IN EXCISION WOUND MODEL. VALUES ARE EXPRESSED AS MEAN ± S.D. (N = 6). STATISTICAL ANALYSIS WAS DONE BY ONE WAY ANOVA FOLLOWED BY DUNNET'S T-TEST. *P < 0.05 COMPARED TO THE NEGATIVE CONTROL GROUP

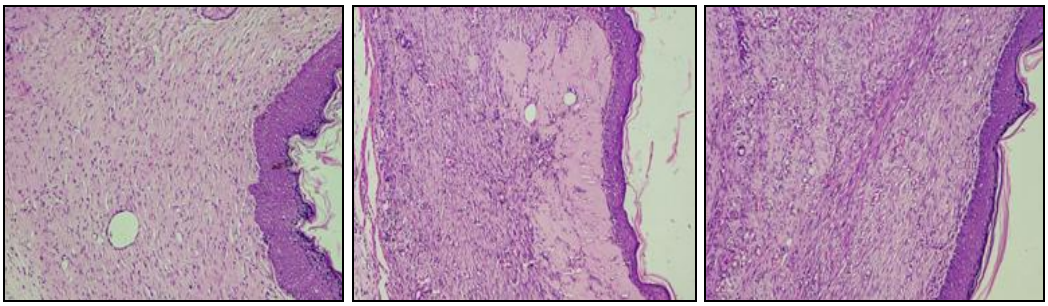


FIG. 8: HISTOLOGICAL EXAMINATION OF HEALED BURN WOUND TREATED WITH (A) PLACEBO (B) SILVER SULFADIAZINE 10% (C) TEST GEL

Effect of Test Gel on Histology of Healed Skin in Burn Wound Model: Fig. 8 shows a representative photomicrograph of skin sections stained with H&E from all groups. Remarkably, inflammatory cells in the wound area were increased in all groups. The highest infiltrations of inflammatory cells were detected in animals from placebo group and test gel and

standard treated animals showed the lowest infiltration.

Effect of Treatments on Pro-Inflammatory (IL-6, TNF-Alpha) and Anti-Inflammatory Cytokine (IL-10) Levels:

Effect on pro-inflammatory (IL-6, TNF-alpha) Cytokines: TNF-alpha and IL-6 are pro-inflammatory cytokines that are elevated during the inflammatory process of wound healing. At low level, they assist in wound healing; however, at high doses they delay the process of wound healing. Thus for rapid healing, their up-regulation should be decreased. TNF-alpha and IL 6 production increased in all the groups initially after 1 day of induction of wounds **Fig. 9** & **Fig 10.** The level of TNF-αin, the placebo group, was significantly ($p < 0.05$) higher than in the test gel and standard treatment groups **Fig. 9** & **10.** On 21st day, the level of TNF-alpha and IL 6 was significantly decreased ($p < 0.05$) in the test gel treated group and standard treatment groups compared to the placebo group.

