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In-vivo Evaluation of the Wound Healing Activity of the *Sesamum Indicum* L. Seed Extract in Novel Ethosomal Vesicular System

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ABSTRACT

Aim: To establish the wound healing activity of *Sesamum indicum L.* of seed extract in novel ethosomal vesicles. **Methods:** The ethosomal vesicles were formulated with prepared seed extract of *Sesamum indicum L.* by solvent evaporation method and characterized it. The optimized ethosomal vesicles then incorporated into gel base for further *in vivo* study in wistar rat. The evaluation of the wound healing activity was performed by using two models i.e. incision and excision models. In excision model percentage wound contraction and period of epithelialization were established for both the extracts. In incision model the parameter which was carried out was breaking strength of wounded skin. **Results:** The results revealed that the percentage wound contraction, period of epithelialization in excision model was enhanced than that of other groups and tensile strength of skin in incision model was similar to that of standard treated group.

Keywords: *Sesamum indicum L.*, Wound Healing, Ethosomal gel.

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INTRODUCTION

Wound may be defined as a disruption of the cellular and anatomic continuity of a tissue.[1] Wounds are inescapable events of life and they arise due to physical injury, chemical injury or microbial infections. Healing of wounds usually takes place in a direction away from its normal course and under-healing, over-healing or no healing of wounds is common. Management of under healing of wounds is a complicated and expensive program and research on drugs that increase wound healing is a developing area in modern biomedical sciences.[2] Wound healing is a process that is characterized by three overlapping phases: inflammation, tissue formation then tissue remodelling. Wound healing studies are mainly aim to detect various means and factor influencing healing process, so they could be either used or avoid in clinical practice to favourably alter the healing process. Although many indigenous tribes around the world have long suspected that this ubiquitous, annual, herbaceous plant might have medicinal wound healing properties, it has not really got the attention of orthodox medical practitioners as a potential source of a healing agent which may prove to be useful in the treatment of wounds.[3]

Numerous growth factors, cytokines and intricate network of blood cells are involved to restore the normal condition of injured skin[4]. Chronic wounds affect 5.7 million patients annually causing both physiological and mental trauma[5-6]. Irrational use of antibiotics, over the counter drugs and environmental pollution are the main culprits for the development of bacterial resistance thereby hindering the process of wound healing. This necessitated the paradigm shift from allopathic medicine

usage to plant medicine. Poly herbal formulations are better compared to pure isolated chemical alone because polyherbals contain various phytoconstituents that possess anti-inflammatory, antioxidant, antimicrobial properties and show synergistic effect on wound healing process. Hence phytoherbals show better safety, efficacy and paucity of adverse reaction. Hence systematic scientific investigation is utmost important to explore the pharmacological activities of herbal medicines and to elucidate the claims made about them in traditional medicines[7].

Present investigation is carried out on herbal extract of seeds of *Sesamum indicum L.* It is belongs to the family Pedaliaceae, is a high altitude medicinal plant having an excellent nutritional content along with a huge pharmacological profile. Sesame is used in villages for auspicious occasions, rituals, religious sacrifices and marriage ceremonies due to its religious and mythological importance. Apart from its religious significance sesame is used as medicine. Sesame is known as the king of oil seeds due to the high oil content (50–60%) of its seed[8]. The seeds of sesame contain a number of important phytochemicals which includes alkaloids, flavonoids, glycosides, phenols, anthraquinones, tannins, carbohydrates and proteins. Sesame reveals the truth that it is a more beneficial plant with anti-pyretic, anti-inflammatory, antioxidant, anti-microbial, anti-hypertensive, anti-cancer, wound healing activity and other properties[9].

The present study was aimed to formulate and investigate improved wound healing activity of novel vesicular carrier in the form of ethosomes which enhances the skin delivery of drugs. Ethosomes are soft malleable lipid vesicles composed mainly of phospholipids, alcohol (10-40%) and water. The physicochemical characteristics of ethosomes allow this vesicular carrier to transport active substances more efficaciously through the skin. Ethosomes has become an area of research interest in herbal formulation because of its enhanced skin permeation and improved entrapment efficiency.[10]

The aim of the present study was to *in vivo* assess the improved wound healing activity of the *Sesamum Indicum* L. seed extract in novel ethosomal vesicular system**.**

MATERIALS AND METHODS

Collection of Plant material

Authenticated seeds of *Sesamum indicum L.* were procured from Nashik, Maharashtra. All the other solvents and reagents were of analytical grade.