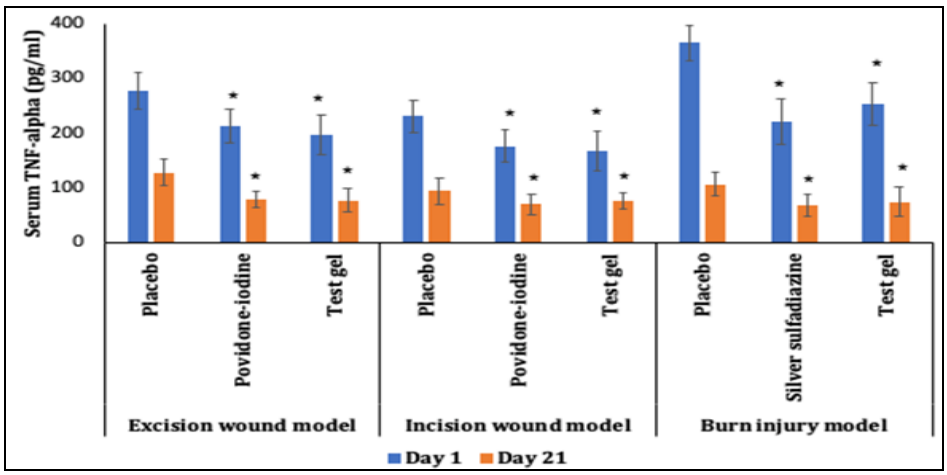


FIG. 9: EFFECT OF TREATMENTS ON PRO-INFLAMMATORY CYTOKINE IL-6 IN DIFFERENT MODELS. VALUES ARE EXPRESSED AS MEAN ± S.D. (N = 6). STATISTICAL ANALYSIS WAS DONE BY ONE-WAY ANOVA FOLLOWED BY DUNNET'S T-TEST. *P < 0.05 COMPARED TO THE NEGATIVE CONTROL GROUP.

Effect on Anti-Inflammatory (IL-10) Cytokines: IL-10, which is an anti-inflammatory cytokine, is a major regulator for suppressing the inflammatory response. It decreases inflammatory cell recruitment by reducing the expression of pro-inflammatory /profibrotic mediators. In this study, the IL-10 level was increased significantly on day 21compared today in all the groups. However, in groups treated with test gel and standard drugs, IL-10 levels were significantly less compared to placebo.

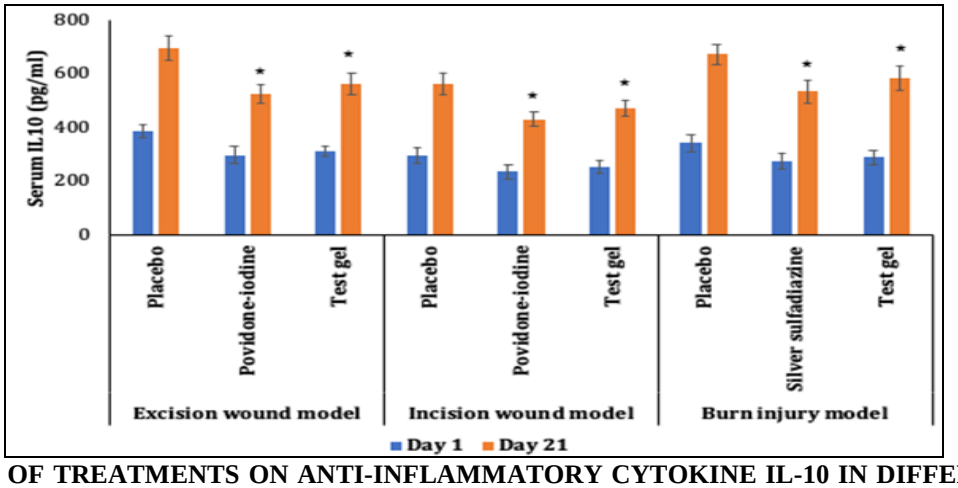


FIG. 10: EFFECT OF TREATMENTS ON ANTI-INFLAMMATORY CYTOKINE IL-10 IN DIFFERENT MODELS. VALUES ARE EXPRESSED AS MEAN ± S.D. (N = 6). STATISTICAL ANALYSIS WAS DONE BY ONE-WAY ANOVA FOLLOWED BY DUNNET'S T-TEST. *P < 0.05 COMPARED TO THE NEGATIVE CONTROL GROUP.

DISCUSSION: The polyherbal test gel contains aloe vera, turmeric, cow ghee, Karanj oil, and rosewater, which were tested and exhibited wound healing properties. The use of herbal medicine and its derivative are gaining importance as an alternative treatment by many countries for various diseases. When the tissue gets damaged, pain arises, and abnormal functioning of the nervous system takes place. If the damaged tissue is not treated or managed properly, it can result in chronic inflammation or serious infection [36]. Pain is also related to the delayed process of wound healing; they dysregulate immune and neuroendocrine function; these two functions are responsible for the mechanism of repairing a wound, so treating the wound with herbal preparations which contain potent wound healing and pain-relieving property, would help in increasing the wound healing activity [37].

The wound healing activity was evaluated using three *in-vivo* models: incision wound, excision wound and burn wound model. The test gel contains ingredients that have been used as folk medicine and have been shown to exhibit wound healing properties and decrease pain. Selecting various ingredients is crucial; it depends on many parameters, including pH, color, appearance, viscosity, density, and many more. Here, in this experiment, the preparation is in gel form. This form of administration of the drug becomes very convenient for applying topically. The reference standard used in this experiment is povidone-iodine, which increases the process of contracting the wound. It reduces migration of fibroblast cell and its proliferation [38].