Preparation of extract

Soxhlet extractor was used for sesame oil extraction for 4 hours by maintaining solvent to solid ratio (25:1). Ethanol had been used as solvent for process, performed in triplicate. The temperature was maintained at 40-50^{0}C. The solvent was removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator to obtain the extract.[11,12]

Preparation of ethosomes

Formulation of the ethosomes (F1 to F5) was followed by solvent dispersion technique. The ethosomal system is comprised of 1-4% phospholipids, 20-40% ethanol and aqueous phase to 100% w/w. Phospholipids were dissolved in ethanol and span 20 in extracted sesame oil. This mixture was heated to 30°C±1°C in a water bath while the aqueous phase was prepared by dissolving tween 20 in double distilled water then heated to 30°C±1°C. The resulting aqueous solution was added slowly in a fine stream to the lipidic solution in the centre of the vessel with constant stirring by using magnetic stirrer at 700 rpm in a closed vessel. The temperature was kept30°C throughout the experiment. The mixing was continued for additionally five minutes[13]. The prepared ethosomes were then sonicated at 4°C by using probe sonicator for three cycles of five minutes each with the interval of five minutes and then refrigerated.

Characterization of Ethosomes

In the present study, the physical properties of the ethosomal vesicles were identified with the optical microscopy, phase contrast microscopy and transmission electron microscopy which showed the shape and surface morphology of the ethosome vesicles. Zeta Potential, vesicle size and PDI were measured by Zetasizer (Malvern Instruments, Malvern).[14,15]

The optimized ethosomal vesicle batch was then added to the carbopol 934 gel base with gentle stirring to obtain the ethosomal gel. This ethosomal gel was used for further *in vivo* studies.

In-Vivo Evaluation

Animals Used

Animal selected for this study are: Wistar male rats weighing 150 g-175 g (approx). These rats were used in laboratory conditions for a period of seven days. The rats were acclimatized at 24± 2^{o}C at a relative humidity of 40-55% and light-dark cycles of 11 and 13 hr resp. They were provided with rodent diet and water *ad libitum* during experiment. All studies were approved by Institutional Animal Ethics Committee of the Department, and the guidelines given by CPCSEA, New Delhi were strictly followed.

Acute dermal toxicity

Acute dermal toxicity study was carried out to determine the dose as per Organisation for Economic Co-operation and Development [OECD guidelines No. 402 (OECD guidelines 1987)][16]. The optimized formulation of ethosomal gel containing sesame oil (7.5% w/w) was evenly applied to a small area (Not less than 10 % of the body surface area) of the closely clipped skin of 10 animals. The site of application was covered with a cotton gauze patch, which was kept in contact with skin by means of semi-occlusive dressing. Toxicity test was carried out followed by Omale James, O. and Ajidahun B. S.[17]

Study Design

Both animal models contain eight groups each and divided into groups; Group 1: Control (Untreated), Group 2: Control (Treated with ointment base (SOB), Group 3: Standard group treated with povidone iodine ointment USP (Betadine ointment), and Group 4: Treated with ethosomal gel. All the treatments were given once daily.

Excision Wound Model

For excision wound four groups of animals each consist of six rats, were shaved using depilatory cream on the dorsum portion followed by anesthetize using ketamine hydrochloride (50mg/kg, i.p.,/body weight).On shaved dorsal region an impression was made and area marked (circular area of 314mm$^{2)}$, wound created along the marking (with tools a surgical blade, toothed forceps, and pointed scissors). Animals were left undressed to the open environment. To them accordingly simple ointment base, Betadine ointment and formulated ethosomal gel were applied o.d from the initial day of wound creation till the complete wound healing. With this model, wound contraction/retrenchment and epithelialization period were examined. Wound retrenchment estimated as % contraction every fourth day following wound creation. Finally all the animals of each group were anesthetized and specimen samples of tissue were collected from healed wounds by leaving a 5mm margin of normal skin around edges. These tissues were stored in formalin solution (10%) followed by histopathological and biochemical analysis.[18]

Incision Wound Model

For incision wound the animals were grouped and treated similarly as excision wound model. Animals were anesthetized with ketamine hydrochloride (50mg/kg, i.p./body weight) followed by paravertebral incision of 6 cm length on either side of the vertebral column of the animals. After hemostasis, the wound were stitched using interrupted sutures placed approx. After stitching, created wound was left undressed and treated daily for next 10 days. After which all animals were anesthetized and tensile strength of cured wound skin was measured using tensiometer by removing the applied sutures.[19]

Wound Healing Evaluation Parameters

Measurement of Wound Contraction and Epithelialization Period

The wound area was measured by tracing the wound with transparent paper sheet on days 0, 4, 8, 12, and 16 for all animal groups. The percentage wound contraction was calculated using the following formula:

$$\% \text{ wound contraction} = \frac{\text{Initial wound area} - \text{Specific day wound area}}{\text{Initial wound area}} X100$$

Epithelialization period is a measure use to determine the number of days required for declining the dead tissue miscellany of the wound.

Measurement of Tensile Strength

The tensile strength during wound healing indicates extent of wound healing. It signifies amount of healed tissue resists to breaking under tension. At the end of 10th day, all the animals were anesthetized by injecting ketamine hydrochloride (50mg/kg, i.p., body weight), the healed tissue was excised from all animals. Tensile strength was measured with the help of tensiometer of the excised tissue.

Hydroxyproline Estimation

Excised wound tissues from all animals were estimated for hydroxyproline. Tissues were dried in a hot air oven at 60^{0}C followed by hydrolyzed in 6N HCL for 4h at 130^{0}C. Then neutralized to pH 7.0 and were subjected to Chloramine-T oxidation for 20min. After 5 min, the reaction was terminated by the addition of 0.4M perchloric acid and developed color with Ehrlich reagent at 60^{0}C. After thorough stirring the samples were analyzed at 290 nm in ultraviolet spectrophotometer (Shimadzu, UV-1800). The hydroxyproline content in the tissue samples was calculated using a standard curve of the pure L-hydroxyproline.[20]

Statistical analysis

The results are expressed as mean ± standard deviation; the differences between the experimental groups compared via ANOVA (one-way analysis of variance). The obtained results were considered statistically significant $P<0.05$. Graphs were prepared using GraphPad Prism 3 (Graph Pad Software, Inc).

Skin Irritation Study

Skin irritation study was carried out to assess the potential of the test articles to produce skin irritation and corrosion when applied to the skin of rats and to assess the potential of the test articles to produce acute changes when applied to the skin of rats.

RESULT AND DISCUSSION

Characterization of the ethosomal vesicles

The vesicles were spherical in shape. Both phase contrast microscopy and TEM studies, confirmed well-identified and spherical shape of vesicles. The polydispersity values of the formulations were very low which indicated uniformity of droplet size within the formulation. The ethosomal vesicles showed negative ZP (−17.0 to 47.7 mV) caused by the net charge of the lipid composition in the formulation as shown in Table 1. The negative value for zeta potential is liable for improved percutaneous penetration of drug. The maximum entrapment efficiency of ethosomal vesicles as determined by ultracentrifugation was 97.26 ± 0.65 % for F5 formulation containing 30% ethanol concentration.

Table 1: Vesicle size, Polydispersity index and Zeta potential of ethosomal vesicles

Formulation Code	Vesicle Size* (nm)	Polydispersity Index (PDI)	Zeta Potential (mV)
F1	139.7 ± 10.55	0.114	-23.9
F2	160.6 ± 11.20	0.259	-17.5
F3	158.1 ± 19.40	0.194	47.7
F4	187.3 ± 08.80	0.209	-31.1
F5	231.8 ± 12.43	0.348	-25.5

*Data represented as mean ± SD, n=3

On the basis of vesicle size, uniform size distribution, and higher EE batch F5 formulation was selected for further studies *in vivo* studies.

In-Vivo Evaluation of optimized formulation

In the present study, the wound healing potential of ethnobiologically popular herbal drug, *Sesamum Indicum L* was enhanced and reframed in ethosomal novel drug delivery system with great advantages over traditional systems like avoidance of first pass metabolism, sustainable duration of action, improved pharmacological response and high patient compliance[21]. Wound healing involves a complex interaction between epidermal and dermal cells, the extracellular matrix, controlled angiogenesis and plasma derived proteins, all co-ordinated by array of cytokines and growth factors.

The optimized ethosomal vesicle was then added to the carbopol 934 gel base with gentle stirring to obtain the ethosomal gel. This ethosomal gel was used for following *in vivo* studies.