In an incision wound, the healing of the wound is measured by checking its tensile strength, in which the amount of force required to break the wound is measured. The strength in breaking the wound increases when there is an enhanced formation and stabilization of collagen fibres [39]. In a burn wound, a thermal burn is caused when the skin of the animal is brought in contact with a hot object; the process is very complex due to the loss of tissues and cells. As compared to an incision wound, there is an extensive loss of cells and tissues in a thermal burn. In an excision wound, the skin of the rat is removed surgically, and the wound formed is examined by measuring the decrease in the wounded area after the application of the gel. When tissue gets damaged, the wound healing process starts, and it tries to restore the integrity of the tissue. In many studies, it has been reported that aloe vera improves wound healing when administered topically [40]. It increases cell migration and wound healing process in human keratinocyte monolayer [41]. There are several mechanisms for aloe vera wound healing activity, which involves keeping the wound moist, increasing migration of epithelial cells, rapid maturation of collagen, and reducing inflammation [42].

The wounds treated with curcumin showed fast healing and improved the rates of inflammatory cells, collagen deposition, angiogenesis, granulation, tissue formation, epithelialization and showed beneficial effects when applied topically that accelerated wound healing [43]. It has been reported to improve wound healing for thousands of years and is applied topically to treat various skin conditions like insect bites and chickenpox. It has been shown to improve wound healing in a rat model of full-thickness injuries and heals the wound by changing cell regeneration and collagen synthesis [44]. Consuming curcumin may be protective against burns or it may be an in-treatment option after burns injury [45]. It has been reported that curcumin possesses potent anti-inflammatory, anti-oxidant, immunomodulatory, and anticancer activity [46].

Cow ghee is obtained from the cow's milk and has been reported for various medicinal properties that include cooling in energy, rejuvenating, enhances luster and beauty, enhances memory and stamina, increases the intellect, promotes longevity, protects the body from various diseases and has been reported for wound healing property in mice and rats [47]. Traditionally prepared cow ghee has been reported to show two types of properties like Samshodhana (detoxifying) and Samshana (palliative) and this ghee alone or when combined with honey has been useful for healing inflammatory swellings, wounds and blisters [48]. It has been used in various Ayurvedic traditional preparations and in gel base. It's a rich source of essential fatty acids that regulates prostaglandin synthesis, which helps heal the wound [49]. Wound healing activity was found when animals were treated with karanj oil. The process of wound

healing is the hemostasis process that involves various other processes like reepithelialization, tissue formation, granulation, and remodeling of the extracellular matrix. Treatment with karanj oil shows wound healing activity mainly due to its mitogenic and angiogenic potential. It regulates pro-inflammatory cytokines and anti-inflammatory cytokines along with other systemic immune pathways involved with it [50]. Honey has proven to exhibit anti-microbial activity against various micro-organisms. The antibiotic activity of honey is mainly due to the enzymes present in it; amongst these, some enzymes convert the complex sugars to glucose and form a supersaturated solution with high osmolarity responsible for inhibiting the growth of bacteria [51]. The glucose gets converted to gluconic acid by glucose oxidase, which produces hydrogen peroxide and lowers the pH of honey, due to which honey exhibits anti-microbial activity [52]. Honey moistens the wound and reduces inflammation, edema and exudation and prevents infection [53]. It also stimulates the immune system and increases the healing process by stimulation the proliferative phase [51]. All the parameters like the tensile strength, reduction in wound area, ELISA, and histopathology observed and studied showed enhanced wound healing activity.

CONCLUSION: The study proved that the polyherbal test gel used in this experiment shows enhanced wound healing property in all three models of wound healing, viz. excision, incision, and burn wound model compared with the reference standard. Our data shows and the polyherbal test gel that contains various ingredients like aloe vera, turmeric, karanj oil, cow ghee, honey and rose water treats wound through various processes that include migration of cells, angiogenesis, development of tissue, *etc*. its increases the contraction of tissue that accelerates the healing process.

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