Acute Dermal Toxicity Studies

The acute skin irritation and toxicity study was performed as per OECD guidelines- 402 (OECD guidelines, 1987) to determine safety of the ethosomal gel of sesame oil (7.5% w/w) was applied once to the shaved portion at the back of the rat and observed for 14 days for an abnormal skin response, including irritation, redness, itching, and other related symptoms. There were no mortality, abnormal clinical signs or remarkable body weight changes were observed in all the experimental animals. No gross pathological observations were recorded in all the experimental animals.

Cage-side observations

Acute application of test formulations did not reveal any abnormal changes in cage side observations.

Mortality

No mortality was observed in any of the groups within the entire period of observation. All the experimental animals survived the entire duration of observation.

Table 2: Mortality data in acute dermal toxicity studies

Group/ Treatment	Mortality	
	Absolute	%
Control group Distilled water	0/5	0
Optimized Ethosomal Gel	0/5	0

LD_{50} Calculation

The LD_{50} by dermal route in rats, of the ethosomal gel of sesame oil could not be determined since the highest dose 7.5% that was employed in this study by dermal route, did not produce any mortality. The LD_{50} of the ethosomal gel of sesame extract on a single dermal application is therefore > 7.5 %. No clinical signs of toxicity were observed after application on skin, of sesame oil loaded formulation in rats throughout the observation period after application.

Body weights

All the experimental animals exhibited similar weight gain throughout the observation period of 14 days following dosing. All the experimental animals showed normal weight during the study period.

Table 3: Mean Body-Weights (g) of animals in acute dermal toxicity studies

Group/ Treatment	00^{th} Day	07^{th} Day	14^{th} Day
Control group Distilled water	150.29	183.45	232.18
Optimized Ethosomal Gel	152.46	195.34	230.77

In-Vivo Wound Healing Activities

The wound healing of formulated gel was evaluated using excision wound animal model. Reduction in wound area of different groups on days 4, 8, 12 and 16th days using excision wound model was calculated, compared and depicted in Figure 1 and table 4. Least rate of wound healing is observed in control groups and faster wound healing was seen in group treated with optimized ethosomal gel.

Table 4: Effect of ethosomal gel on % wound contraction of wound in excision wound model

Group	% wound contraction			
	4thday	8thday	12thday	16thday
Group 1: Control (Untreated)	3.9±0.82	18.62±0.69	2.62±2.56	68.87±0.91
Group 2: Control (Treated with ointment base (SOB).	2.1±0.19	19.8±0.93	2.76±1.36	71.2±0.93
Group 3: Standard group treated with povidone iodine ointment USP (Betadine ointment).	1.55±0.87	38.39±0.6	67.38±2.01 a***,b***	76.68±0.3 a***,b***
Group 4: Treated with ethosomal gel.	13.33±0.9 a***,b***	31.82±0.67 a***,b***	59.6±1.18 a***,b***,c*	93.58±0.76 a***,b***

All values are represented as mean ± SEM, n = 6 animals in each group. Data were analyzed by one-way ANOVA, followed by Tukey-Kramer Multiple Comparisons Test. a: significant difference as compared to untreated group (group I); b: significant difference as compared to ointment base treated group (group II); c: significant difference as compared to standard group (group III), and **P < 0.01, ***P <0.001.

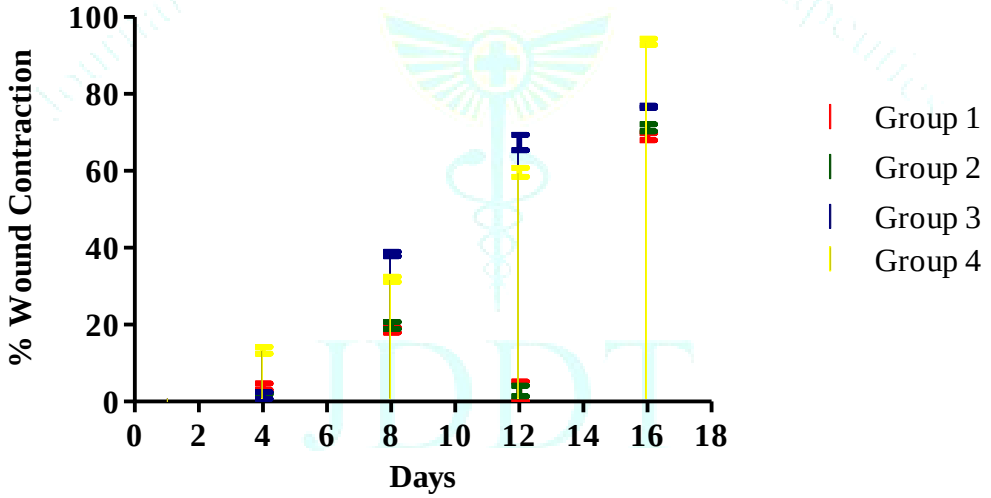

Figure 1: Effect of ethosomal gel on % wound contraction and epithelialization period

All values are represented as mean ± SEM, n = 6 animals in each group. Data were analyzed by one-way ANOVA, followed by Tukey-Kramer Multiple Comparisons Test. a: significant difference as compared to untreated group (group I); b: significant difference as compared to ointment base treated group (group II); c: significant difference as compared to standard group (group III), and **P < 0.01, ***P <0.001.

Effect of ethosomal gel on % wound contraction and epithelialization period

Wound healing is a complex, multifactorial process differs pathologically and making it difficult to understand the underlying mechanism. Perfect wound healing is demarcated with complete closure of wounds in less span of time without any adverse effect. In the present investigation it was observed that new vesicular ethosomal gel (optimized formulation) on topical application showed potent wound healing property, indicating that it enhances various phases of healing process. The potent wound healing property of ethosomal gel may be due to the synergistic activity of phytochemical constituents. Phytoconstituents such as flavonoids, triterpenoids possess free radical scavenging property; modulate the immune system which is complementary to wound healing process.

Wound healing is a natural and inbuilt process but delayed by oxidative stress, diabetes mellitus and by microbial infection. Wound healing process involves cell proliferation, inflammation suppression and contraction of the collagen. Antioxidant activity helps in the release of oxygen radicals, reduces oxidative stress thus controlling microbial infection and clearing the wound fibrin matrix, thus enhancing the healing

process[7]. The optimized ethosomal gel formulation shown better wound healing activity and it may be due to antioxidant activity of flavonoids.

Wound healing process involves several steps such as hemostasis, coagulation, inflammation, granulation tissue formation, matrix formation, connective tissue remodeling, collagenization and acquisition of wound strength[22]. In excision wound, all three phases coexist.

The results of the excision wound model manifest that the percentage of wound contraction is 68.87±0.91% in control untreated group on the 16th day, 71.2±0.93% in control untreated group and in standard treated group it is 76.68±0.30%; whereas it is 93.58±0.76% in optimized ethosomal gel treated group. Wound contraction on the 16th day itself is increased in ethosomal gel formulation when compared with control and standard groups.

The epithelialization was observed from the first day. The epithelialization time was found to be lesser in group treated with optimized ethosomal gel when compared with the other three groups. The results of the epithelialization period in excision wound model marked that the epithelialization period of wound is 19.16±0.7 days in control untreated group, 19.66±0.66 days in control untreated group and in standard treated group it is 17.33±0.58 days; whereas it is 17.66±0.7 days in ethosomal gel formulation treated group shown in Figure 2. Epithelialization period of wound itself is decreased in ethosomal gel formulation when compared with control and standard groups.

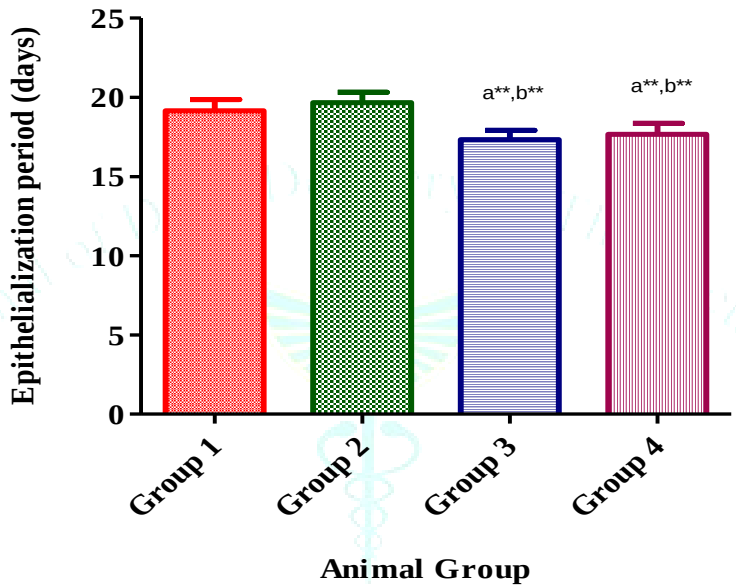

Figure 2: Effect of ethosomal gel on epithelialization period of wound in excision wound model

All values are represented as mean ± SEM, n = 6 animals in each group. Data were analyzed by one-way ANOVA, followed by Tukey-Kramer Multiple Comparisons Test. a: significant difference as compared to untreated group (group I); b: significant difference as compared to ointment base treated group (group II); and $^{**}P < 0.01$, $^{***}P < 0.001$.

Re-epithelialization is a process of epidermal restoration and it involves proliferation and migration of keratinocytes. Dermal and epidermal regeneration in optimized ethosomal gel treated rats is increased indicating that the gel had a constructive effect towards cellular proliferation, granular tissue formation and epithelialization.

Throughout the course of treatment the ethosomal gel was establish its prelude effect from day 4 up to day 16 (Figure 3). The assessment of on day 16 incognito errand the impending healing effect, it shows the maximum substantial consequence by ever-increasing wound contraction with respect to control ($P < 0.001$) and standard groups ($P<0.01$) and ointment base treated ($P < 0.001$) that proportionally confer healing process. As indicated by rate of epithelialization for the ethosomal gel it proves ointment base treated group.

The present investigation its contributing role in the hasten epithelialization rate and required lesser time to complete epithelialization process ($P <0.01$) as compared to control and the confirmed that the rate of percentage wound contraction was higher and epithelialisation period was shorter in optimized ethosomal gel treated groups of rats. In conclusion, topical administration of optimized ethosomal gel accelerated the scar formation and promotes various phases of wound healing such as collagen synthesis, wound contraction and epithelialization.

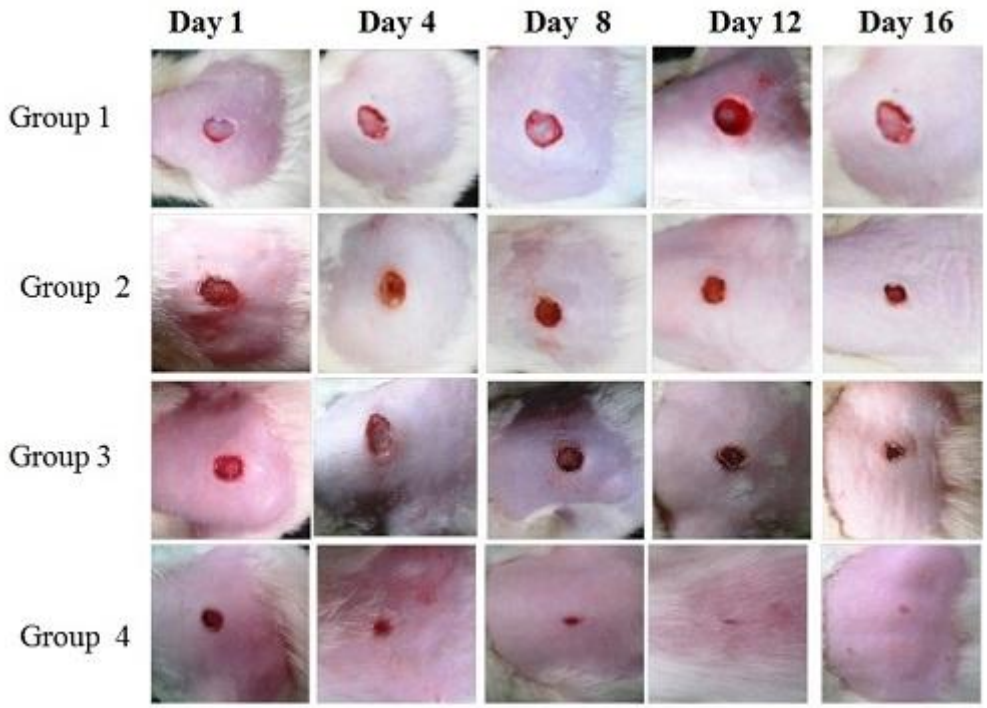

Figure 3: Photographs of wound repair at different time interval in excision wound model

Effect of ethosomal gel on tissue hydroxyproline content

Wound healing is dynamic and there is an interactive process involving soluble mediators, blood cells, extracellular matrix, and parenchymal cells. Though the healing process be accomplished by itself and does not need much help, but different risk factors such as infection and delay in healing has brought attention to promote this process. There is almost unanimous settlement that collagen has a major role in restoring strength and remodeling scar tissue. Biochemical analyses have showed that increased hydroxyproline content is a reflection of increased collagen production. Collagen is one of the most dominant extracellular matrix proteins in the granulation tissue and is manifested to be significantly high by the fifth day after skin injury. After day 7, collagen production is further progressive[20].

In this study, ethosomal gel was evaluated for hydroxyproline content shown in Figure 4.

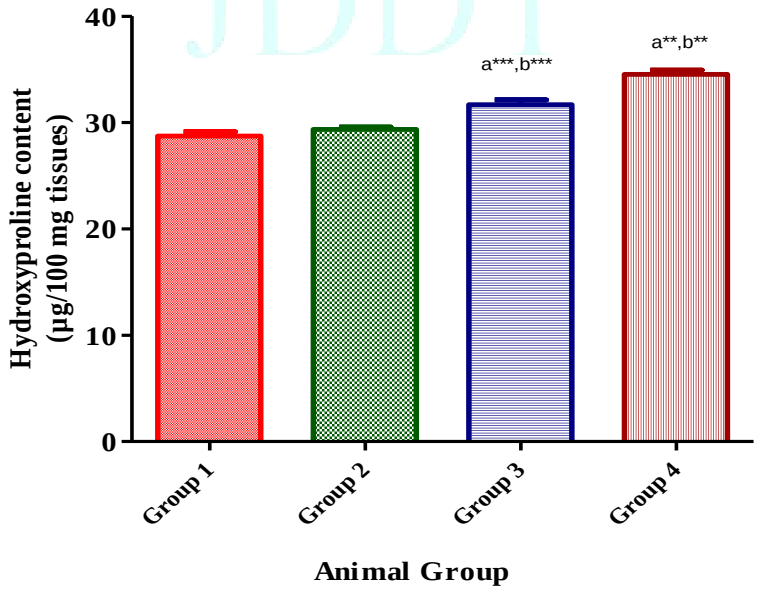

Figure 4: Effect of ethosomal gel on tissue hydroxylproline content in excision wound model

All values are represented as mean ± SEM, n = 6 animals in each group. Data were analyzed by one-way ANOVA, followed by Tukey-Kramer Multiple Comparisons Test. a: significant difference as compared to untreated group (group I); b: significant difference as compared to ointment base treated group (group II) and **P <0.01, ***P <0.001.

The collagen synthesized gets deposited at the wound site and undergoes cross linking to form fibres. Collagen not only offers strength and integrity to the tissue matrix but also plays an imperative role in homeostasis and in epithelialization at the later phase of healing. Hydroxyproline, the major constituent of collagen serves as a marker of collagen synthesis at the wound site. Increased hydroxyproline content ultimately responsible for increasing the collagen level confirmed the increased viability or microcirculation of collagen fibrils around the wound area. The amount of collagen content in granulation tissues of control and experimental induced wounds was measured suggesting that optimized ethosomal gel formulation enhanced collagen synthesis and deposition and it is due to increased cell division.

The hydroxyproline level was found to be significantly elevated ($p < 0.01$) in treated group animals in a concentration dependent manner in comparison to control and ointment base treated group (Figure 4). The relative order for different groups in accordance to collagen stability or wound strength was at ethosomal gel containing sesame oil > standard group > ointment base treated > control.

Effect of ethosomal gel on tensile strength of wound in incision wound model

An ideal wound healing agent must have the property of increasing the viability of collagen fibrils around the wound area that increases the tensile strength of the wound that was assessed by evaluating the tensile strength of the healed wound using tensiometer. Comparison of the tensile strength of the healed skin of the rats in different groups is shown in Figure 5. Minimum tensile strength was noticed in control untreated group. Among the tensile strength of the tissues treated with ethosomal gel; standard group and control treated group exerted more or less the same strength.

In incision wound model the tensile strength of the tissues was measured on 16th day and in control untreated group it was 159.32 ± 3.53, in control untreated group was 171.36 ± 1.32 and in standard treated group was 221.12 ± 6.53 while in optimized ethosomal gel treated group it was 193.36 ± 4.89. From the above results it is inferred that the tensile strength was increased in optimized ethosomal gel treated rats in comparison with control groups (untreated and treated) and this augment in tensile strength of optimized ethosomal gel treated group may be due to the increase in collagen, hexosamine content and stabilization of the fibres by increased protein concentration[23].

The ethosomal gel was found to possess significant concentration dependent action in increasing the tensile strength as compared to control and ointment base treated group ($P < 0.01$ and $P < 0.001$).

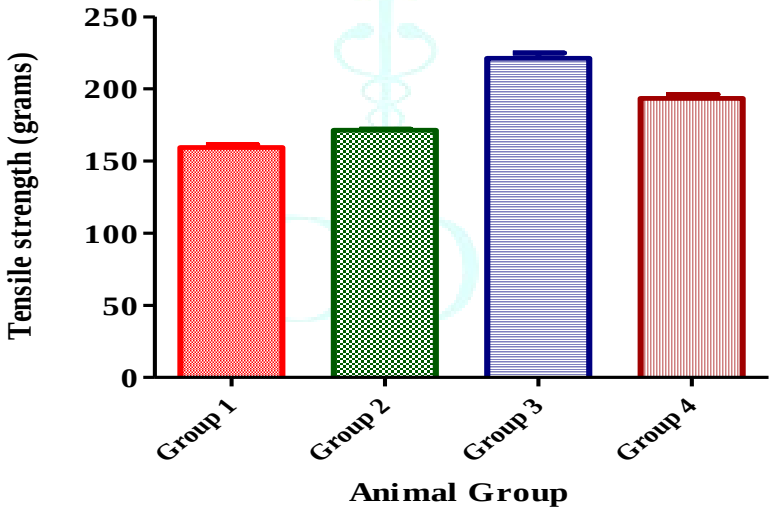

Figure 5: Effect of ethosomal gel on tensile strength of wound in incision wound model

All values are represented as mean ± SEM, n = 6 animals in each group. Data were analyzed by one-way ANOVA, followed by Tukey-Kramer Multiple Comparisons Test.

Skin Irritation Studies

The skin irritation testing was carried out to evaluate the primary skin irritation potential of the developed ethosomal formulations.

A. Mortality/Morbidity

There were no deaths or evidence of impending death during the in-life period.

B. Clinical Observations

During the study, reduction in severity of skin irritation was also assessed. Severity of skin irritation was rated at five different levels. The skin irritation was almost absent in case of optimized formulation of ethosomal gel where as for standard treated group was rated at slightly patchy erythema (Table 5). The level of skin irritation was rated severe for control group.

Table 5: Skin irritation studies of Ethosomal Gel formulation

Group	No. of Hours			
	1 hr	24 hrs	48 hrs	72 hrs
Group 1: Control (Untreated)	---	+	+++	+++
Group 2: Standard group treated with povidone iodine ointment USP (Betadine ointment).	---	+	±	±
Group 3: Treated with ethosomal gel.	---	---	+	---

Severity:

1. No reaction : ---
2. Slight, patchy erythema : ±
3. Slight but confluent or moderate but patchy erythema : +
4. Moderate erythema : ++
5. Severe erythema with or without edema : +++

This improved performance in countering skin irritation levels by ethosomal formulations may be due to higher skin deposition of drugs from ethosomal gels. Some solvents, such as alcohol, DMSO, propylene glycol may extract lipids from skin thereby forming aqueous channels within the stratum corneum which increases permeability. Unfortunately many of such skin penetration enhancers that act on lipid bilayer cause skin irritation there by limiting their clinical application[24], but during this study it was very much evident that ethosome formulations did not cause any type of skin irritation, rather it has reduced the level of skin irritation caused by wound infection which makes ethosomal gel as one of the best vehicles to administer wound healing or anti-inflammatory drugs.

CONCLUSION

In vivo studies revealed that *Sesamum Indicum L* seed extract containing ethosomal vesicles incorporated into gel based system provided appropriate physiological environment for re-epithelialization and revascularization at wound site. The fabricated formulation provides not only a synergistic carriage of therapeutic agents, but also a familiar as well as anti-infection environment to support the cell proliferation and healing process. The novel ethosomal vesicles showed promising improved wound healing activity in rats.

Conflict of interest

The authors declare no conflict of interest.

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